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**Taxonomy of Genus *Puntius* (Hamilton, 1822) and *Pethia*  
(Hamilton, 1822) in the Lakes of Pokhara Valley**

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**A dissertation submitted**

**In partial fulfilment of the requirements for the award of the degree  
of Master of Science in Zoology with special paper Fish Biology and Aquaculture**

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**May 2025**



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**Dissertation submitted in partial fulfilment of the requirements for the degree of  
Master of Science in Zoology with special paper Fish Biology and Aquaculture**

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**May 2025**

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May 2025

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## Declaration

I hereby declare that the work presented in this dissertation “Taxonomy of Genus *Puntius* (Hamilton, 1822) and *Pethia* (Hamilton, 1822) in the Lakes of Pokhara Valley” has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

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### Recommendation

This is to recommend that the dissertation entitled “Taxonomy of the Genus *Puntius* (Hamilton, 1822) and *Pethia* (Hamilton, 1822) in the Lakes of Pokhara Valley” has been carried out by Anjali Poudel for the partial fulfilment of Master’s Degree of Science in Zoology with special paper Fish Biology and Aquaculture. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institutions.

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**Certificate of acceptance**


This dissertation work submitted by Anjali Poudel entitled “Taxonomy of *Puntius* (Hamilton, 1822) and *Pethia* (Hamilton, 1822) in the Lakes of Pokhara” has been accepted as a partial fulfilment for the requirements of Master’s Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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Anjali Poudel

## Abstract

This study investigates the taxonomy, morphology, and morphometric characteristics of two *Puntius* species (*Puntius sophore* and *Puntius chola*) and one *Pethia* species (*Pethia conchoni*) inhabiting the three lakes of Pokhara Valley, namely, Phewa, Begnas and Rupa lake. A total of 65 specimens were analyzed for 38 morphometric characters and 12 meristic counts, with their morphological features compared to earlier descriptions. Morphometric analysis revealed significant interspecies differences: *P. chola* exhibited the largest total length (TL) at 8.32 cm, followed by *P. sophore* (6.17 cm) and *P. conchoni* (4.97 cm). Similarly, *P. chola* had the greatest standard length (SL) at 6.46 cm, while *P. conchoni* was the smallest at 3.90 cm. Body depth (BD) was highest in *P. chola* (2.31 cm), followed by *P. sophore* (1.71 cm) and *P. conchoni* (1.31 cm), reflecting potential ecological niche differentiation. Meristic traits further supported species differentiation, with *P. chola* displaying larger fin measurements across dorsal, pectoral, pelvic, and anal fins. Statistical analyses, including correlation and regression, revealed varying degrees of predictability in morphometric traits relative to total length (TL) and head length (HL). *P. chola* exhibited the strongest correlations (e.g.,  $R = 0.83$  for SL-TL), while *P. sophore* and *P. conchoni* showed moderate to weak correlations ( $R$  values ranging from -0.62 to 0.56), suggesting environmental or genetic influences on variability. The PCA and truss analyses reveal clear morphological differentiation among the three species. *Puntius chola* is distinctly separated while *P. sophore* and *P. conchoni* show closer similarity. PCA analysis revealed that PC1 accounted for 98.7% of the total variation, clearly separating *P. chola* (score: 6.65) from *P. sophore* (-1.23) and *P. conchoni* (-5.41). PC2 explained only 1.3%, reflecting minor differences, with *P. conchoni* at 0.52, *P. chola* at 0.28, and *P. sophore* at -0.79. PCA plot shows AFL and PFL strongly influenced by PC1, aligning with *P. chola*'s distinct score, while DFH and DPA contributed negatively, corresponding to the lower scores of *P. sophore* and *P. conchoni*. The dendrogram based on truss analysis showed that *P. sophore* and *P. conchoni* clustered closely, while *P. chola* branched off at a much higher distance, confirming its distinct morphology. In the dendrogram, the vertical height represents the level of difference between the species' body shapes. These findings confirm the effectiveness of multivariate morphometric methods in species discrimination. This study provides the foundational data crucial for the future investigations concerning taxonomy of the fish species found in lakes of Pokhara valley.

## शोध सारांश

यो अध्ययन पोखरा उपत्यकाका तालहरू, फेवा, बेगनास र रूपा तालमा पाइने तीन प्रजातिका माछाहरू *Puntius sophore*, *Puntius chola* र *Pethia conchoni* को morphological, morphometric and meristic विशेषताहरूको आधारमा वर्गीकरण गरिएको छ। कुल ६५ वटा नमुनाहरू संकलन गरि ३८ वटा morphometric विशेषता र १२ वटा meristic गन्तीहरूको विश्लेषण गरेर प्राप्त मापनहरूलाई अघिल्ला अध्ययनहरूसँग तुलना गरिएको छ। Morphometric विश्लेषणमा *P. chola* सबैभन्दा ठूलो प्रजातिको देखियो, जसको औसत कुल लम्बाइ ८.३२ से.मी., मानक लम्बाइ ६.४६ से.मी. र शरीरको गहिराइ २.३१ से.मी. थियो। त्यसैगरी *P. sophore* को कुल लम्बाइ ६.१७ से.मी. र शरीरको गहिराइ १.७१ से.मी. पाइयो, र अन्त्यमा *P. conchoni* सबैभन्दा सानो थियो, जसको कुल लम्बाइ ४.९७ से.मी. र गहिराइ १.३१ से.मी. थियो। अतः यी माछाहरू आआफ्नो फरक आकार अनुसार फरक पारिस्थितिक स्थान मा बस्न सक्ने संकेत गर्छ। Meristic विवरणमा पनि केही फरकहरू देखिए, जहाँ *P. chola* मा dorsal, pectoral, pelvic र anal fin को संख्या र लम्बाइ अरू दुई प्रजातिभन्दा बढी पाइए। सांख्यिकीय विश्लेषण (correlation र regression) मा *P. chola* का मापनहरूमा कुल लम्बाइ (TL) तथा टाउकोको लम्बाइ (HL) बीच सकारात्मक सम्बन्ध ( $r = 0.८३$ ) पाइयो, जसले समग्रमा बलियो सम्बन्ध देखाउँछ। *P. sophore* र *P. conchoni* मा भने कमजोर वा मध्यम सम्बन्ध देखियो ( $r = -0.६२$  देखि  $0.५६$ ), जसले वंशज तथा वातावरणीय प्रभावको संकेत दिन्छ। PCA विश्लेषणबाट PC1 ले ९८.७% भिन्नताको व्याख्या गर्‍यो, जसमा *P. chola* को प्राप्ताङ्क ६.६५ रहेको थियो, जुन *P. sophore* (-१.२३) र *P. conchoni* (-५.४१) भन्दा धेरै फरक छ। PC2 ले १.३% भिन्नता व्याख्या गर्‍यो, जसमा *P. conchoni* को प्राप्ताङ्क ०.५२, *P. chola* को ०.२८, र *P. sophore* को -०.७९ थियो। यसबाट *P. chola* अरू दुई प्रजाति भन्दा स्पष्ट रूपमा फरक छ भन्ने देखाउँछ। PCA ले देखायो कि AFL र PFL ले PC1 मा धेरै प्रभाव पार्ने गर्दछ, जसले *P. chola* को फरक प्राप्ताङ्कलाई समर्थन गर्छ। DFH र DPA ले नकारात्मक प्रभाव पारेका छन्, जुन *P. sophore* र *P. conchoni* को प्राप्ताङ्कसँग मेल खान्छ। Truss विश्लेषणमा आधारित dendrogram ले *P. sophore* र *P. conchoni* लाई एउटै cluster मा राखेको छ भने *P. chola* लाई धेरै टाढा छुट्टिएको देखाएको छ, जसले उसको फरक बनावटलाई पुष्टि गर्छ। Dendrogram मा दूरी भिन्नताको सूचक हो। यस अध्ययनले देखाउँछ कि PCA र Truss जस्तो बहुपरिमाणीय विश्लेषण (multivariate analysis) प्रविधिहरू माछाका प्रजाति छुट्याउनका लागि प्रभावकारी छन्। यसले पोखरा उपत्यकाका माछाका प्रजातिहरूको वर्गीकरणका बारेमा उपयोगी जानकारी दिन्छ र भविष्यमा हुने अनुसन्धानका लागि आधार तयार पार्दछ।

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## **List of abbreviations**

### **Abbreviated form**

### **Details of abbreviations**

masl

Meters Above Sea Level

r

Coefficient of Correlation

$r^2$

Coefficient of Determination

PCA

Principal Component Analysis

# 1. Introduction

## 1.1 Background

Taxonomy involves organizing species based on shared characteristics and assigning them universally accepted names. Its primary objectives are to identify, describe, and classify species to better understand biological diversity and relationships among organisms (Jayaram, 1991). This systematic approach is particularly critical for groups like fishes, which exhibit remarkable diversity and play essential roles in aquatic ecosystems.

Pisces, a diverse collection of aquatic chordates, includes finned bony fishes, sharks, rays, chimaeras, hagfishes, and lampreys. While many of these groups are primarily marine, bony fishes dominate freshwater environments and represent the most varied group within Pisces (Lévêque et al., 2008). With an altitudinal range of 50 meters to the highest peaks in the world at over 8000 meters, Nepal has some of the world's most abundant freshwater resources (Khatri et al., 2020). These watery ecosystems are home to a diverse range of fish species, including those belonging to the biggest and most varied family of freshwater fishes, the Cyprinidae.

Cyprinidae is a member of the Cypriniformes Order, which is the most varied group of freshwater fishes, with approximately 4,400 species identified (Eschmeyer & Fong, 2017). Many cypriniform fishes are valued for their food and recreational qualities and are widely traded as ornamental pets worldwide (Tan & Armbruster, 2018). Members of the Cyprinidae Family exhibit remarkable diversity in size, ranging from 12 mm to 3 meters, and are distributed across Asia, Africa, Europe, and North America (Plamoottil & Maji, 2020). Within this family, the genus *Puntius* stands out as a group of small to medium-sized fishes often used as food or kept as ornamental species (Plamoottil & Maji, 2020).

Historically, the genus *Puntius* has been subject to significant taxonomic revisions. Hamilton (1822) initially classified some species now recognized as *Puntius* under the genus *Cyprinus*, while others were categorized directly under *Puntius*. Later, Jerdon (1849) assigned these fishes to the genus *Systemus*. Over time, various traits such as coloration (Taki et al., 1978), fin ray counts (Hamilton, 1822), geography (Günther, 1868), body proportions, meristic counts (Day, 1865, 1878, 1889; Jerdon, 1849), and structural characteristics like osteology, barbel presence, and karyotypes (Magtoon &

Arai, 1939) have been employed to classify *Puntius*. Morphometric analysis has also played a crucial role in understanding the diversity within this genus (Jayaram, 1991).

There is significant morphological variation among the species in *Puntius*. For example, their standard length (SL) ranges from around 20 mm to 500 mm (Menon, 1999; Jayaram, 2019). They possess 18 to 47 lateral-line scales, with the lateral line being complete, incomplete, or interrupted depending on the species. Barbels may be absent, present in one pair, or present in two pairs. Additionally, the dorsal fin's last unbranched ray can be smooth or serrated, strong or weak. Variations exist in body coloration as well; some species have lateral stripes, bars or blotches down the body, spots or blotches at the base of the caudal fin, or no pattern at all (Jayaram, 1991).

