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# Integrative Morphological Analysis of Berod Fish (*Mastacembelus unicolor*) in the Brantas River, East Java

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**Abstract:** *Mastacembelus sp.*, a member of the Mastacembelidae family, is distributed across Java, Sumatera, and Kalimantan. Mastacembelus, locally named berod fish in the Brantas River lacks population's descriptions and morphological analyses. Morphological analyses, including morphometric and meristic descriptions, are important for precise identification and classification of kinship relationships, serving as a cornerstone in the fields of biology, taxonomy, and conservation. This study aimed to conduct a comprehensive morphometric and meristic identification of the berod fish population in the Brantas River, East Java. A total of 37 samples were collected from 6 different locations: Blitar, Tulungagung, Kediri1, Kediri2, Nganjuk, and Mojokerto. The morphometric measurements were based on 25 characters, while the meristic calculations were based on 6 characters. The data analyzed with PCA and CA using SPSS and PAST programs. The results revealed that berod fish in the Brantas River had morphological characters belongs to the *Mastacembelus unicolor*. However, notable disparities were observed in meristic characters, particularly in the Dorsal Fin Spines (DFS). PCA analysis showed significant differences in morphometric characters but not supported by meristic characters. These results underscore the importance of integration both morphological and meristic analyses for a comprehensive understanding of the berod fish population.

**Keywords:** Mastacembelidae; *Mastacembelus unicolor*; Meristic; Morphological differentiation; morphometric

## Introduction

Meristic and morphometric measurements have been used to identify species and determine morphological differentiation of fish populations in recent years. Morphometric characters play an important role in stock structure studies, as long-term geographic isolation leading to unusual breeding pattern that resulting changes in fish body shapes. Morphometric analysis provides precise identification on interspecific disparities, detailed depiction on morphological diversities among populations or species, and an effective classification method of kinship relationships (Mahfuj et al., 2019). Morphological analysis can be relied upon to provide statistical evidence of differences in form and important in distinguishing between two species due to its ability to assess physical characteristics and structural traits unique to each species (Cakmak et al., 2010). By examining morphological features such as body shape,

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size, coloration, fin configuration ns, and other external attributes, it can discern subtle but significant differences that exist between species (Petrellis, 2021). These morphological disparities often reflect underlying genetic variations and adaptations to specific ecological niches or environmental conditions (Wilson et al., 2021). Furthermore, morphological analysis enables the establishment of diagnostic features serve as reliable identifiers, facilitating accurate species identification even in cases where genetic or molecular analysis data may be unavailable or inconclusive (Astuti et al., 2022; Littman et al., 2021). Additionally, morphological analysis contribute to the taxonomic classification of organisms, aiding in the development of comprehensive species descriptions and classification systems.

The Mastacembelidae family of spiny eels is distributed throughout Indonesia, including Java, Sumatera and Kalimantan. Within Java Island, two genera, *Macrognathus* and *Mastacembelus*, have been recorded. Among these, *Macrognathus aculeatus* and *Mastacembelus unicolor* have been identified (Kottelat et al., 1993; Kusuma et al., 2023). Within East Java, *Mastacembelus* genus can be found in major river systems like the Brantas River with the local name berod fish. However, this genus has not yet been thoroughly studied morphologically and meristically. The study of morphological characters is the basic data of a comprehensive species identification and is a prerequisite needed in providing information for stock assessment (Kusrini et al., 2009). Morphological disparities serve as potential indicators of genetic variance or kinship associations among fish populations, thus offering valuable insights for the development of effective aquatic resource management strategies (Astuti et al., 2021; Irmawati, 2016).

The importance of morphological analysis push this research especially for *Mastacembelus genus* or berod fish. The purpose of this study was to identify the meristic and morphometric characters of berod fish (*Mastacembelus* sp.) and determine the morphological differences between populations in the Brantas River. Overall, the rigorous application of morphological analysis provides a fundamental framework for species identification and classification, serving as a cornerstone in biological research, taxonomic study, conservation efforts, and ecosystem management.

## Method

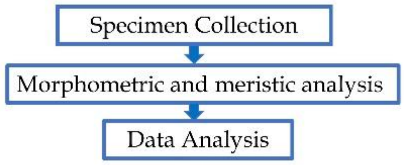


**Figure 1.** Research flowchart

*Specimen Collection*

Specimen collection was conducted by purposive random sampling in the Brantas River, East Java. 37 fish were captured across six distinct locations: Blitar/BTR (n=5), Tulungagung/TLG (n=3), Kediri 1/KD1 (n=9), Kediri 2/KD2 (n=10), Nganjuk/NGA (n=5), and Mojokerto/MJK (n=5) (Fig. 1), using both bubu traps and fishing rods. Samples were obtained alive and dead. Samples obtained alive were euthanized using the method of Wilson et al. (2009) with rapid cooling. The cooling process is carried out using ice cubes, while dead samples are immediately preserved using 96% ethanol and then taken to the laboratory for further analysis.

