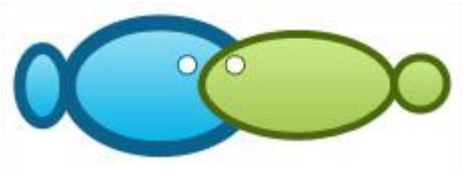

# DNA barcoding reveals possible misidentification of selected barb species (Cypriniformes) from Klawing River, Central Java, Indonesia

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**Abstract**. Previous studies overlappingly used *Barbonymus balleroides*, *Systomus orphoides*, and *Systomus rubripinnis* as scientific names for three barb species living in Klawing River, i.e., for 'brek', 'lukas', and 'maracoca' fish. This taxonomic chaos can be solved by applying DNA barcoding, using the cytochrome c oxidase 1 gene for precise species identification. This study evaluated the taxonomic status of barb species (Cyprinidae) living in Klawing River, Purbalingga Regency, Central Java, Indonesia. Fish samples were randomly collected from the upper stream to downstream using spread nets targeting consumable adult individuals. Pectoral fin clips were cut off using scissors and preserved in ethanol 96%. Morphological identification was carried out by comparing the fish samples to images available in references. The genetic identity was determined based on similarity, homology tests, and monophyly to reference species in databases. The current study genetically identified barb species from Klawing River as *Labiobarbus fasciatus* (100% and 100%) for lukas, *Barbonymus schwanefeldii* (>96.80 and >96.80) for brek, and *Leptobarbus melanopterus* (>99.80 and >99.80) for maracoca. There was high genetic identity, genetic similarity, and monophyly with their conspecific references in BOLD systems and GenBank, respectively. None of the barb samples were identified as *B. balleroides*, *S. orphoides*, or *S. rubripinnis*, as previously utilized scientifically for the three barb species from the Klawing River. Instead of validating *B. balleroides*, *S. orphoides*, and *S. rubripinnis*, as previously employed scientific names for barb species, this research alleged possible misidentification of barb species and validates that the barb fishes in Klawing River, Central Java, Indonesia belong to *B. schwanefeldii, L. fasciatus*, and *Leptobarbus melanopterus*.
**Key Words**: genetic distance, homology, Javaen barb, monophyly, morphology.

**Introduction**. *Systomus rubripinnis* (Valenciennes, 1842), often known as the Javaen barb, is locally known in central Java as 'maracoca' fish (Suryaningsih et al 2020). Maracoca is also identified as *S. orphoides* (Pramono et al 2018). At the same time, *Barbonymus balleroides* is also called Javaen barb, but locally known as 'brek' (Mote et al 2014). There is also another Javaen barb species, locally called 'lukas' fish, *Labiobarbus leptocheilus* (Pramono et al 2018). These three Cyprinidae species have broad geographic and overlap range in the tropical rivers of Asia, in India, Thailand, Cambodia, Peninsular Malaysia, and Indonesia (Jutagate et al 2016; Hayati et al 2017; Ng et al 2017; Mogalekar & Canciyal 2018; Mohammad et al 2018; Pin et al 2020; Fricke et al 2023; Froese & Pauly 2023).

There is overlapping utilization of different scientific names during the identification of these three Javaen barbs. Some studies utilized *B. balleroides* to refer to fish specimens called brek (Arifin et al 2017; Haryono et al 2017). At the same time, some studies also used *S. rubripinnis* and *S. orphoides* to refer to brek specimens (Suryaningsih et al 2012; Susatyo et al 2016; Susilo et al 2022; Susatyo et al 2022). Similarly, Suryaningsih et al (2020) also used *S. rubripinnis* to identify brek fish. Currently, *S. rubripinnis* is known as the synonym of *S. orphoides* (Fricke et al 2023). In contrast, other studies utilized *S. orphoides* for maracoca (Pramono et al 2018). Froese & Pauly (2023) also summarized that *B. balleroides* is used to refer to fish specimens called

lukas. However, lukas is also identified as *L. leptocheilus*, especially for specimens from Java (Froese & Pauly 2023).