Recent taxonomic studies have reclassified many species previously grouped under *Puntius* into new genera such as *Systemus* and *Pethia*, based on molecular and morphological analyses (Pethiyagoda et al., 2012). This reclassification reflects advancements in our understanding of evolutionary relationships and highlights the complexity of classifying species within this genus. Such taxonomic revisions are essential for ensuring accurate identification and classification, which are prerequisites for biodiversity assessment and conservation planning.

The Pokhara Valley in central Nepal is renowned for its scenic lakes Phewa, Begnas, and Rupa which are unique freshwater ecosystems harboring rich biodiversity. These lakes provide critical habitats for a variety of fish species, including members of the genus *Puntius* and the closely related genus *Pethia*. Recent taxonomic revisions have separated *Pethia* from *Puntius*, underscoring the need for comparative studies to understand better their evolutionary and ecological relationships (Pethiyagoda et al., 2012; Katwate et al., 2013). For instance, *Puntius sophore* and *Puntius chola* are common inhabitants of Nepalese freshwater systems, while *Pethia conchoniis* (formerly classified under *Puntius*) is another prominent species found in these lakes. Despite their ecological and economic significance, there is a rare taxonomic detail focusing on these species in the Pokhara Valley.

Taxonomic ambiguity remains a significant challenge in ichthyology, particularly for genera like *Puntius* and *Pethia*, where overlapping morphological traits often lead to misidentification (Eschmeyer et al., 2010; Froese & Pauly, 2001). Morphometric and meristic analyses, which involve the measurement of physical features and counts of

anatomical structures, respectively, are essential tools for resolving such ambiguities. These methods provide precise data that can distinguish between closely related species and reveal intraspecific variations influenced by environmental factors (Talwar & Jhingran, 1991; Hubbs & Lagler, 2004). Nevertheless, little is known about the morphometric and meristic traits of *Puntius* and *Pethia* species in the lakes of the Pokhara Valley, which leaves gaps in our knowledge of their diversity and distribution.

## **1.2 Statement of problem**

Although species within the genus *Puntius*, including *Puntius sophore*, and *Puntius chola*, and genus *Pethia* including *Pethia conchonius* are globally classified as "Least Concern," their populations in the Pokhara Valley lakes face localized threats such as habitat degradation, pollution, invasive species, and overfishing (Pant et al., 2019). These pressures could lead to declines in their abundance or genetic diversity, even if they remain stable elsewhere. Furthermore, taxonomic ambiguities and a lack of detailed morphological and meristic studies hinder accurate species identification and biodiversity assessment in these unique freshwater ecosystems. This study addresses this critical gap by conducting a thorough taxonomic analysis of *Puntius* species in the Pokhara Valley, aiming to establish baseline data for biodiversity monitoring, resolve taxonomic uncertainties, and inform strategies for the long-term preservation of these ecologically significant fish species and their habitats.

## **1.3 Objectives**

### **1.3.1 General objective**

- To describe the taxonomy of genus *Puntius* and *Pethia* in the lakes of Pokhara valley.

### **1.3.2 Specific objectives**

- To analyze the morphological, morphometric and meristic characteristics of genus *Puntius*.
- To examine the morphological, morphometric and meristic characteristics of genus *Pethia*.

## **1.4 Significance of the study**

This study has significantly enhanced the understanding of the taxonomic details of the *Puntius* and *Pethia* species in the lakes of Pokhara valley. Through detailed

morphological, morphometric, and meristic analyses of *Puntius* species, it has provided accurate taxonomic identification, which is essential for ecological studies and biodiversity assessments in the region. The research has clarified the species composition of genus *Puntius* and *Pethia*, particularly in light of recent taxonomic revisions, and has contributed valuable data for distinguishing closely related species. Furthermore, the findings will support local conservation efforts by offering critical insights for targeted management practices in this ecologically significant Ramsar site. By promoting the sustainable use and preservation of freshwater ecosystems, this study has laid a foundation for future research and conservation initiatives in the region.

### **1.5 Limitations of the study**

- All samples were collected from a limited geographic range and during one season, which may not reflect the full morphological diversity of the species across its entire distribution or at different times of the year.
- Morphometric and meristic traits can be influenced by environmental factors (e.g., water temperature, food availability), which were not measured in this study.
- The age and sex of specimens were not considered, which could influence morphological variation in some fish species.

## 2. Literature review

The lakes of Pokhara Valley represent a unique ecosystem characterized by diverse aquatic habitats and endemic fish species. Previous studies have highlighted the significance of morphometric and meristic analyses in documenting biodiversity and assessing conservation status. Timilsina et al. (2019) documented 34 fish species in the lakes of Pokhara Valley, including four species of *Puntius*: *Puntius sophore*, *Puntius sarana*, *Puntius chola*, and *Puntius conchoni*. Among these, *Puntius sophore*, *Puntius chola*, and *Puntius conchoni* were found in multiple lakes such as Phewa, Begnas, Rupa, Dipang, and Maldi, indicating a broad distribution across various water bodies. In contrast, *Puntius sarana* was reported only in Phewa, Begnas, and Rupa Lakes, suggesting a more limited habitat range. These findings offer important insights into the distribution patterns of *Puntius* species in Pokhara Valley.

The morphological and taxonomic distinctions among *Puntius chola*, *Puntius sophore*, and *Pethia conchoni* highlight key differences that justify their generic classifications. *Puntius chola* (Hamilton, 1822) and *P. sophore* (Hamilton, 1822) share a smooth last unbranched dorsal-fin ray and complete lateral line (22–28 scales), but *P. chola* is distinguished by its weak maxillary barbels and black dorsal-fin spot, while *P. sophore* lacks barbels entirely. In contrast, *Pethia conchoni* (Hamilton, 1822) exhibits a stiff, serrated last dorsal-fin ray and often an incomplete lateral line, key traits separating it from *Puntius sensu stricto*. These differences, particularly the dorsal-fin ray structure, barbel presence, and lateral-line completeness validate the reclassification of *Pethia conchoni* into *Pethia* while retaining *P. chola* and *P. sophore* within *Puntius* (Pethiyagoda et al., 2012; Plamoottil & Maji, 2020).

Morphometric and meristic characters are widely used to differentiate fish species and populations. According to Azadi and Rahman (2013), morphometric measurements are essential in understanding evolutionary adaptations and ecological preferences of fish species. They studied *Gudusia chapra* and *Gonialosa manmina* and reported significant variations in body dimensions influenced by environmental factors. Similarly, Jan and Ahmed (2020) emphasized that morphometric traits such as standard length, pre-dorsal length, and fin measurements are crucial for species identification. Their study on *Schizothorax labiatus* highlighted the role of these parameters in differentiating fish populations from different water systems. Meristic counts provide discrete data on fin rays, scales, and gill rakers, which remain relatively stable within species. Sidiq et al.

(2021) studied *Crossocheilus diplochilus* and found that meristic characters such as the number of fin rays and lateral line scales were effective in distinguishing species. Plamoottil (2018) conducted a comprehensive taxonomic assessment of *Puntius dorsalis*, highlighting the significance of both morphometric and meristic traits in identifying species and their variations. These findings underscore the importance of integrating both approaches in taxonomic studies of *Puntius* species.

Regression analysis is a valuable tool in examining relationships between morphometric variables and total length. Paulinkar and Kaneez (2023) used regression models to establish linear relationships between morphometric traits in Cypriniformes species from Song River, Dehradun. They found strong correlations between total length and other body dimensions, emphasizing the role of regression analysis in taxonomic studies. Parvin et al. (2018) also applied regression techniques to study *Salmostoma bacila* from the Ganges River, confirming that morphometric traits show significant growth relationships with total body length. Regression analysis can further aid in identifying allometric growth patterns. Hossen et al. (2020) analyzed three species from the family Ambassidae and reported differential growth rates in specific body parts due to environmental conditions. Similarly, Safi and Khan (2014) studied *Pomadasys stridens* and found variations in morphometric traits due to ecological influences.

Mir and Pandey (2014) conducted a detailed regression analysis on *Botia birdi* from the Poonch River, Jammu and Kashmir, India. They reported highly significant positive correlations between total length (TL) and various morphometric traits, indicating consistent allometric growth across individuals. For example, fork length (FL) showed the strongest correlation with total length ( $r = 0.999$ ), followed by standard length (SL) ( $r = 0.996$ ), pre-dorsal length (PrDL) ( $r = 0.991$ ), and post-dorsal length (PoDL) ( $r = 0.992$ ). Even head-related measurements such as head length (HL) ( $r = 0.977$ ) and pre-orbital length (PrOL) ( $r = 0.989$ ) exhibited strong correlations, underscoring the predictability of body proportions in this species. The morphometric and meristic analysis of four *Puntius* species from central India revealed significant insights into their characteristics. The study found that the coefficients of correlation ( $r$ ) for various characters ranged from 0.240–0.994 for *Puntius conchonius*, 0.567–0.996 for *P. chola*, 0.786–0.997 for *P. sophore*, and 0.237–0.997 for *P. ticto* (Saroniya et al., 2013). These results indicate that *P. chola* and *P. sophore* exhibit a high degree of interdependence among their

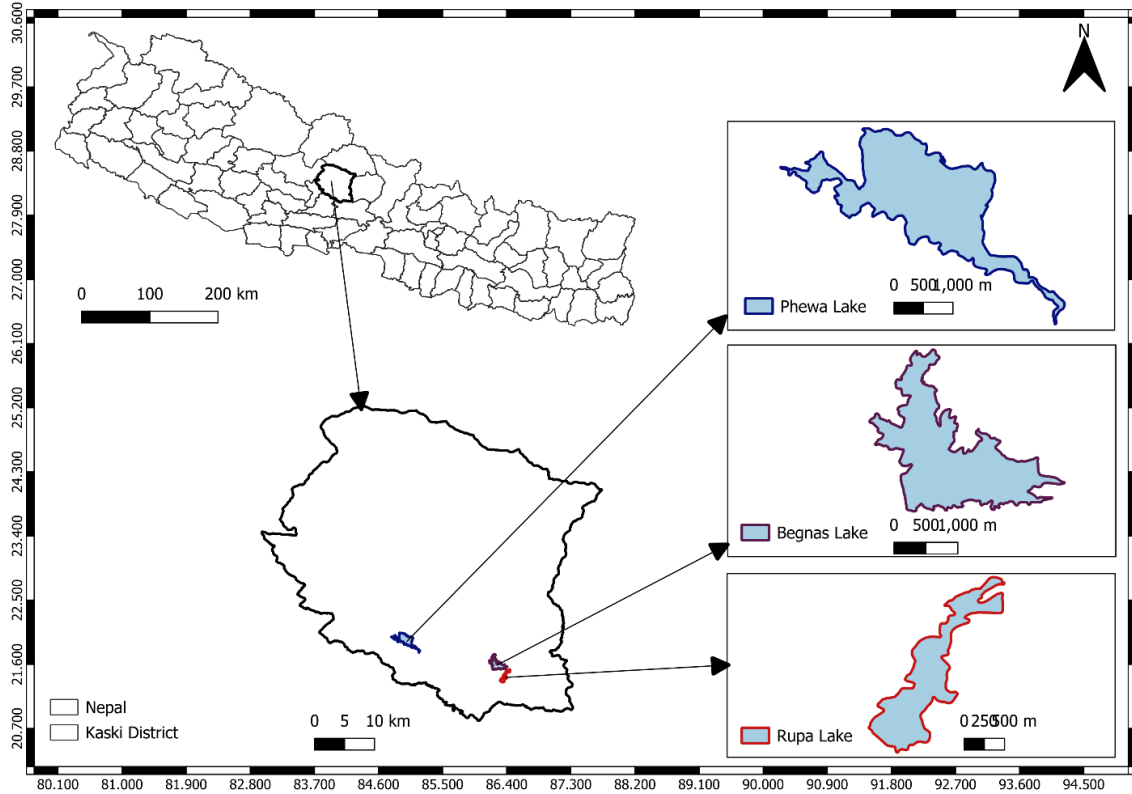
morphological traits, while *P. conchoni* and *P. ticto* demonstrate lower interdependence (Saroniya et al., 2013).