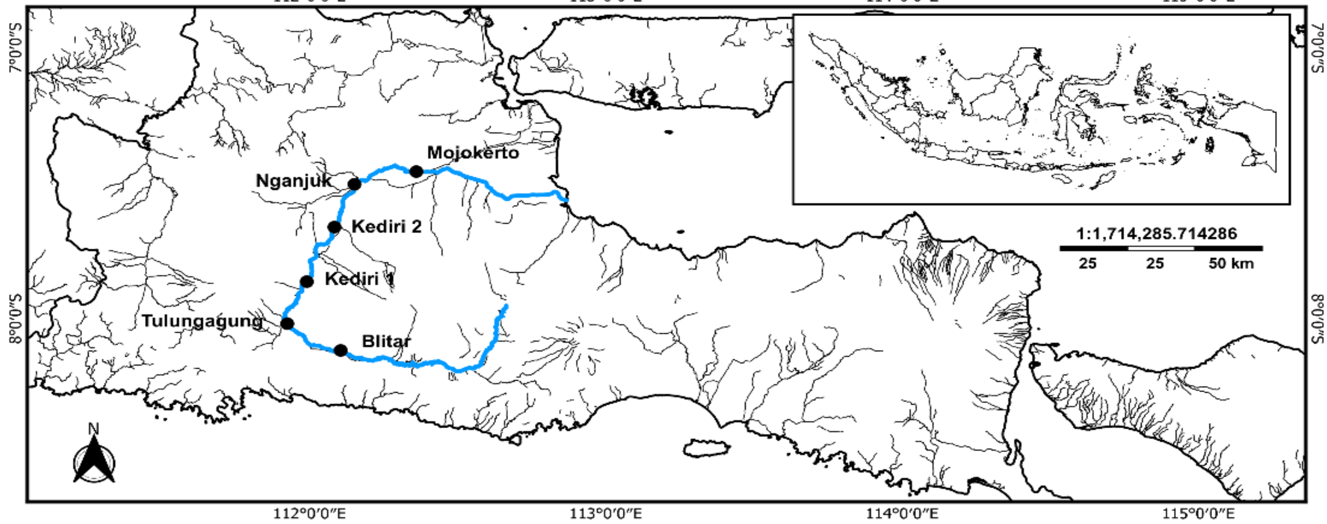


**Figure 2.** A map shows sampling locality of *M. unicolor*, the line with blue color indicates Brantas River

*Morphometric and Meristic Analysis*

Morphological analysis encompassed morphometric measurements and meristic calculations. Morphological identification refers to Kottelat et al. (1993) with subsequent morphometric measurements involving assessment of 25 distinct characters in each sample. Measurements were conducted using precise tools including a digital caliper (accuracy: 0.01 mm) and a ruler (accuracy: 0.1 mm). Morphometric character measurements of the *Mastacembelus* genus refer to Cakmak et al. (2010), Plamoottil et al. (2013), Mahfuj et al. (2019) and Rashid et al. (2019) which have been modified. Morphometric measurements include total length (TL), body depth (BD), body width (BW), pectoral fin length (PFL), height of soft dorsal fin (HSD), height of dorsal spine (HDS), height of soft anal fin (HSA), height of anal spine (HAS), caudal fin length (CL), dorsal spine base length (DSB), dorsal fin base length (DFB), anal spine base length (ASB), anal fin base length (AFB), pectoral fin base (PFB), pre dorsal length (PDL), pre anal length (PAL), upper jaw length (UJL), lower jaw length (LJL), width of gape of mouth (WGM), head length (HL), head depth (HD), head width (HW), eye diameter (ED), and inter orbital width (IO) (Figure 3A). Meristic characteristics represent the count of elements within distinct segments of the fish's body. Measurement of meristic characteristics is based on Plamoottil and Abraham (2013) and Gholamhosseini et al. (2022) which have been modified. A total of six meristic characters were counted included dorsal fin spines (DFS), dorsal fin rays (DFR), anal fin spines (AFS), anal fin rays (AFR), pectoral fin rays (PFR), caudal fin rays (CFR) (Fig 3B).

*Data Analysis*

Morphological data analysis refers to Gholamhosseini et al. (2022). Measurement of meristic characters yield directly usable data as they are independent of the fish's size. The data from morphometric measurements undergo standardization initially. This involves dividing the results of morphometric measurements (excluding head characters) by either the total length (TL) or standard length (SL) and then multiplying by 100%. Conversely, for head characters, the data is divided by head length (HL) and multiplied by 100%. This process ensures the attainment of the ratio for each character. This step was taken to eliminate the effect of variation in fish size or age. Gelsano et al. (2022) explained that the standardization aimed to understand how the relationship is between each body parts of different sexes. The differences of the standardization result also can explain that as the fish grow, morphometric characters may grow symmetrically. To identify the pattern of diversity between populations, the principal component analysis (PCA) method was used, followed by cluster analysis (CA) to determine the grouping of each population and see how far the differences and morphological similarities between populations. PCA and CA analyses were conducted using the Statistical Package for Social Science (SPSS) and PAleontological STatistics (PAST) programs.

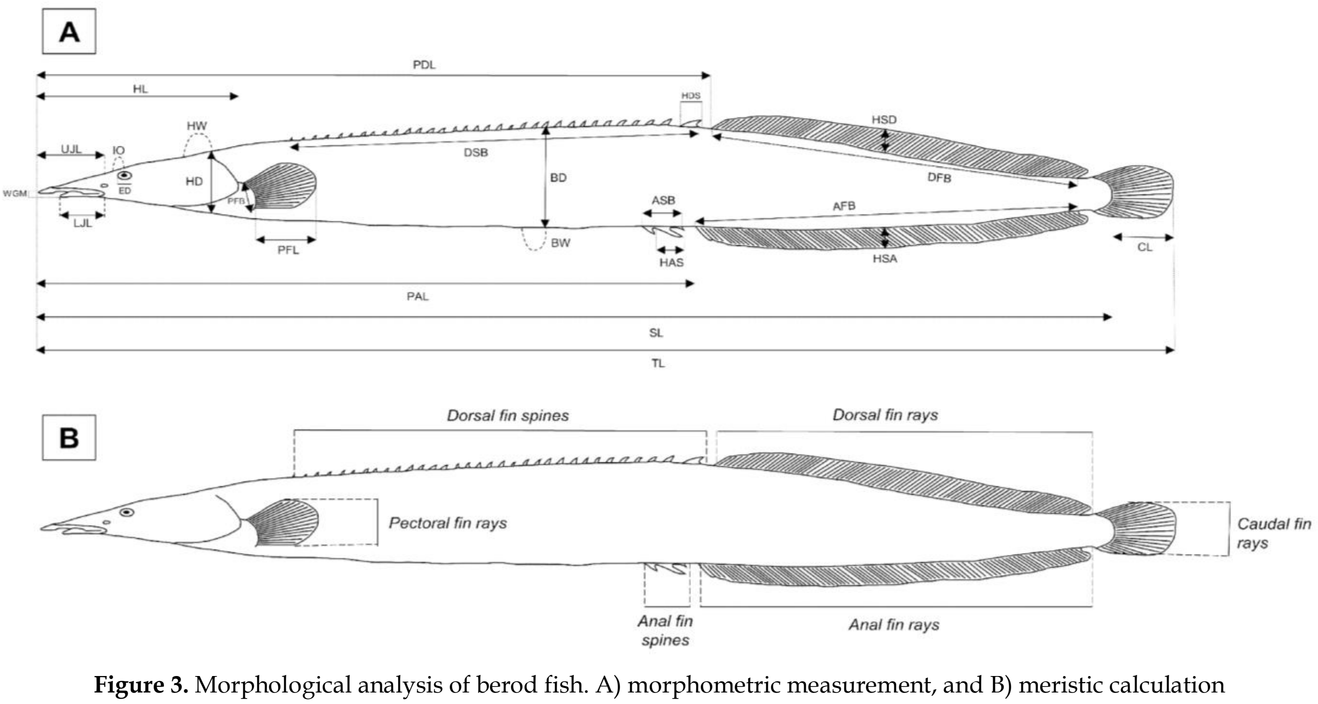


**Figure 3.** Morphological analysis of berod fish. A) morphometric measurement, and B) meristic calculation

## Result and Discussion

The morphological analysis, encompassing color patterns and specific characteristics, as referenced in Kottelat et al. (1993), provides distinctive features for the specimen. The acquired berod fish exhibits an elongated body with a rounded tail, has spines along the dorsal fin and there is a row of short spines in front of the anal fin (Fig. 4). This fish does not have a ventral fin but possesses a snout adorned with two fimbriae located anterior to the mouth (Fig. 5A), dorsal and anal fins seamlessly connected to the tail, accompanied by small spines adorning the preopercular operculum (Fig. 5B), has a blackish brown color on the head and body and brownish-white on the abdomen, has a rounded pattern without any red lines (Fig. 6).