PCA is a widely used statistical technique for reducing the dimensionality of morphometric datasets while retaining key information about variability. Gupta et al. (2018) employed PCA to analyze morphometric traits of Barbinae species from the Ganga River system, successfully differentiating species based on their morphological characteristics. Their study demonstrated that PCA effectively captures major morphological trends and aids in species classification. Mir and Pandey (2015) used PCA to study *Botia birdi*, identifying principal components that explained the majority of the variance in morphometric data. Similarly, Kamboj et al. (2019) applied PCA to four freshwater fish species from the Ganga River and found distinct clustering patterns based on morphological traits. The study by Roy et al. (2023), focusing on barbs from Koraput district, Eastern Ghats, southern Odisha, India, utilized LDA but referenced PCA findings from Choudhury and Dutta (2013), which grouped *P. chola* and *P. conchoni* together while distinguishing *P. sophore*. These findings suggest that PCA can be instrumental in explaining population-level differences in *Puntius* species in the lakes of Pokhara Valley.

### 3. Materials and methods

#### 3.1 Study area

The Phewa, Begnas, and Rupa lakes, the three biggest in the Pokhara Valley, are all included in the research area. Phewa Lake's watershed spans approximately 123 km<sup>2</sup>, situated between latitudes 28°11'39" N and 28°17'25" N, and longitudes 83°47'51" E to 83°59'17" E, with elevations ranging from 789 to 2508 meters above sea level (Regmi et al., 2017). The lake is primarily fed by perennial, spring-sourced streams such as Harpan Khola and Andheri Khola, which show increased flow during the monsoon season. Covering an area of 4.43 km<sup>2</sup>, Phewa Lake reaches a maximum depth of 23.5 meters and has an average depth of 8.6 meters (Gurung et al., 2006; Khadka & Ramanathan, 2021). Begnas Lake, situated approximately 13 kilometers east of Pokhara, lies between the Mahabharat Range and the Greater Himalayas, with an elevation range of 600 m to 1,410 m. With a water volume of  $17.96 \times 10^6$  m<sup>3</sup> and average and maximum depths of 6.6 m and 10 m, respectively, it covers an area of 3.28 km<sup>2</sup> (Rai et al., 1995; Oli, 1996). Rupa Lake, located at 600 masl in the Kaski district, is oriented north to south and is primarily fed by the Talbesi stream. Its watershed covers an area of 30 km<sup>2</sup>, with the Dhovan Khola stream acting as a significant feeder that eventually joins the Seti Gandaki River at Sistani Ghat (Kafle et al., 2008). These lakes are vital for the local ecosystem and support agriculture, tourism, and fisheries, making them crucial for the region's socio-economic development. However, they face challenges such as sedimentation, pollution, and eutrophication, which threaten their ecological balance and sustainability (Timilsina et al., 2019; Kafle et al., 2008).



**Figure 1.** Map of the study area showing Phewa, Begnas, and Rupa lakes

### 3.2 Fish sampling and preservation

Fieldwork was conducted in October and November 2024. Fish samples were collected primarily in the morning (7–11 AM), with each lake visited three times. A local fisherman was hired to collect fish using a gill net with a mesh size of 10 mm. The nets were set up the night before and retrieved the following morning. The fish other than *Puntius* and *Pethia* species were released. Cooperative markets were visited for a cross-sectional survey study. They were then kept in 10% formalin for additional research. The samples were brought to Tribhuvan University's Central Department of Zoology lab for additional examination. Each sample species was photographed using an iPhone 12 Pro Max.

### 3.3 Fish identification

The specimens were identified up to species level following Hamilton (1822), Shrestha (1981,2001), Shrestha (2008), and Jayaram (1999). Fish morphology, such as its body shape, size, coloration, structure, etc., was noted down in detail.

### **3.4 Data collection**

A total of 38 morphometric characteristics (total length (TL), standard length (SL), fork length (FL), body depth (BD), weight (W), dorsal fin length (DFL), dorsal fin height (DFH), pectoral fin height (PFH), pectoral fin length (PFL), pre dorsal length (PDL), post dorsal length (PODL), pre pectoral length (PPL), post pectoral length (POPL), pelvic fin height (VFH), pelvic fin length (VFL), anal fin height (AFH), anal fin length (AFL), pre pelvic length (PVL), post pelvic length (POVL), pre anal length (PAL), post anal length (POAL), caudal fin length (CFL), caudal fin height (CFH), distance between pelvic and anal fin (DPA), distance between pectoral and pelvic fin (DPP), length of caudal peduncle (LCP), depth of caudal peduncle (DCP), head depth (HD), head width (HW), head length (HL), snout length (SNL), eye diameter (ED), post-orbital length (POL), internarial width (INW), interorbital length (IOL), distance from snout to occiput (DSO), width of gape of mouth (WGM), distance from occiput to dorsal fin (DOD)) were taken for each specimen in the laboratory (Plamoottil & Vineeth, 2022). Body weight (W) was measured using the digital weight scale and other measurements were taken with a Vernier caliper accurate to 0.1mm. Whenever feasible, measurements and counts were conducted on the left side of the fish, following established standard procedures.

Twelve meristic characters were counted which includes pectoral fin rays (PFR), ventral fin rays (VFR), dorsal fin rays (DFR), caudal fin rays (CFR), anal fin rays (AFR), pre dorsal scales (PDS), lateral line (LL), scales between lateral line and ventral fin (SLV), scales between lateral line and anal fin (SLA), scales between lateral line and dorsal fin (SLD), pre anal scales (PAS) and pre pelvic scales (PPS) (Katwate et al., 2018; Plamoottil, 2018; Hossen et al., 2020). The same observer counted the meristic characters twice for accuracy. A magnifying glass was used to count the fin rays, and a stereomicroscope was employed to examine the lateral lines when necessary. Fish specimens were handled carefully to ensure no damage was done to the specimens.

### **3.5 Statistical analysis**

Descriptive statistics were employed to perform the general morphometric analysis. Morphometric measurements were analyzed using the regression method, applying the formula  $Y = a + bx$ , where Y represents the dependent variable, a is a constant to be determined, b is the coefficient representing the rate of change, and x is the independent variable. Principal Component Analysis (PCA) was employed to assess morphometric

variation. Descriptive statistics, regression analysis, PCA and truss analysis was conducted using morphometric data with the assistance of R software version 4.3.1 (R Core Team, 2023).

## 4. Results

A study conducted in Pokhara's three major lakes Phewa, Begnas, and Rupa identified two *Puntius* species (*Puntius sophore* and *Puntius chola*) and one *Pethia* species (*Pethia conchonius*). A total of 65 specimens from these species were analyzed to assess their morphological, morphometric characteristics, and meristic features.

### 4.1 Morphological, morphometric and meristic characteristics of *Puntius sophore*

Twenty-seven specimen of *Puntius sophore* irrespective of sex were used to study the morphometric and meristic characters.

#### 4.1.1 Morphological description of *Puntius sophore*

*Puntius sophore* (Hamilton, 1822)

##### Senior synonyms

*Cyprinus sophore*, Hamilton, Fish. Ganges, 1-405, pls. 1-39. 1822.

*Barbus sophore*, Gudger, Journal and Proceedings of the Asiatic Society of Bengal (New Series) v.19 (no.4): 121-136. 1924.

*Systemus sophore*, Roberts, Archives of Natural History v.25 (no.2): 267-280. 1988.

##### Junior synonyms

*Systemus chrysopterus*, McClelland, Asiatic Researches, v.19 (pt 2): 217-471, pls. 37-61. 1839.

*Barbus chrysopterus*, McClelland, Annals and Magazine of Natural History, v.8 (nos 48,49,50): 35-46, 108-121, 192-203. 1841.

*Puntius chrysopterus*, McClelland, Journal of the Asiatic Society of Bengal v.7:941-948, Pls. 55-56. 1838.

*Leuciscus duvancelli*, Valenciennes, Histoire Naturelle des Poissons. v.17: i-xxiii+1-497+ 2p.p., pls. 487-519.1844.

*Puntius modestus*, Kner, Fishes. Vienna. Zool, Theil. v.1 (pt 3): 275-433, pls. 12-16. 1867.

*Barbus annandalei*, Fowler. Proceedings of the Academy of Natural Sciences of Philadelphia v.76: 67-101. 1924.

**Description:** A deep-bodied fish with a distinct black blotch at the base of the dorsal fin and on the caudal peduncle. The back is olive green, while the belly is silvery. The fins have reddish tips, and a faint black band runs along the complete lateral line. The dorsal fin is relatively high, with 8–9 branched rays and a soft dorsal spine. The head is small, with comparatively large eyes, and barbels are absent. The opercles are golden-red, and the mouth is terminal, with a slightly larger upper lip.

#### **4.1.2 Morphometric characters of *Puntius sophore***

The minimum, maximum, range difference, mean and standard deviation of morphometric characteristics for *Puntius sophore* are presented in Table 1. The mean values of the morphometric measurements for *Puntius sophore* indicated an average weight (W) of 3.67 grams, total length (TL) of 6.17 cm, fork length (FL) of 5.55 cm, and standard length (SL) of 4.84 cm. The body depth (BD) averaged at 1.71 cm, and the dorsal fin height (DFH) and length (DFL) were 1.19 cm and 0.84 cm, respectively. The post-dorsal length (PDL) and pre-dorsal length (PODL) were 3.26 cm and 2.42 cm, respectively. The pectoral fin height (PFH) was 0.91 cm with a length (PFL) of 0.24 cm. Pre-pectoral length (PPL) and post-pectoral length (POPL) averaged at 1.32 cm and 1.56 cm, respectively. The pelvic fin height (VFH) and length (VFL) were 0.90 cm and 0.29 cm, respectively, whereas pre-pelvic length (PVL) and post-pelvic length (POVL) were 2.35 cm and 2.64 cm. The anal fin height (AFH) and length (AFL) were 0.75 cm and 0.49 cm, respectively. Post-anal length (PAL) and pre-anal length (POAL) averaged at 3.92 cm and 3.43 cm, respectively. Caudal fin height (CFH) and length (CFL) were 1.32 cm and 0.65 cm. The distance between the pelvic and anal fins (DPA) was 1.17 cm, and the distance between pectoral and pelvic fins (DPP) was 1.08 cm. The depth (DCP) and length (LCP) of the caudal peduncle were 0.78 cm and 0.84 cm, respectively. The head depth (HD), head width (HW) and head length (HL) were 1.07 cm, 0.72 cm and 1.33 cm, respectively. The eye diameter (ED) was 0.44 cm, snout length (SNL) was 0.37 cm, post-orbital length (POL) was 0.61 cm, interorbital length (IOL) was 0.63 cm, internarial width (INW) was 0.27 cm, and the width of the gape of the mouth (WGM) was 0.35 cm. The distance from the snout to the occiput (DSO) was 1.16 cm, and the distance from the occiput to the dorsal fin (DOD) was 1.28 cm.

**Table 1.** Minimum, maximum, range difference, mean and standard deviation of morphometric characteristics of *Puntius sophore* (Weight is in grams (g); all other measurements are in centimeters (cm).)

S.N.	Morphometric measurements	Code	Min	Max	Range difference	Mean±standard deviation
1	Weight	W	1.69	7.54	5.85	3.67±1.49
2	Total length	TL	4.8	7.6	2.8	6.17±0.81
3	Fork length	FL	4.4	7	2.6	5.55±0.73
4	Standard length	SL	3.8	6.2	2.4	4.84±0.63
5	Body depth	BD	1.3	2.24	0.94	1.71±0.24
6	Dorsal fin height	DFH	0.94	1.53	0.59	1.19±0.16
7	Dorsal fin length	DFL	0.68	1.06	0.38	0.84±0.11
8	Pre dorsal length	PDL	2	2.9	0.9	2.42±0.27
9	Post dorsal length	PODL	2.68	3.96	1.28	3.26±0.36
10	Pectoral fin height	PFH	0.61	1.16	0.55	0.91±0.11
11	Pectoral fin length	PFL	0.17	0.4	0.23	0.24±0.05
12	Pre pectoral length	PPL	1.08	1.7	0.62	1.32±0.17
13	Post pectoral length	POPL	1.26	2.1	0.84	1.56±0.20
14	Pelvic fin height	VFH	0.6	1.17	0.57	0.90±0.14
15	Pelvic fin length	VFL	0.2	0.42	0.22	0.29±0.06
16	Pre pelvic length	PVL	1.87	3.03	1.16	2.35±0.31
17	Post pelvic length	POVL	2.07	3.33	1.26	2.64±0.33
18	Anal fin height	AFH	0.56	0.96	0.4	0.75±0.09
19	Anal fin length	AFL	0.33	0.74	0.41	0.49±0.08
20	Pre anal length	PAL	2.76	4.34	1.58	3.43±0.47
21	Post anal length	POAL	3.23	5.08	1.85	3.92±0.52
22	Caudal fin height	CFH	1.04	1.58	0.54	1.32±0.15
23	Caudal fin length	CFL	0.46	0.98	0.52	0.65±0.12
24	Distance between pectoral and pelvic fin	DPP	0.79	1.52	0.73	1.08±0.20
25	Distance between pelvic and anal fin	DPA	0.9	1.59	0.69	1.17±0.17

26	Depth of caudal peduncle	DCP	0.55	1.04	0.49	0.78±0.12
27	Length of caudal peduncle	LCP	0.54	1	0.46	0.84±0.11
28	Head length	HL	1.12	1.66	0.54	1.33±0.15
29	Head depth	HD	0.82	1.36	0.54	1.07±0.12
30	Head width	HW	0.57	0.95	0.38	0.72±0.09
31	Eye diameter	ED	0.31	0.57	0.26	0.44±0.05
32	Snout length	SNL	0.28	0.53	0.25	0.37±0.06
33	Post orbital length	POL	0.44	0.87	0.43	0.61±0.10
34	Interorbital length	IOL	0.44	0.89	0.45	0.63±0.14
35	Internarial width	INW	0.17	0.36	0.19	0.26±0.05
36	Width of gape of mouth	WGM	0.24	0.47	0.23	0.35±0.06
37	Distance from snout to occiput	DSO	0.87	1.46	0.59	1.16±0.17
38	Distance from occiput to dorsal fin	DOD	1	1.66	0.66	1.28±0.19

#### 4.1.3 Meristic counts of *Puntius sophore*

The meristic characteristics of *Puntius sophore* (Table 2) revealed the following average counts: the dorsal fin rays (DFR) consisted of 10 rays in total (ii,8), while the pectoral fin rays (PFR) averaged 12 (i,11). The ventral fin rays (VFR) showed a mean of 9 (i,8), and the anal fin rays (AFR) were recorded with an average of 7 (iii,4). In the caudal fin rays (CFR), an average of 20 rays was observed (i,18,i). Typically, the lateral line (LL) ranged from 20 to 25 scales. The count for pre-dorsal scales (PDS) was between 7 to 11. Between the lateral line and the dorsal fin (SLD), 5½ scales were found; 4½ scales were located between the lateral line and ventral fin (SLV), and 3½ scales between the lateral line and anal fin (SLA). The pre-pelvic scales (PPS) ranged from 7 to 11, whereas the pre-anal scales (PAS) ranged from 13 to 20.

**Table 2.** Meristic counts of *Puntius sophore*

S.N.	Characters	Code	Range
1	Dorsal fin rays	DFR	ii,8
2	Pectoral fin rays	PFR	i,11
3	Ventral fin rays	VFR	i,8
4	Anal fin rays	AFR	iii,4
5	Caudal fin rays	CFR	i,18,i
6	Lateral line	LL	20-25
7	Pre-dorsal scales	PDS	7-11
8	Scales between lateral line and dorsal fin	SLD	5 ½
9	Scales between lateral line and ventral fin	SLV	4 ½
10	Scales between lateral line and anal fin	SLA	3 ½
11	Pre-pelvic scales	PPS	7-11
12	Pre anal scales	PAS	13-20

#### **4.2 Morphological, morphometric, and meristic characteristics of *Puntius chola***

Thirteen specimens of *Puntius chola* irrespective of sex were used to study the morphometric and meristic characters.

##### **4.2.1 Morphological description of *Puntius chola***

*Puntius chola* (Hamilton, 1822)

##### **Senior synonyms**

*Cyprinus chola*, Hamilton, Fish, Ganges, pp. 312, 389. 1822.

*Capoeta chola*, Gudger, Journal and Proceedings of the Asiatic Society of Bengal, v.19 (no. 4): 121-136, 1924.

*Barbus chola*, Roberts, Archives of Natural History v.25 (no. 2): 121-136, 1924.

##### **Junior synonyms**

*Systemus unimaculatus*, Blyth, Journal of the Asiatic Society of Bengal, v.9 (no. 2): 138-174. 1860.

*Systemus tetrapagus*, McClelland, Asiatic Researches, v.19 (pt.2): 217-471, pls. 37-61. 1839.

*Puntius titius*, Hamilton, Fishes, Ganges, 1-405, pls. 1-39, 1822.

*Puntius tetrarupagus*, McClelland, Asiatic Researches, v.19 (pt.2): 217-471. 1839.

*Barbus unimaculatus*, Blyth, Journal of the Asiatic Society of Bengal, v.29 (no. 2): 138-174. 1860.

*Barbus titius*, Hamilton, Fishes, Ganges, 1-405, pls. 1-39. 1822.

*Barbus tetrarupagus*, McClelland, Asiatic Researches, v.19 (pt.2): 217-471, pls. 37-61. 1839.

**Description:** This fish has a silvery body with a darker shade along the upper side and features two prominent dark spots: one located on the caudal peduncle and a smaller one just behind the opercle. The dorsal fin is serrated, while the tips of the anal fin are marked with black. It has a terminal mouth, golden-colored opercle, and noticeably large eyes. A single pair of small barbels is also present.

#### **4.2.2 Morphometric characters of *Puntius chola***

The minimum, maximum, range difference, mean and standard deviation of morphometric characteristics for *Puntius chola* are presented in Table 3. The mean weight (W) was 9.66 grams, with a total length (TL) of 8.32 cm, fork length (FL) of 7.40 cm, and standard length (SL) of 6.46 cm. The body depth (BD) averaged at 2.31 cm, and the dorsal fin height (DFH) and length (DFL) were 1.45 cm and 1.21 cm, respectively. The post-dorsal length (PDL) and pre-dorsal length (PODL) were 4.23 cm and 3.02 cm, respectively. The pectoral fin height (PFH) was 1.28 cm with a length (PFL) of 0.30 cm. Pre-pectoral length (PPL) and post-pectoral length (POPL) averaged at 1.88 cm and 2.18 cm, respectively. The pelvic fin height (VFH) and length (VFL) were 1.29 cm and 0.38 cm, respectively, whereas pre-pelvic length (PVL) and post-pelvic length (POVL) were 3.20 cm and 3.59 cm. The anal fin height (AFH) and length (AFL) were 0.95 cm and 0.68 cm, respectively. Post-anal length (PAL) and pre-anal length (POAL) averaged at 5.36 cm and 4.68 cm, respectively. Caudal fin height (CFH) and length (CFL) were 1.77 cm and 0.88 cm. The distance between the pelvic and anal fin (DPA) was 1.55 cm, and the distance between pectoral and pelvic fins (DPP) was 1.43 cm. The depth (DCP) and length (LCP) of the caudal peduncle were 1.13 cm and 1.01 cm, respectively. The head depth (HD), head width (HW) and head length (HL) were 1.54 cm, 1.03 cm and 1.85 cm respectively. The eye diameter (ED) was 0.48 cm, snout length (SNL) was 0.57 cm, post-orbital length (POL) was 0.87 cm, interorbital length (IOL) was 0.91 cm, internarial

width (INW) was 0.36 cm, and the width of the gape of the mouth (WGM) was 0.46 cm. The distance from the snout to the occiput (DSO) was 1.46 cm, and the distance from the occiput to the dorsal fin (DOD) was 1.88 cm.

**Table 3.** Minimum, maximum, range difference, mean and standard deviation of morphometric characteristics of *Puntius chola* (Weight is in grams (g); all other measurements are in centimeters (cm).)

S.N.	Morphometric measurements	Code	Min	Max	Range difference	Mean±standard deviation
1	Weight	W	5.03	18.53	13.50	9.66±4.95
2	Total length	TL	6.70	10.50	3.80	8.32±1.39
3	Fork length	FL	5.80	9.50	3.70	7.40±1.32
4	Standard length	SL	5.10	8.50	3.40	6.46±1.25
5	Body depth	BD	1.89	2.85	0.96	2.31±0.37
6	Dorsal fin height	DFH	1.29	1.69	0.40	1.45±0.12
7	Dorsal fin length	DFL	0.99	1.49	0.50	1.21±0.16
8	Pre dorsal length	PDL	2.30	3.90	1.60	3.02±0.59
9	Post dorsal length	PODL	3.37	5.37	2.00	4.23±0.74
10	Pectoral fin height	PFH	0.89	1.59	0.70	1.28±0.22
11	Pectoral fin length	PFL	0.23	0.42	0.19	0.30±0.06
12	Pre pectoral length	PPL	1.54	2.27	0.73	1.88±0.24
13	Post pectoral length	POPL	1.80	2.69	0.89	2.18±0.30
14	Pelvic fin height	VFH	1.09	1.58	0.49	1.29±0.17
15	Pelvic fin length	VFL	0.29	0.48	0.19	0.38±0.06
16	Pre pelvic length	PVL	2.62	3.98	1.36	3.20±0.50
17	Post pelvic length	POVL	2.91	4.41	1.50	3.59±0.54
18	Anal fin height	AFH	0.70	1.16	0.46	0.94±0.14
19	Anal fin length	AFL	0.45	0.86	0.41	0.68±0.12
20	Pre anal length	PAL	3.78	6.00	2.22	4.68±0.84
21	Post anal length	POAL	4.26	6.86	2.60	5.36±0.94
22	Caudal fin height	CFH	1.50	2.23	0.73	1.77±0.25
23	Caudal fin length	CFL	0.67	1.10	0.43	0.88±0.15
24	Distance between pectoral and	DPP	1.07	1.88	0.81	1.43±0.26

pelvic fin						
25	Distance between pelvic and anal fin	DPA	1.00	2.11	1.11	1.55±0.35
26	Depth of caudal peduncle	DCP	0.93	1.42	0.49	1.13±0.15
27	Length of caudal peduncle	LCP	0.75	1.36	0.61	1.01±0.19
28	Head length	HL	1.53	2.34	0.81	1.85±0.26
29	Head depth	HD	1.23	1.93	0.70	1.54±0.21
30	Head width	HW	0.81	1.35	0.54	1.03±0.15
31	Eye diameter	ED	0.40	0.57	0.17	0.48±0.05
32	Snout length	SNL	0.50	0.67	0.17	0.57±0.06
33	Post orbital length	POL	0.70	1.06	0.36	0.87±0.10
34	Interorbital length	IOL	0.77	1.13	0.36	0.91±0.13
35	Internarial width	INW	0.29	0.44	0.15	0.36±0.05
36	Width of gape of mouth	WGM	0.37	0.60	0.23	0.46±0.07
37	Distance from snout to occiput	DSO	1.23	1.77	0.54	1.46±0.17
38	Distance from occiput to dorsal fin	DOD	1.43	2.55	1.12	1.88±0.40