Mastacembelidae is freshwater fishes with anguilliform body. Mastacembelidae has unique characteristic in the rostral and the gill opening. They have two tubular anterior nostrils on the left and right side of the rostral, while the gill opening is reducted because of the opercular membrane is connected to the body's lateral wall (Vreven, 2005; Ng et al., 2020). Morphological analyses of the specimens in this study is in accordance with Sudarto (2010) who explained that *Mastacembelus* fish, a member of mastacembelidae generally have long bodies with flat tails and rows of small spines along the back, precisely in front of the dorsal fin rays. This fish does not have pelvic fins, but has an elongated snout like a trunk with nostrils located on the side (Sudarto, 2010).

There are three species of *Mastacembelus* in Indonesia, including *Mastacembelus erythrotaenia, Mastacembelus notophtalmus* and *Mastacembelus unicolor*. *M. erythrotaenia* has a red band on the head (yellow or white when dead), has 1-15 caudal fin fingers, connected dorsal fins and anal fins. *M. notophtalmus* has an upright dark band under the eye and *M. unicolor* has caudal fin spokes 19-21 that are slightly separated from the dorsal and anal fins with no red band (Kottelat et al., 1993; Dahruddin et al., 2016). The *Mastacembelus* in Indonesia compared to the berod fish in the Brantas River can be seen in Fig 6. The berod fish in the Brantas River based on Fig 6, morphologically has many similarities with the *M. unicolor* described by Kottelat et al. (1993). *M. unicolor* has a special characteristic: the absence of red bands, along with caudal fins slightly set apart from the dorsal and anal fins. Therefore, based on morphological characteristics, the specimen found in the Brantas River are classified as *M. unicolor*. *M. unicolor* fish can reach a size of 1 meter in accordance with Vreven's statement (2005) which explains that the *Mastacembelus* genus can reach a maximum length of 1 meter.

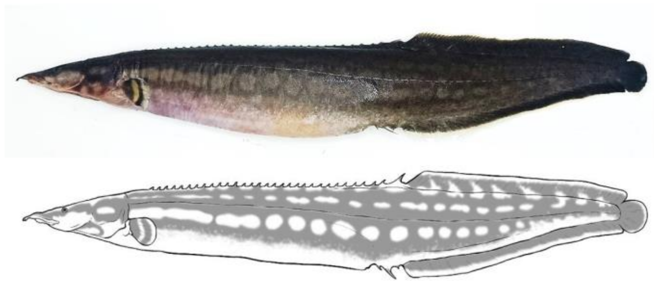

**Figure 4.** Berod fish in Brantas River

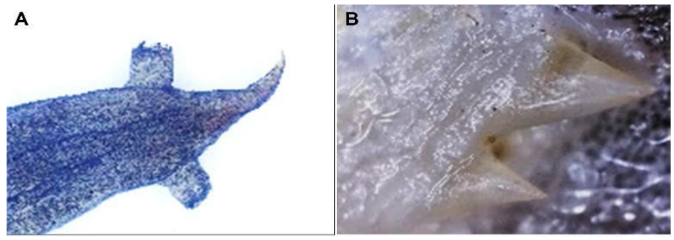

**Figure 5.** Morphology of Berod fish. (A) Fimbriae, (B) Preopercle spines

**Figure 6.** The spiny eel *Mastacembelus* genus in Indonesia, (A) *M. erythrotaenia* (Duong et al., 2020), (B) *M. notophtalmus* (Hasan et al., 2023), (C) *M. unicolor* (Dahruddin et al., 2016)

Meristic identification was carried out by calculating several characters of Berod fish. The results of meristic calculations of all samples are similar to *M. unicolor* but there are discrepancies in dorsal fin spines (DFS) (Table 1). Berod fish in the Brantas River have DFS XXXII-XXXIV, DFR 79-81, AFS III, AFR 71-79, PFR 18-21, CFR 19-21, while Kottelat et al. (1993) described *M. unicolor* fish as having DFS XXXIV-XXXV, DFR 79-90; AFS III, AFR 73-86; CFR 19-21. Broadly speaking, the observation of meristic characters shows that Berod fish belongs to the *M. unicolor* species although there are deviations in DFS. Berod fish has a DFS of 32 - 34, while *M. unicolor* has a DFS of 34 - 35. The difference in DFS is thought to be caused by pollution or as a form of adaptation to environmental changes for a long time. The differences in dorsal fin spines among fish populations also can be attributed to a variety of factors, including genetic variation, environmental influences, and evolutionary adaptations (Caiger et al., 2021). Environmental conditions such as water temperature, pH levels, food availability, and habitat structure can influence the development of dorsal fin spines (Astuti et al., 2020; Francis, 2013). Fish living in different environments may experience varying selective pressures that favor certain traits, including differences in morphology (Wiadnya et al., 2023; Gomes et al., 2008). Another pressure is about predation. Predation can play a significant role in shaping the morphology of fish, including the dorsal fin spines. Populations facing different levels of types of predation may evolve different spine structures as adaptations for defense or predator avoidance. Human activities such as pollution, habitat destruction, overfishing, and introduction of invasive species can disrupt natural selection pressure and genetic flow among fish populations, potentially leading to differences in dorsal fin spines over time (Astuti et al., 2023; Fulton et al., 2013). The results of meristic calculations of berod in the Brantas River can be seen in Table 1.