#### 4.2.3 Meristic counts of *Puntius chola*

The meristic characteristics of *Puntius chola* (Table 4) revealed the following average values: dorsal fin rays (DFR) had a mean count of 10 (ii,8), while pectoral fin rays (PFR) averaged 12 (i,11). Ventral fin rays (VFR) showed a mean of 9 (i,8), and anal fin rays (AFR) averaged 6 (iii,3). The caudal fin rays (CFR) had an average count of 19 (i,17,i). The lateral line (LL) ranged from 21 to 26 scales. Pre-dorsal scales (PDS) ranged between 7 to 10. Between the lateral line and the dorsal fin (SLD), 5½ scales were observed; 4½ scales were found between the lateral line and both the ventral fin (SLV) and the anal fin (SLA). The counts for pre-pelvic scales (PPS) and pre-anal scales (PAS) were 8 to 12 and 15 to 22, respectively.

**Table 4.** Meristic counts of *Puntius chola*

S.N.	Meristic characteristics	Code	Range
1	Dorsal fin rays	DFR	ii,8
2	Pectoral fin rays	PFR	i,11
3	Ventral fin rays	VFR	i,8
4	Anal fin rays	AFR	iii,3
5	Caudal fin rays	CFR	i,17,i
6	Lateral line	LL	21-26
7	Pre-dorsal scales	PDS	7-10
8	Scales between lateral line and dorsal fin	SLD	5 ½
9	Scales between lateral line and ventral fin	SLV	4 ½
10	Scales between lateral line and anal fin	SLA	4 ½
11	Pre pelvic scales	PPS	8-12
12	Pre anal scales	PAS	15-22

### 4.3 Morphological, morphometric and meristic characteristics of *Pethia conchoni*

Twenty-five specimens of *Pethia conchoni* irrespective of sex were used to study the morphometric and meristic characters.

#### 4.3.1 Morphological description of *Pethia conchoni*

*Pethia conchoni* (Hamilton, 1822)

##### Senior Synonyms

*Systemus conchoni*, Gudger, Journals and Proceedings of Asiatic Society of Bengal v.19 (no.4): 121-136. 1924.

*Puntius conchoni*, Roberts, Archives of Natural History v.25 (no.2): 267-280. 1998.

*Cyprinus conchoni*, Britz, Fishes. Ganges, Ray Society, 1-48, i-vii, 1-405, i-vii, pls. 1-228. 2019.

*Barbus conchoni*, Hamilton, Fishes. Ganges, i-vii+ 1-405, pls. 37-61. 1822.

##### Junior Synonyms

*Systemus pyropterus*, McClelland, Asiatic Researches, v.19 (pt 2): 217-471, pls. 37-61. 1839.

*Puntius conchoni* *Khagariensis*, Dutta Munshi, J.S. and M.P. Srivastava, Natural History of Fishes & Systematics of Freshwater Fishes of India: i-xvii+1-403. Pls. 1-50. 1988.

**Description:** A deep-bodied silvery fish with a dark back and a silvery belly. Its scales have dark bases, and a black blotch is present in the caudal region. The lateral line is incomplete. The pectoral and pelvic fins are transparent, while the dorsal and caudal fins have dark tips. It has a serrated dorsal spine shorter than the first soft ray. Barbels are absent.

#### 4.3.2 Morphometric characters of *Pethia conchoni*

The minimum, maximum, range difference, mean and standard deviation of morphometric characteristics for *Pethia conchoni* are presented in Table 5. In *Pethia conchoni*, the mean weight (W) was 2.03 grams, with a total length (TL) of 4.97 cm, standard length (SL) of 3.90 cm, and fork length (FL) of 4.45 cm. The body depth (BD) averaged at 1.31 cm, and the dorsal fin height (DFH) and length (DFL) were 0.81 cm and 0.69 cm, respectively. The post-dorsal length (PDL) and pre-dorsal length (PODL) were 2.60 cm and 2.00 cm, respectively. The pectoral fin height (PFH) was 0.81 cm with a length (PFL) of 0.22 cm. Pre-pectoral length (PPL) and post-pectoral length (POPL) averaged at 1.11 cm and 1.32 cm, respectively. The pelvic fin height (VFH) and length (VFL) were 0.73 cm and 0.26 cm, respectively, whereas pre-pelvic length (PVL) and post-pelvic length (POVL) were 1.97 cm and 2.24 cm. The anal fin height (AFH) and length (AFL) were 0.60 cm and 0.47 cm, respectively. Pre-anal length (PAL) and post-anal length (POAL) averaged at 2.78 cm and 3.25 cm, respectively. Caudal fin height (CFH) and length (CFL) were 1.11 cm and 0.52 cm. The distance between the pelvic and anal fins (DPA) was 0.81, and the distance pectoral and pelvic fins (DPP) was 0.93 cm, and the distance between the pelvic and anal fins (DPA) was 0.81 cm. The depth (DCP) and length (LCP) of the caudal peduncle were 0.63 cm and 0.69 cm, respectively. The head depth (HD), head width (HW), and head length (HL) were 0.86 cm, 0.58 cm, and 1.08 cm. The eye diameter (ED) was 0.36 cm, snout length (SNL) was 0.29 cm, post-orbital length (POL) was 0.48 cm, interorbital length (IOL) was 0.51 cm, internarial width (INW) was 0.21 cm, and the width of the gape of the mouth (WGM) was 0.26 cm. The distance from the snout to the occiput (DSO) was 0.92 cm, and the distance from the occiput to the dorsal fin (DOD) was 0.99 cm.

**Table 5.** Minimum, maximum, range difference, mean and standard deviation of morphometric characteristics of *Pethia conchonius* (Weight is in grams (g); all other measurements are in centimeters (cm).)

S.N.	Morphometric measurements	Code	Min	Max	Range difference	Mean±Standard deviation
1	Weight	W	1.24	2.72	1.48	2.03±0.38
2	Total length	TL	4.50	5.90	1.40	4.97±0.31
3	Fork length	FL	4.00	5.30	1.30	4.45±0.29
4	Standard length	SL	3.50	4.70	1.20	3.90±0.25
5	Body depth	BD	1.13	1.61	0.48	1.31±0.12
6	Dorsal fin height	DFH	0.68	0.97	0.29	0.81±0.06
7	Dorsal fin length	DFL	0.59	0.81	0.22	0.69±0.06
8	Pre dorsal length	PDL	1.70	2.30	0.60	1.91±0.16
9	Post dorsal length	PODL	2.29	3.01	0.72	2.61±0.18
10	Pectoral fin height	PFH	0.67	0.97	0.30	0.81±0.09
11	Pectoral fin length	PFL	0.15	0.27	0.12	0.22±0.03
12	Pre pectoral length	PPL	0.98	1.29	0.31	1.11±0.07
13	Post pectoral length	POPL	1.15	1.48	0.33	1.32±0.08
14	Pelvic fin height	VFH	0.54	0.88	0.34	0.73±0.08
15	Pelvic fin length	VFL	0.19	0.38	0.19	0.26±0.05
16	Pre pelvic length	PVL	1.83	2.32	0.49	1.97±0.12
17	Post pelvic length	POVL	2.07	2.85	0.78	2.24±0.17
18	Anal fin height	AFH	0.43	0.96	0.53	0.60±0.10
19	Anal fin length	AFL	0.37	0.55	0.18	0.47±0.05
20	Pre anal length	PAL	2.55	3.22	0.67	2.78±0.17
21	Post anal length	POAL	2.95	3.71	0.76	3.25±0.19
22	Caudal fin height	CFH	0.95	1.30	0.35	1.11±0.09
23	Caudal fin length	CFL	0.46	0.63	0.17	0.52±0.05
24	Distance between pectoral and pelvic fin	DPP	0.69	1.15	0.46	0.93±0.10
25	Distance between pelvic and anal fin	DPA	0.70	0.98	0.28	0.81±0.08
26	Depth of caudal peduncle	DCP	0.53	0.80	0.27	0.63±0.07
27	Length of caudal peduncle	LCP	0.52	0.85	0.33	0.69±0.08
28	Head length	HL	0.93	1.23	0.30	1.08±0.07
29	Head depth	HD	0.75	0.96	0.21	0.86±0.06
30	Head width	HW	0.53	0.65	0.12	0.58±0.03

31	Eye diameter	ED	0.31	0.42	0.11	0.36±0.03
32	Snout length	SNL	0.23	0.36	0.13	0.29±0.04
33	Post orbital length	POL	0.38	0.63	0.25	0.48±0.05
34	Interorbital length	IOL	0.43	0.64	0.21	0.51±0.05
35	Internarial width	INW	0.16	0.28	0.12	0.21±0.03
36	Width of gape of mouth	WGM	0.21	0.31	0.10	0.26±0.02
37	Distance from snout to occiput	DSO	0.78	1.08	0.30	0.92±0.08
38	Distance from occiput to dorsal fin	DOD	0.70	1.38	0.68	0.99±0.14

### 4.3.3 Meristic counts of *Pethia conchoni*

The meristic characteristics for *Pethia conchoni* are given in Table 6. The meristic characteristics showed the following average counts: dorsal fin rays (DFR) were 9 (formatted as ii,7), pectoral and ventral fin rays (PFR and VFR) both averaged 9 (i,8), and anal fin rays (AFR) were 7 (iii,4). The caudal fin rays (CFR) had an average of 19 (i,17,i), while lateral line scales (LL) ranged between 5-8. The pre-dorsal scales (PDS) ranged between 7-11, with 4.5 scales between the lateral line and both the dorsal (SLD) and ventral fins (SLV). Additionally, pre-pelvic scales (PPS) and pre-anal scales (PAS) ranged between 7 to 11 and 13 to 17, respectively.

**Table 6.** Meristic counts of *Pethia conchoni*

S.N.	Meristic measurements	Code	Range
1	Dorsal fin rays	DFR	ii,7
2	Pectoral fin rays	PFR	i,8
3	Ventral fin rays	VFR	i,8
4	Anal fin rays	AFR	iii,4
5	Caudal fin rays	CFR	i,17,i
6	Lateral line	LL	5-8
7	Pre-dorsal scales	PDS	7-11
8	Scales between lateral line and dorsal fin	SLD	4 1/2
9	Scales between lateral line and ventral fin	SLV	4 1/2
10	Pre pelvic scales	PPS	7-11
11	Pre anal scales	PAS	13-17

#### 4.4 Correlation analysis of *Puntius sophore*, *Puntius chola* and *Pethia conchonius*

In the present study, twenty-six characteristics were analyzed with total length as an independent variable and nine parameters were analyzed as head length as an independent variable.

##### 4.4.1 Correlation analysis of *Puntius sophore*

The slope, intercept, coefficient of correlation ( $r$ ), coefficient of determination ( $r^2$ ) and regression equation for *Puntius sophore* are presented in Table 7. The correlation analysis of *Puntius sophore* revealed the following  $r$  values: PDL ( $r = 0.94$ ), FL ( $r = 0.92$ ), PAL ( $r = 0.92$ ), SL ( $r = 0.91$ ), PVL ( $r = 0.91$ ), POAL ( $r = 0.91$ ), PPL ( $r = 0.90$ ) and PODL ( $r = 0.93$ ) showed very strong positive correlations with TL, while BD ( $r = 0.84$ ), PPL ( $r = 0.90$ ), POPL ( $r = 0.86$ ), POVL ( $r = 0.88$ ), and CFL ( $r = 0.82$ ) demonstrated strong positive correlations. Weaker positive correlations with TL were observed for VFL ( $r = 0.20$ ) and, PFL ( $r = 0.38$ ). For HL measurements, HW ( $r = 0.79$ ), POL ( $r = 0.81$ ), and WGM ( $r = 0.75$ ) exhibited moderately strong positive correlations.

**Table 7.** Slope, intercept, coefficient of correlation ( $r$ ), coefficient of determination ( $r^2$ ) and regression equation of *Puntius sophore*

S.N.	Dependent	Independent	Slope	Intercept	$r$	$r^2$	Equation
1	FL	TL	0.84	0.38	0.92	0.85	$0.84x + 0.38$
2	SL	TL	0.71	0.47	0.91	0.83	$0.71x + 0.47$
3	BD	TL	0.25	0.15	0.84	0.71	$0.25x + 0.15$
4	DFH	TL	0.15	0.25	0.77	0.59	$0.15x + 0.25$
5	DFL	TL	0.10	0.20	0.79	0.62	$0.10x + 0.20$
6	PDL	TL	0.31	0.49	0.94	0.88	$0.31x + 0.49$
7	PODL	TL	0.42	0.69	0.93	0.87	$0.42x + 0.69$
8	PFH	TL	0.09	0.35	0.68	0.46	$0.09x + 0.35$
9	PFL	TL	0.02	0.11	0.38	0.14	$0.02x + 0.11$
10	PPL	TL	0.20	0.12	0.90	0.81	$0.20x + 0.12$
11	POPL	TL	0.22	0.23	0.86	0.74	$0.22x + 0.23$
12	VFH	TL	0.12	0.16	0.71	0.50	$0.12x + 0.16$
13	VFL	TL	0.01	0.20	0.20	0.04	$0.01x + 0.20$
14	PVL	TL	0.35	0.19	0.91	0.82	$0.35x + 0.19$

15	POVL	TL	0.36	0.41	0.88	0.77	$0.36x + 0.41$
16	AFH	TL	0.05	0.43	0.47	0.22	$0.05x + 0.43$
17	AFL	TL	0.06	0.13	0.62	0.39	$0.06x + 0.13$
18	PAL	TL	0.53	0.16	0.92	0.84	$0.53x + 0.16$
19	POAL	TL	0.59	0.29	0.91	0.83	$0.59x + 0.29$
20	CFH	TL	0.12	0.57	0.67	0.45	$0.12x + 0.57$
21	CFL	TL	0.12	-0.12	0.82	0.67	$0.12x - 0.12$
22	DPP	TL	0.18	-0.04	0.75	0.56	$0.18x - 0.04$
23	DPA	TL	0.16	0.21	0.74	0.54	$0.16x + 0.21$
24	DCP	TL	0.11	0.08	0.73	0.54	$0.11x + 0.08$
25	LCP	TL	0.07	0.41	0.50	0.25	$0.07x + 0.41$
26	DOD	TL	0.12	0.53	0.51	0.26	$0.53x + 0.12$
27	HD	HL	0.58	0.30	0.71	0.50	$0.58x + 0.30$
28	HW	HL	0.49	0.06	0.79	0.62	$0.49x + 0.06$
29	ED	HL	0.22	0.15	0.62	0.38	$0.22x + 0.15$
30	SNL	HL	0.28	-0.0008	0.67	0.45	$0.28x - 0.0008$
31	POL	HL	0.54	-0.11	0.81	0.65	$0.54x - 0.11$
32	IOL	HL	0.60	-0.16	0.62	0.39	$0.60x - 0.16$
33	INW	HL	0.19	0.02	0.61	0.37	$0.19x + 0.02$
34	WGM	HL	0.31	-0.07	0.75	0.57	$0.31x - 0.07$
35	DSO	HL	0.68	0.25	0.60	0.36	$0.68x + 0.25$

#### 4.4.2 Correlation analysis of *Puntius chola*

The slope, intercept, coefficient of correlation ( $r$ ), coefficient of determination ( $r^2$ ) and regression equation for *Puntius chola* are presented in Table 8. The correlation analysis of *Puntius chola* revealed extremely strong relationships between total length (TL) and several body measurements, including FL ( $r = 0.99$ ), SL ( $r = 0.99$ ), PDL ( $r = 0.99$ ), PODL ( $r = 0.99$ ), PVL ( $r = 0.99$ ), POVL ( $r = 0.99$ ), PAL ( $r = 0.99$ ), and POAL ( $r = 0.99$ ), with corresponding  $r^2$  value indicating near-perfect linear relationships. Similarly, strong correlations were observed for PPL ( $r = 0.98$ ), POPL ( $r = 0.98$ ), VFH ( $r = 0.98$ ), DOD ( $r = 0.98$ ), BD ( $r = 0.97$ ), DFL ( $r = 0.94$ ), CFH ( $r = 0.93$ ), CFL ( $r = 0.95$ ), DPP ( $r = 0.95$ ), DPA ( $r = 0.95$ ), and DCP ( $r = 0.91$ ). The weakest correlations with TL were found for DFH ( $r = 0.36$ ). For head measurements versus head length (HL), the strongest

correlations were observed with HW ( $r = 0.91$ ), IOL ( $r = 0.91$ ), and POL ( $r = 0.87$ ), while the weakest were with ED ( $r = 0.60$ ) and WGM ( $r = 0.76$ ).

**Table 8.** Slope, intercept, coefficient of correlation ( $r$ ), coefficient of determination ( $r^2$ ) and regression equation of *Puntius chola*

S.N.	Dependent	Independent	Slope	Intercept	$r$	$r^2$	Equation
1	FL	TL	0.95	-0.49	0.99	0.98	$0.95x - 0.49$
2	SL	TL	0.90	-1.01	0.99	0.98	$0.90x - 1.01$
3	BD	TL	0.26	0.18	0.97	0.94	$0.26x - 0.18$
4	DFH	TL	0.03	1.20	0.36	0.13	$0.03x + 1.20$
5	DFL	TL	0.11	0.29	0.94	0.88	$0.11x + 0.29$
6	PDL	TL	0.42	-0.46	0.99	0.97	$0.42x - 0.46$
7	PODL	TL	0.53	-0.16	0.99	0.98	$0.53x - 0.16$
8	PFH	TL	0.14	0.08	0.92	0.85	$0.14x + 0.08$
9	PFL	TL	0.04	-0.02	0.89	0.79	$0.04x - 0.02$
10	PPL	TL	0.17	0.46	0.98	0.97	$0.17x + 0.46$
11	POPL	TL	0.21	0.45	0.98	0.95	$0.21x + 0.45$
12	VFH	TL	0.12	0.31	0.98	0.95	$0.12x + 0.31$
13	VFL	TL	0.03	0.14	0.70	0.48	$0.03x + 0.14$
14	PVL	TL	0.35	0.26	0.99	0.99	$0.35x + 0.14$
15	POVL	TL	0.38	0.40	0.99	0.99	$0.38x + 0.40$
16	AFH	TL	0.08	0.28	0.80	0.64	$0.08x + 0.28$
17	AFL	TL	0.07	0.10	0.80	0.64	$0.07x + 0.10$
18	PAL	TL	0.60	-0.31	0.99	0.99	$0.60x - 0.31$
19	POAL	TL	0.67	-0.22	0.99	0.98	$0.67x - 0.22$
20	CFH	TL	0.17	0.39	0.93	0.86	$0.17x + 0.39$
21	CFL	TL	0.10	0.04	0.95	0.91	$0.10x + 0.04$
22	DPP	TL	0.18	-0.03	0.95	0.90	$0.18x - 0.03$
23	DPA	TL	0.24	-0.42	0.95	0.90	$0.24x - 0.42$
24	DCP	TL	0.10	0.32	0.91	0.82	$0.10x - 0.32$
25	LCP	TL	0.09	0.24	0.68	0.47	$0.09x - 0.24$
26	DOD	TL	0.28	-0.46	0.98	0.96	$0.28x - 0.46$
27	HD	HL	0.67	0.29	0.83	0.68	$0.67x - 0.29$

28	HW	HL	0.54	0.04	0.91	0.83	0.54x + 0.04
29	ED	HL	0.12	0.25	0.60	0.36	0.12x + 0.25
30	SNL	HL	0.18	0.23	0.84	0.70	0.18x + 0.23
31	POL	HL	0.32	0.28	0.87	0.75	0.32x + 0.28
32	IOL	HL	0.45	0.09	0.91	0.82	0.45x + 0.09
33	INW	HL	0.15	0.08	0.81	0.66	0.15x + 0.08
34	WGM	HL	0.20	0.10	0.76	0.57	0.20x + 0.10
35	DSO	HL	0.53	0.48	0.81	0.65	0.53x + 0.48

#### 4.4.3 Correlation analysis of *Pethia conchonius*

The slope, intercept, coefficient of correlation (r), coefficient of determination ( $r^2$ ) and regression equation for *Pethia conchonius* are presented in Table 9. The correlation analysis of *Pethia conchonius* revealed markedly different scaling patterns between body and head dimensions. Total length (TL) showed exceptionally strong positive correlations with key body measurements including FL ( $r = 0.96$ ), SL ( $r = 0.95$ ), PAL ( $r = 0.95$ ), PVL ( $r = 0.89$ ), and POAL ( $r = 0.94$ ). In contrast, head length (HL) exhibited only moderate to weak correlations with other cranial measurements: HD ( $r = 0.59$ ), HW ( $r = 0.54$ ), and notably poor correlations with ED ( $r = 0.08$ ), SNL ( $r = 0.07$ ), WGM ( $r = 0.08$ ), and IOL ( $r = 0.20$ ) when HL served as the independent variable. This variation was similarly observed in selective body traits such as PFL which showed  $r = 0.07$ , and AFH with  $r$  value 0.14 showed minimal correlation. The strong TL-body correlations versus weak HL-head correlations suggest differential growth control mechanisms.