**Table 1.** Meristic Measurements of Berod Fish

| | Blitar (n=5) | Tulungagung (n=3) | Kediri 1 (n=9) | Kediri 2 (n=10) | Nganjuk (n=5) | Mojokerto (n=5) |
|---|---|---|---|---|---|---|
| DFS | 32-34 | 32-33 | 32-33 | 32-33 | 32-33 | 33-34 |
| DFR | 79 | 79 | 79-81 | 79-80 | 79-81 | 79-80 |
| AFS | 3 | 3 | 3 | 3 | 3 | 3 |
| AFR | 73 | 73 | 73-75 | 73-79 | 73-75 | 73-76 |
| PFR | 18 | 18-19 | 18-21 | 18-20 | 18-20 | 19-21 |
| CFR | 19 | 19-20 | 19-21 | 18-21 | 19-21 | 19 |

Meristic characters were subjected to PCA to classify correlated data into several independent groups. This analytical approach is used to determine size and shape variations among populations based on meristic characters, with the outcomes visualized through scattergrams, simplifying the planning and determination of group number (Aryantojati et al., 2022). PCA analysis refers to AnvariFar et al. (2011) and Gholamhosseini et al. (2022). The PCA results on meristic characters have 2 factors with eigenvalues > 1 which explained 71% of the component variation. PC 1 accounted for 41% of the variation, and PC 2 explained 30%. Notably, in PC 1, PFR exhibited the most significant loading at 0.8, followed by AFR at 0.5. Conversely, in PC 2, AFR held the highest loading at 0.8 Lombarte et al. (2012), highlighted that loadings exceeding 0.30 are deemed significant.

**Table 2.** Meristic characters of Berod fish compared to Mastacembelus in Kottelat et al. (1993)

| | Berod fish | *M. unicolor* | *M. erythrotaenia* | *M. notophthalmus* |
|---|---|---|---|---|
| DFS | 32 – 34 | 34 – 35 | 32 – 35 | 37 – 39 |
| DFR | 79 – 81 | 79 – 90 | 68 – 76 | 73 – 86 |
| AFS | 3 | 3 | 3 | 3 |
| AFR | 73 – 76 | 73 – 86 | 68 – 73 | 69 – 85 |
| CFR | 19 – 21 | 19 – 21 | 14 – 15 | - |

PC 1 and PC 2 scores were visualized to produce a scatter plot that overlapped and separated among the 6 populations (Fig. 7B). These results indicate that not all individuals have meristic similarities. The overlapping results explain the presence of individuals with similar meristic characters and the scattered results indicate the difference in meristic between one individual and another.

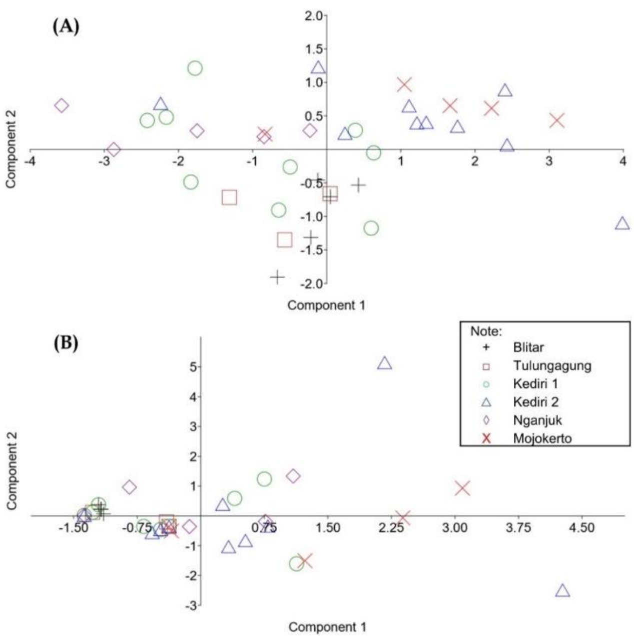


**Figure 7.** Scatter plot meristic and morphometric berod fish (A) Scatter plot of morphometric in six population representing 71% of the data variation, (B) Scatter plot of meristic in six population representing 84,1% of the data variation

The meristic characters underwent cluster analysis to elucidate the relationships between individuals (Fig. 8A). Cluster analysis uses the Euclidean distance algorithm with the unweighted pair group method with arithmetic mean (UPGMA) to show the clustering algorithm among species (Rahman et al., 2022). Cluster Analysis used to generate dendrogram illustrating the morphological relationships among fish based on both morphological and morphometric characters (Mwita, 2005). This is indicated by almost all individuals spreading and not clustering in each location.

*Morphometric characters*

The total length (TL) of Berod fish in the Brantas River was 21.3 - 57.3 cm. Blitar specimens ranged from 21.3 to 26.3 cm; Tulungagung specimens ranged from 26.3 to 38.5 cm; Kediri 1 specimens ranged from 21.3 to 37.8 cm; Kediri 2 ranged from 24.6 to 39.7 cm; Nganjuk specimens ranged from 25.8 to 57.3 cm and Mojokerto specimens ranged from 29.2 to 42.5 cm. The identification results obtained the mean and standard deviation in Table 3, then statistically tested using ANOVA on 25 characters resulted in four characters that have a significant effect (sig <0.05) namely on SL, BD, PFL, and HDS. The PCA results on morphometric characters have 2 factors with eigenvalues >1 which explained 84.1% of the component variation (Fig. 8A).

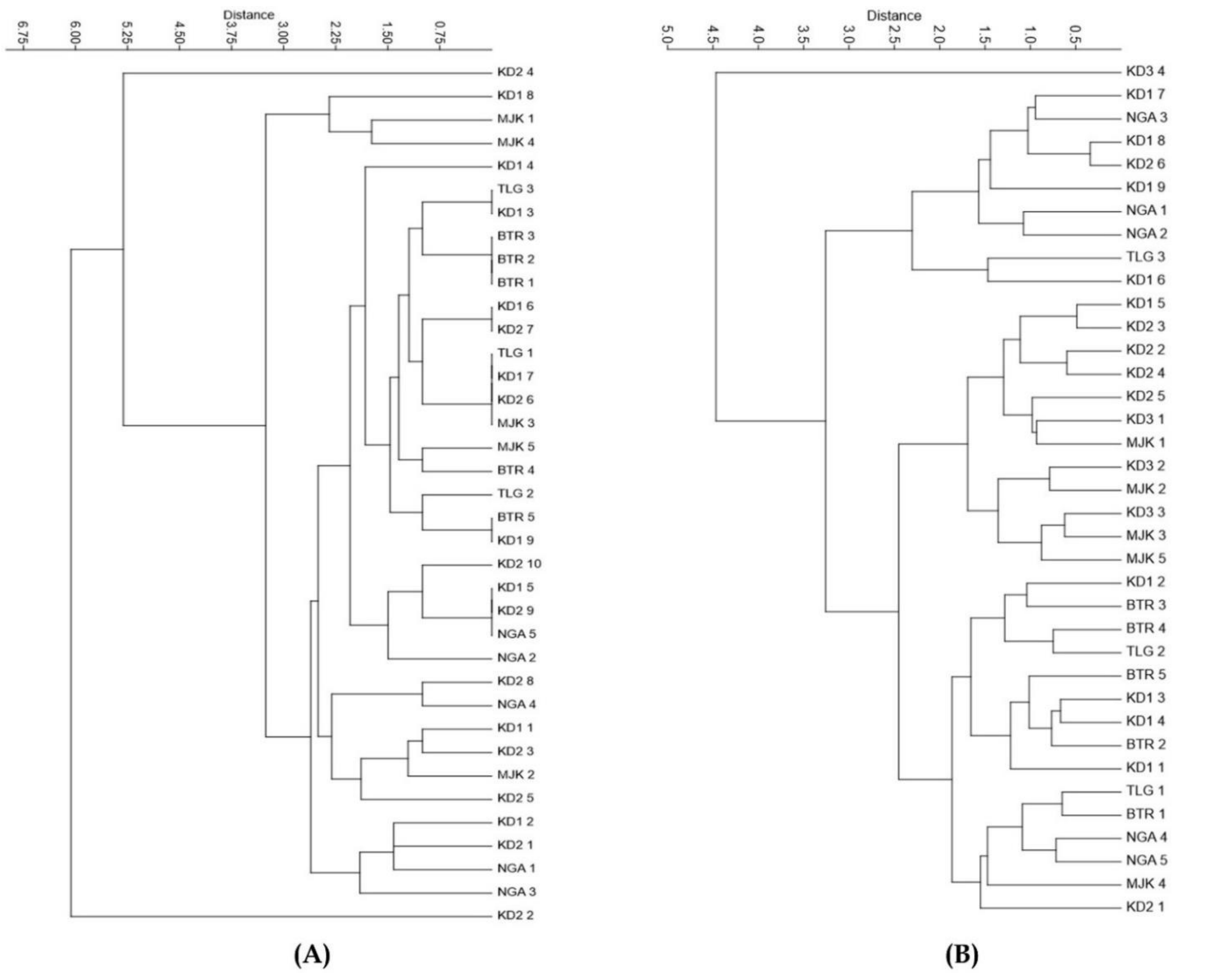


**Figure 8.** Cluster analysis using the UPGMA method (A) meristic characters, (B) morphometric characters

PC 1 explains the proportion of variation by 52.34% and PC 2 explains the proportion of variation by 31.77% with the value of the first main component (PC1) and the second main component (PC2) showing positive results. Positive values on SL, BD, PFL, and HDS characters indicate that these characters can be used as distinguishing characters in the Berod fish population. Aryantojati et al. (2022) explained the results of principal component analysis obtained positive and negative values. A positive value indicates that the character can be used as a distinguishing character between populations. The most significant loading in PC 1 is SL with a value of 0.5 and BD has a value of 0.5 while in PC 2 is PFL with a value of 0.5 and HDS 0.5. Values greater than 0.30 are considered significant, 0.40 is more important, and 0.50 or greater is highly significant (AnvariFar et al., 2011). PC 1 and PC 2 scores were then visualized resulting in clearly separated scatter plots across six populations (Fig. 7B). The separate scatter plots indicate that there are variations in characters in each of the observed individuals.

Cluster analysis was also conducted to determine the relationship between individuals. Cluster analysis using the Euclidean distance algorithm with the unweighted pair group method with arithmetic mean (UPGMA). UPGMA results of morphometric characters are the same as meristic characters. Berod fish at all location not clustered at each location, so that between one individual and another individual at the same location does not have a close kinship relationship (Fig. 8B).

Species grouping using UPGMA has been carried out on fish of the *Cobitis* genus (Cobitidae family) (Mousavi-Sabet et al., 2013) and subfamily Barbinae (Gupta et al., 2018). UPGMA is proven to be able to separate a population under study based on morphological characteristics, so that based on the results of UPGMA on meristic and morphometric characters, significant differences are found in each individual in one location and tend to cluster in individuals in other locations.