**Table 9.** Slope, intercept, coefficient of correlation (r), coefficient of determination ( $r^2$ ) and regression equation of *Pethia conchonius*

S.							
N.	Dependent	Independent	Slope	Intercept	r	$r^2$	Equation
1	FL	TL	0.89	0.05	0.96	0.93	0.89x + 0.05
2	SL	TL	0.77	0.06	0.95	0.91	0.77x + 0.06
3	BD	TL	0.30	-0.17	0.76	0.57	0.30x - 0.17
4	DFH	TL	0.08	0.41	0.40	0.16	0.080x + 0.41
5	DFL	TL	0.10	0.21	0.51	0.26	0.10x + 0.21
6	PDL	TL	0.39	-0.01	0.77	0.60	0.39x - 0.01
7	PODL	TL	0.49	0.20	0.85	0.71	0.49x + 0.20

8	PFH	TL	0.16	0.03	0.56	0.32	$0.16x + 0.03$
9	PFL	TL	0.01	0.18	0.07	0.01	$0.01x + 0.18$
10	PPL	TL	0.11	0.55	0.53	0.28	$0.11x + 0.55$
11	POPL	TL	0.12	0.71	0.49	0.24	$0.12x + 0.71$
12	VFH	TL	0.12	0.15	0.45	0.20	$0.12x + 0.15$
13	VFL	TL	0.05	0.01	0.34	0.12	$0.05x + 0.01$
14	PVL	TL	0.33	0.31	0.89	0.79	$0.33x + 0.31$
15	POVL	TL	0.35	0.51	0.62	0.39	$0.35x + 0.51$
16	AFH	TL	0.04	0.38	0.14	0.02	$0.04x + 0.38$
17	AFL	TL	0.05	0.23	0.33	0.11	$0.05x + 0.23$
18	PAL	TL	0.52	0.19	0.95	0.90	$0.52x + 0.19$
19	POAL	TL	0.57	0.41	0.94	0.88	$0.57x + 0.41$
20	CFH	TL	0.11	0.54	0.40	0.16	$0.11x + 0.54$
21	CFL	TL	0.07	0.18	0.46	0.21	$0.07x + 0.18$
22	DPP	TL	0.22	-0.16	0.71	0.50	$0.22x - 0.16$
23	DPA	TL	0.17	-0.03	0.65	0.42	$0.17x - 0.03$
24	DCP	TL	0.19	-0.31	0.79	0.62	$0.19x - 0.31$
25	LCP	TL	0.06	0.41	0.23	0.05	$0.06x + 0.41$
26	DOD	TL	0.20	0.0029	0.46	0.21	$0.20x + 0.0029$
27	HD	HL	0.47	0.35	0.59	0.35	$0.47x + 0.35$
28	HW	HL	0.25	0.31	0.54	0.30	$0.25x + 0.31$
29	ED	HL	0.03	0.33	0.08	0.01	$0.03x + 0.33$
30	SNL	HL	0.04	0.25	0.07	0.01	$0.04x + 0.25$
31	POL	HL	0.30	0.16	0.44	0.20	$0.30x + 0.16$
32	IOL	HL	0.14	0.36	0.20	0.04	$0.14x + 0.36$
33	INW	HL	0.20	-0.01	0.42	0.17	$0.20x - 0.01$
34	WGM	HL	0.03	0.23	0.08	0.01	$0.03x + 0.23$
35	DSO	HL	0.31	0.58	0.29	0.09	$0.31x + 0.58$

#### 4.5 Multivariate analysis of morphometric data

##### 4.5.1 Principal component analysis (PCA)

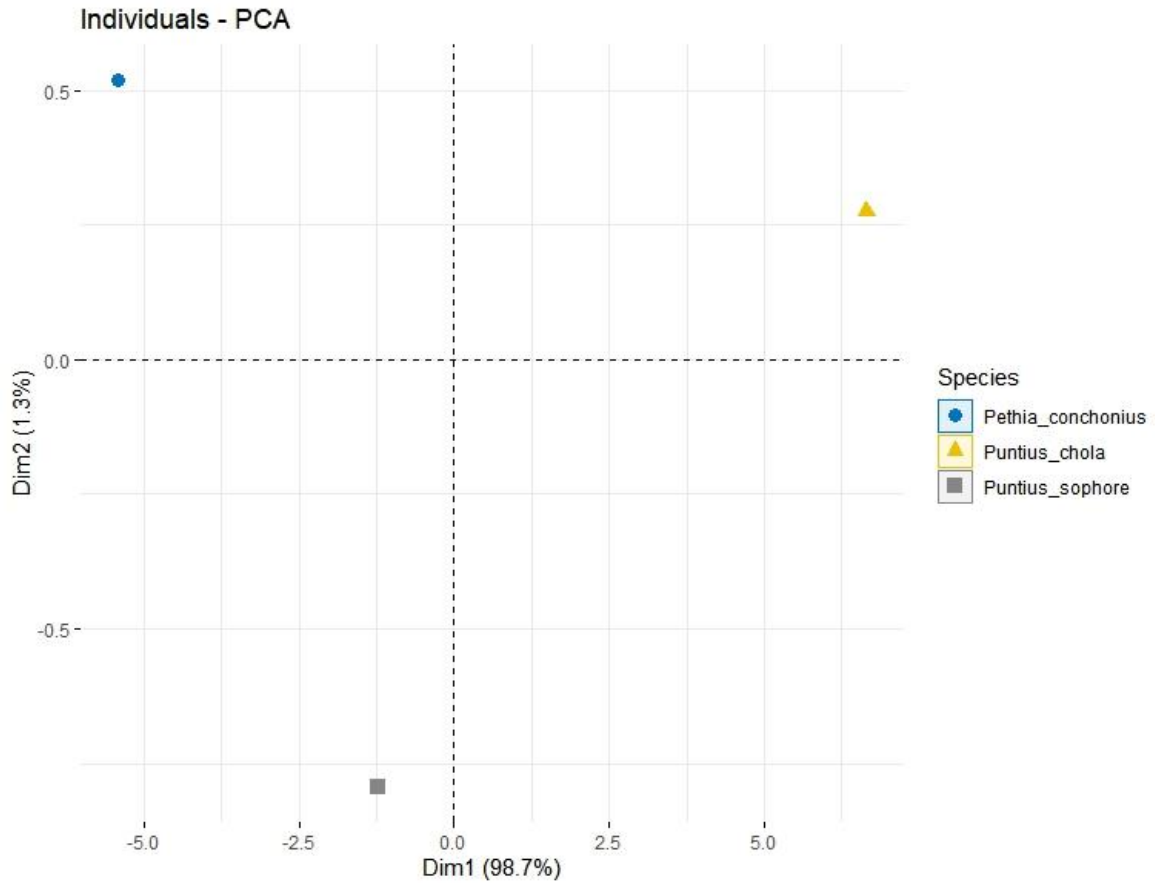
Principal Component Analysis (PCA) was conducted to evaluate morphological variations among three fish species: *Puntius sophore*, *Puntius chola*, and *Pethia*

*conchonius*, based on body part measurements. The PCA scores for the first three principal components (PC1, PC2, and PC3) are presented in Table 10. PC1 accounted for 98.7% of the total variance, indicating it captures the majority of morphological differences among the species, while PC2 explained 1.3% of the variance, providing additional but minor differentiation. Notably, *Puntius chola* exhibited a high positive PC1 score (6.647421), distinctly separating it from *Pethia conchonius* (-5.414635) and *Puntius sophore* (-1.232786), both of which had negative PC1 scores. Along PC2, *Pethia conchonius* had a slightly positive score (0.5183201), while *Puntius sophore* had a negative score (-0.7933809), suggesting subtle differences in secondary morphological traits.

**Table 10.** PCA scores of *Puntius sophore*, *Puntius chola* and *Pethia conchonius*

Species	PC1	PC2
<i>Puntius sophore</i>	-1.232786	-0.7933809
<i>Puntius chola</i>	6.647421	0.2750608
<i>Pethia conchonius</i>	-5.414635	0.5183201

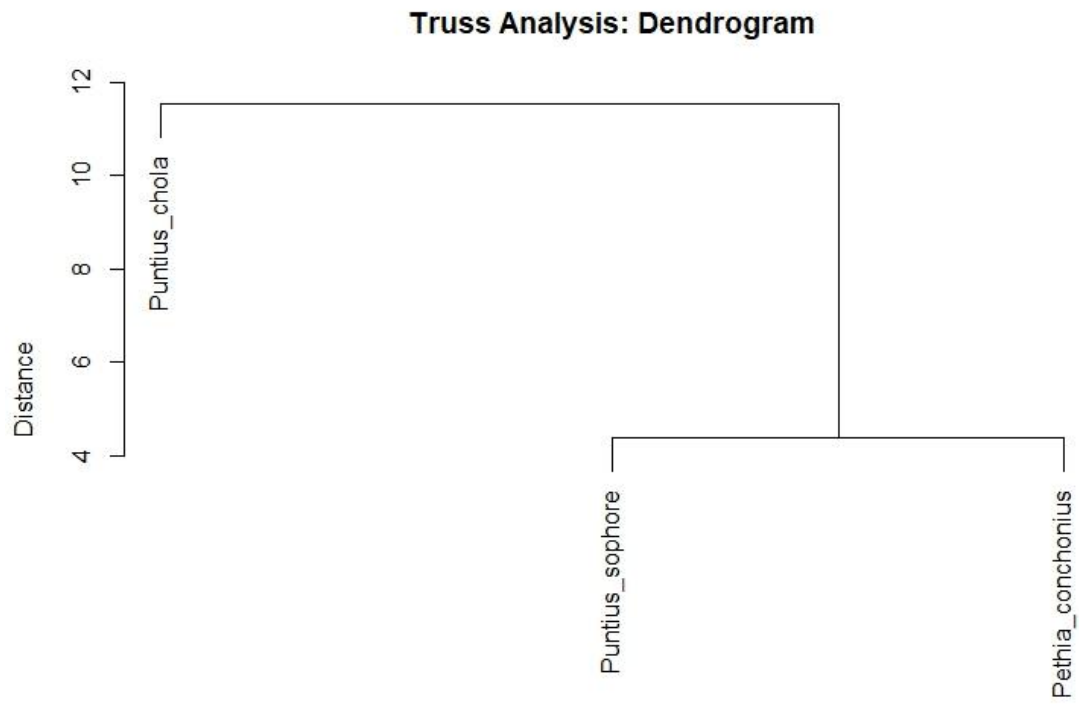
The distribution of the three species in the PC1 vs. PC2 space is visualized in Figure 2, which plots the scores from Table 10. PC1 (Dim1, 98.7%) is represented on the x-axis, and PC2 (Dim2, 1.3%) on the y-axis, collectively explaining 100% of the variance relevant to this two-dimensional representation. The scatter plot clearly illustrates the separation observed in the scores: *Puntius chola* (yellow triangle) is positioned far on the positive side of PC1 (approximately 6.6), while *Pethia conchonius* (blue circle) and *Puntius sophore* (gray square) are on the negative side, with PC1 scores of approximately -5.4 and -1.2, respectively. Along PC2, *Pethia conchonius* is slightly positive (~0.5), *Puntius chola* is near zero (~0.3), and *Puntius sophore* is negative (~-0.8), indicating additional differentiation among the species. This visual representation confirms that PC1 is the primary axis of variation, with *Puntius chola* being morphologically distinct from the other two species.