**Table 3.** Mean and Standard Deviation of Morphometric Measurements of Berod Fish

| | BTR (n=5) X ± SD | TLG (n=3) X ± SD | KD1 (n=9) X ± SD | KD2 (n=10) X ± SD | NGA (n=5) X ± SD | MJK (n=5) X ± SD |
|---|---|---|---|---|---|---|
| TL (cm) | 22.8 ± 2 | 32 ±6.1 | 28.2 ±5.9 | 34 ±2.9 | 41.4 ± 12.5 | 37.4 ±5 |
| SL | 11.7±0.6 | 10.7±0.7 | 11.2±1.2 | 12.6±1.4 | 10.1±1 | 12.9±1 |
| BD/SL | 6.7±0.4 | 7.2±0.3 | 6.3±0.8 | 7.9±1 | 6.1±1 | 8±1.2 |
| BW/SL | 4.7±0.5 | 5±0.4 | 4.7±0.8 | 4.7±0.4 | 4.4±0.4 | 4.9±0.4 |
| PFL/SL | 2.9±0.4 | 3.2±0.3 | 3.7±0.7 | 3.9±0.6 | 4.2±0.1 | 4.1±0.4 |
| HSD/SL | 3.1±0.2 | 3.3±1.1 | 3.2±0.4 | 3.6±1.4 | 3.7±0.4 | 3 ±0.2 |
| HDS/SL | 2.3±0.5 | 1.8±0.2 | 2.9±0.5 | 2.8±0.6 | 2.9±0.5 | 2.8±0.3 |
| HSA/SL | 3.7±0.2 | 3.3±0.8 | 3.7±0.7 | 3.5±0.5 | 3.5±0.2 | 3.3±0.5 |
| HAS/SL | 5.7±0.3 | 5.9±0.4 | 5.9±0.4 | 5.3±1.1 | 5.6±0.4 | 5.4±0.8 |
| CL/SL | 43.8±0.9 | 43.8±1 | 43.1±1.1 | 43.6±1.8 | 42.1±1.3 | 41.5±2.7 |
| DSB/SL | 37.9±1.2 | 37.3±1.6 | 36.8±1.2 | 36.8±1.1 | 37 ±1.2 | 37.5±1.5 |
| DFB/SL | 4.7±0.2 | 5±0.5 | 4.6±0.5 | 4.4±0.7 | 4.6±0.5 | 4.3±0.4 |
| ASB/SL | 37.8±1 | 38.1±0.7 | 37.2±0.7 | 37.3±0.7 | 38.4±2.2 | 37.8±1.3 |
| AFB/SL | 2.5±0.2 | 2.2±0.2 | 2.4±0.3 | 2.6±0.3 | 2.4±0.2 | 2.5±0.2 |
| PFB/SL | 64.8±0.8 | 64.5±0.1 | 64.3±0.7 | 64.3±1.7 | 63.5±2.5 | 63.6±1.2 |
| PDL/SL | 62.8±1.4 | 64.4±0.2 | 63.2±1.7 | 63.2±1.5 | 62.5±1.7 | 62.9±1.4 |
| PAL/SL | 40.3±3.7 | 39.7±2.1 | 38.2±7.9 | 39.8±2.6 | 41.5±4.6 | 37.7±2.6 |
| HD/HL | 27.2±2.3 | 28.7±3.1 | 26.7±6 | 26.7±2.7 | 23.7±1.9 | 25.2±1.2 |
| HW/HL | 12.1±2.3 | 11.4±2.1 | 10.5±2.7 | 10.4±1.2 | 10.2±2.2 | 9.3±1.1 |
| ED/HL | 11.6±1.9 | 16.9±1.5 | 13.8±4.6 | 12.7±2.4 | 11.5±1.7 | 12.5±0.6 |
| IO/HL | 32.6±1.1 | 37.5±4.7 | 35.3±8.2 | 32.5±5.9 | 35.6±2.8 | 31.9±3.5 |
| UJL/HL | 22.4±2.9 | 22.8±1.1 | 21.9±4.9 | 20.4±4.5 | 22.6±2 | 17.8±2.4 |
| LJL/HL | 15.8±1.7 | 14.1±1.1 | 15.5±4.9 | 13.3±3.2 | 12.6±3.3 | 18.2±3.8 |
| WGM/HL | 22.4±2.9 | 22.8±1.1 | 21.9±4.9 | 20.4±4.5 | 22.6±2 | 17.8±2.4 |
| HD/HL | 15.8±1.7 | 14.1±1.1 | 15.5±4.9 | 13.3±3.2 | 12.6±3.3 | 18.2±3.8 |

Note: X is mean, SD is standar deviation, and n is total samples

The results of PCA analysis on meristic characters show overlapping and separate patterns, while on morphometric characters are clearly separated, so there are significant differences in morphometric characters but not supported by meristic characters. The same thing also happened in the research of Cakmak et al. (2010) on *Mastacembelus mastacembelus* in Karakaya Reservoir, small rivers in Tahoma and Tigris River which showed differences in morphometric