**Figure 2.** PCA Scatter Plot of Three Fish Species in PC1 (Dim1, 98.7%) vs. PC2 (Dim2, 1.3%) Space

To understand the morphological basis of the species' separation, the contributions of body part measurements to PC1 and PC2 were analyzed using a loadings plot (Figure 3). Each vector represents a body part measurement, with its direction and length indicating its contribution to the principal components. Body parts such as anal fin length (AFL) and pectoral fin length (PFL) exhibit strong positive loadings on PC1, suggesting they are key drivers of the variation along this axis, which aligns with the high positive PC1 score of *Puntius chola*. Conversely, dorsal fin height (DFH) and distance between pelvic and anal fin (DPA) show negative loadings on PC1, contributing to the negative PC1 scores of *Pethia conchonius* and *Puntius sophore*. Along PC2, AFL and PFL also have positive loadings, while DFH has a negative loading, reflecting the subtle differences observed in the species' PC2 scores. The clustering of other body parts near the origin indicates their balanced or moderate contributions to both components.





**Figure 4.** Hierarchical Clustering Dendrogram Based on Truss Morphometric Data of *Puntius chola*, *Puntius sophore*, and *Pethia conchonius*

## 5. Discussion

### 5.1 Morphological and meristic analysis of *Puntius sophore*

*Puntius sophore*, originally described by Hamilton (1822) as *Cyprinus sophore*, is a small and elegant freshwater fish characterized by its greenish-silver hue on the dorsal side and a silvery underside. It features a diffuse golden spot on the gill covers and on the base of a dorsal fin, there is a presence of distinctive black blotch, traits that remain consistent with recent studies.

Hamilton (1822) documented the meristic counts of *Puntius sophore* as follows: the dorsal fin has 10 rays, with the second ray being twice as long as the first; the pectoral fins consist of 14 rays; the ventral fins, smaller than the pectorals, have 9 rays; the anal fin is slightly arched with 7 rays; and the caudal (tail) fin comprises 19 rays.

In contrast, Shrestha (2019) reported slightly different meristic counts, describing the species as having 11 dorsal fin rays, 15–16 pectoral fin rays, 9 ventral fin rays, 8 anal fin rays, and 19 caudal fin rays. However, the present aligns closely with Hamilton's original description, reporting 10 dorsal fin rays, 8–15 pectoral fin rays, 7–11 ventral fin rays, 7 anal fin rays, and 17–24 caudal fin rays. These variations in fin ray counts highlight potential regional or population-level differences within the species.

### 5.2 Morphological and meristic analysis of *Puntius chola*

*Puntius chola*, originally described by Hamilton (1822) under the name *Cyprinus chola*, is characterized by its opaque body with a greenish-silver hue on the dorsal side and a silvery underside. Distinctive features include a golden spot on the gill covers, a small black spot near the tail, and large scales. In this study, the noted features are similar to those of the original description except the small black spot near the opercle, which was documented later. Later, a fish with similar characteristics but bearing a small dark spot behind the operculum was classified as *Puntius titius* (Hamilton, 1822). However, this classification was eventually considered a junior synonym for *Puntius chola*. Interestingly, Shrestha (1981) also proposed *Puntius titius* as a distinct species. The present study shows a similar variation, characterized by two distinct black blotches: one on the base of the tail and one behind the opercle.

The meristic counts of *Puntius chola* have been documented differently across historical and recent studies, highlighting potential variability within the species or differences in

observation methods. Hamilton (1822) originally described the species with 10 rays in the dorsal fin, 13 rays in the pectoral fins, 9 rays in the ventral fins, 7 rays in the anal fin, and 19 rays in the caudal fin, emphasizing that the first dorsal fin ray is notably short. In contrast, Shrestha (2019) reported slightly higher counts, including 11 rays in the dorsal fin, 14–15 rays in the pectoral fins, 9 rays in the ventral fins, 8 rays in the anal fin, and 19 rays in the caudal fin. The present study aligns more closely with Hamilton's original description but introduce a range for some counts, reporting 10 rays in the dorsal fin, 11–14 rays in the pectoral fins, 7–9 rays in the ventral fins, 6 rays in the anal fin, and 18–20 rays in the caudal fin.

### **5.3 Morphological and meristic analysis of *Pethia conchonius***

*Pethia conchonius*, originally described by Hamilton (1822) as *Cyprinus conchonius* but later classified under the genus *Puntius*, is a small, elegant fish native to the freshwater systems of northern Bengal, including the Kosi and Ami rivers within the Gangetic provinces. This species exhibits remarkable variability in coloration and fin patterns across different regions. In Bengal, it is characterized by clear, spotless fins, a greenish back, and a silvery underside. Specimens from Behar display blackish fins, while those from the weed-filled Ami River have a black-stained belly, along with yellow dorsal and tail fins that lack blackish ends. Despite these regional differences in appearance, the observed variations likely represent intraspecific diversity, and all populations are believed to belong to the same species. In the current study, *Pethia conchonius* exhibited darkish back and silvery belly with pelvic fins being transparent and but dorsal and caudal fins with dark tips, showing similarity to the original description.

Hamilton (1822) documented the meristic counts of *P. conchonius*, noting that its dorsal fin contains 10 rays, the pectoral fins have 10 rays each, the ventral fins consist of 9 rays each, the anal fin has 8 rays, and the caudal fin comprises 19 distinct rays. In contrast, Shrestha (2019) reported slightly different counts, describing the species as having 11 dorsal fin rays, 13–15 pectoral fin rays, 9 ventral fin rays, 8 anal fin rays, and 19 caudal fin rays. The present study provides further variation, reporting 9 dorsal fin rays, 7–11 pectoral fin rays, 8–10 ventral fin rays, 7 anal fin rays, and 16–22 caudal fin rays.

### **5.4 Correlation analysis of *Puntius sophore*, *Puntius chola* and *Pethia conchonius***

The correlation analysis revealed distinct scaling patterns among the three species. *Puntius chola* exhibited the strongest linear relationships, with near-perfect correlations ( $r$

= 0.98–0.99) between total length (TL) and most body measurements (e.g., FL, SL, PDL, PODL). *Puntius sophore* showed very strong correlations ( $r = 0.90–0.94$ ) for key metrics like PDL, PODL, and PAL, but weaker associations for VFL ( $r = 0.20$ ) and PFL ( $r = 0.38$ ). In contrast, *Pethia conchonius* displayed strong TL-body correlations (e.g., FL  $r = 0.96$ , PAL  $r = 0.95$ ) but notably weak head-related scaling, with HL showing minimal correlations ( $r \leq 0.20$  for ED, SNL, IOL, WGM). This suggests *P. chola* has the most consistent allometric growth, while *P. conchonius* exhibits divergent growth control between body and head dimensions.

The correlation analysis for *Puntius chola*, *Puntius sophore*, and *Pethia conchonius* reveals both consistencies and subtle variations when comparing the summarized results in Saroniya et al. (2013) with the detailed findings of this study. For *Puntius chola*, both datasets consistently indicate very high correlations ( $r > 0.95$ ) for key morphometric traits such as fork length (FL), standard length (SL), pre-anal length (PAL), pre-dorsal length (PODL), and pre-ventral length (POVL). However, weaker correlations are observed for traits like dorsal fin height (DFH), with  $r = 0.36$ , aligning across both sources.

Similarly, for *Puntius sophore*, both Saroniya et al. (2013) and this study highlight strong correlations for FL, SL, PAL, PODL, and POVL, though slight differences emerge in the exact  $r$  values. For instance, the correlation for FL is reported as  $r = 0.995$  in Saroniya et al. (2013), while this study shows a slightly lower value of  $r = 0.92$ .

In the case of *Pethia conchonius*, the datasets align in demonstrating high correlations for FL, SL, PAL, and PVL, but notable differences are observed for head-related traits. For example, this study reports markedly weaker correlations for eye diameter (ED,  $r = 0.08$ ) and snout length (SNL,  $r = 0.07$ ) compared to the moderate correlations mentioned in Saroniya et al. (2013). These discrepancies underscore the variability in cranial measurements and suggest potential differences in sample sizes or measurement techniques between the two analyses.

## 5.5 Multivariate analysis of morphometric data

When comparing morphometric variation using PCA across studies, notable consistency emerges in the distribution of *Puntius chola*, *Puntius sophore*, and *Pethia conchonius* within principal component space. The present analysis showed a remarkably high proportion of variance explained by the first principal component (PC1 = 98.7%), with species scores indicating clear separation. In Gupta et al. (2018), PCA extracted four

components that together explained 96.45% of the total variance (PC1: 48.39%, PC2: 20.12%, PC3: 15.65%, PC4: 12.28%), effectively differentiating the species using truss measurements. *P. chola* consistently appeared as the most distinct in their PCA plots. Similarly, Roy et al. (2023) reported clear species-level separation among *P. sophore*, *P. conchoni*, and *P. ticto* using PCA, though exact component scores were not detailed. In Roy et al. (2024), PCA also confirmed notable interspecific variation. Across all three studies, *P. chola* emerges as morphometrically distinct, while *P. sophore* and *P. conchoni* often show closer clustering, validating the effectiveness of PCA in identifying shape-based species divergence.

Truss-based hierarchical clustering analysis across studies supports the PCA findings. In the present study, the dendrogram showed *P. sophore* and *P. conchoni* clustering at a lower linkage distance, while *P. chola* branched off at a much greater distance (~12), indicating higher morphological divergence. This pattern closely mirrors the results of Gupta et al. (2018), where dendrograms constructed from 91 truss measurements also placed *P. chola* in a separate cluster, far from *P. sophore* and *P. conchoni*. These repeated clustering patterns across independent datasets further support the unique body shape of *P. chola* and the relative morphometric similarity between *P. sophore* and *P. conchoni*. The agreement between PCA and truss-based clustering across multiple studies emphasizes the reliability of multivariate morphometric tools in resolving species boundaries within cyprinid fishes.

## 6. Conclusions and recommendations

### 6.1 Conclusions

This study provides a detailed taxonomic analysis of *Puntius sophore*, *Puntius chola*, and *Pethia conchonius* in the Pokhara Valley lakes, highlighting their distinct morphological, morphometric, and meristic characteristics. The findings align with previous descriptions while revealing variations that may reflect environmental or seasonal influences. The correlation analysis reveals distinct allometric patterns among the three species. *Puntius chola* exhibits the most consistent linear growth, while *Puntius sophore* shows slight variations in fin measurements. *Pethia conchonius* demonstrates strong body-length correlations but weak head-related scaling, indicating divergent growth regulation. The PCA and truss analyses reveal clear morphological differentiation among the three species. *Puntius chola* is distinctly separated while *P. sophore* and *P. conchonius* show closer similarity. These findings confirm the effectiveness of multivariate morphometric methods in species discrimination.

### 6.2 Recommendations

- Future research should include samples from a wider geographic range and across multiple seasons to better capture the full morphological diversity of the species throughout its distribution.
- Researchers are encouraged to consider measuring environmental factors such as water temperature, food availability, and habitat conditions, as these may influence morphometric and meristic characteristics.
- It is suggested that further molecular and taxonomic studies be conducted to verify whether *Puntius chola* belongs to a different genus.

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## 8. Photographs



Photograph 1: Begnas Lake



Photograph 2: Rupa Lake



Photograph 3: Fresh specimen of *Puntius sophore*



Photograph 4: *Puntius sophore*



Photograph 5: Fresh specimen of *Puntius chola*



Photograph 6: *Puntius chola*



Photograph 7: Fresh specimen of *Pethia conchonius*



Photograph 8: *Pethia conchonius*



Photograph 9: Cooperative visit in Phewa lake



Photograph 10: Cooperative visit in Begnas lake



Photograph 11: Interacting with the fisherman



Photograph 12: Making of cast net