characters not supported by differences in meristic characters. Each species has morphological characteristics with special characteristics that can differentiate one species from another (Akmal et al., 2018). Organisms have a potential ability to changes they anatomical traits. This changes called morphological plasticity or phenotype plasticity. Morphological plasticity can occurred because of organisms adaptations due to varying environmental. Plasticity can be one of the adaptation mechanism to increase organism survival, performance and cope with stress (West-Eberhard, 2008).

The differences in morphological characteristics of each species can be an indication of the fish's habitat and adaptation style to the environment (Bhagawati et al., 2013). But, the causes of morphological differences between populations are often quite difficult to explain, but it is known that morphometric characters can show a high degree of flexibility in response to environmental conditions (Wimberger, 2008). Morphological differences between different populations are closely related to differences in habitat factors such as temperature, turbidity, food availability, water depth and flow (Allendorf, 1988; Swain et al., 1991; Wimberger, 2008; Kelley et al., 2017). Moyle et al. (1988) stated that there is often variation in morphological characters within one species. These variations can be caused by the development conditions of fish larvae, environmental factors and food availability. Morphological differences are also suggested that fish morphological characteristics are determined by genetics, environment and the interaction between genetics and environment (Poulet et al., 2004; Pinheiro et al., 2005; AnvariFar et al., 2011). In general, morphometric studies have three benefits, namely: distinguishing between sexes and species, describing patterns of morphological diversity between populations or species, and classifying and inferring phylogenic relationships (Muhotimah et al., 2013).

## Conclusion

The results showed that Berod fish in the Brantas River had morphological characters belongs to the species *M. unicolor*, but there was a discrepancy in the meristic characters in the dorsal fin spines (DFS). Meristic and morphometric PCA analysis of berod fish showed significant differences in morphometric characters but was not supported by meristic characters.

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**Conflicts of Interest**
The author declares no conflict of interest in this research.

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