Overlapping utilization of scientific names occurred due to morphological similarity among closely related species, which appear similar. Currently, *S. orphoides* and *S. rubripinnis* are synonyms that refer to Javaen barb, locally known as maracoca, while *B. balleroides* is a scientific name for another Javaen barb, called brek (Anita et al 2019) and *L. leptocheilus* for lukas from Java (Pramono et al 2018; Fricke et al 2023). The placement of maracoca into *S. orphoides* and brek into *B. balleroides* were based on their slightly different color pattern. *S. orphoides* has black bands on the back side of the operculum and caudal fin. Meanwhile, *B. balleroides* does not have this black color (Froese & Pauly 2023). However, the black color does not appear at certain life stages in these two species. According to Ko et al (2013), fish from different species at certain stages can have the same morphology, leading to misidentification. Errors often occur, especially in closely related species (Nuryanto et al 2021). Identification errors using morphological characters can be overcome through molecular identification or DNA barcoding (Dahruddin et al 2016; Fotedar et al 2019; Ahmed et al 2020; Eissa et al 2021; Nuryanto et al 2021). The cytochrome c oxidase 1 gene can be used for species barcoding. Several studies have proven that the cytochrome c oxidase 1 gene is a good genetic marker for identifying members of fish species (Nuryanto et al 2018; Ceruso et al 2018; Caputi et al 2021; Ceruso et al 2021; Setyaningrum et al 2022; Abdalwahhab et al 2020; Mohammed-Geba et al 2021). This technique has been successfully used to overcome the problem of cryptic species, species that have similar morphology, but are different species (Bilgin et al 2015; Becker et al 2015; Muchlisin et al 2017).

The Klawing River is a tributary of the Serayu River (Nuryanto et al 2019). The river flows along Purbalingga Regency, Central Java, before emptying into the Serayu River (Suryaningsih et al 2018). Previous studies have reported the diversity of fish species from the Klawing River (Suryaningsih et al 2018; Serdiati et al 2022). *B. balleroides* fish are reported to live in the Serayu River (Haryono et al 2014; Haryono et al 2017). However, both studies did not report the presence of *S. rubripinnis* in the Klawing River (Suryaningsih et al 2018; Serdiati et al 2022). *S. rubripinnis* is also reported to inhabit several rivers in Java (Hayati et al 2017; Hayati et al 2019; Rasyad et al 2020). Meanwhile, Suryaningsih et al (2020) states that *S. rubripinnis* also lives in several rivers in Purbalingga, Central Java, Indonesia. The presence of *S. rubripinnis* fish in the Klawing River has been reported by Pramono et al (2018). Meanwhile, Susilo et al (2022) reported the performance of digestive enzymes on *S. rubripinnis*, especially protease and amylase. However, Susilo et al (2022) refer to studies (Suryaningsih et al 2012; Susatyo et al 2016) in which the researchers worked on the fish *S. orphoides*. The use of different names for the same species can cause misinformation and confusion because the taxonomic status of the fish studied by Suryaningsih et al (2012), Susatyo et al (2016), and Susilo et al (2022) is the same, but has different scientific names.

This study evaluated the taxonomic status of three Javaen barbs (brek, lukas, and maracoca) living in Klawing River, Purbalingga Regency, Central Java, Indonesia.

## Material and Method

***Description of the study sites***. The research was conducted in the Klawing River in Purbalingga Regency, Central Java, Indonesia. Sampling was conducted at six locations along the river from upstream to downstream (Figure 1). Sampling stations were determined based on the ease of access to the location and previous research (Saprudin et al 2022). Station I was located in Palumbungan Village (07016'39''S; 109022'06”E), Station II was located in Tangiksan Village (07019'36''S; 109022'47”E), Station III was in Banjaran Village (07021'40''S; 109022'33”E), Station IV in Lamongan Village (07024'30''S; 109023'53"E), Station V in Jetis Village (07025'41''S; 109023'07"E), and Station VI found in Kedungbenda Village (07028'20''S; 109019'36”E) (Pramono et al 2018).

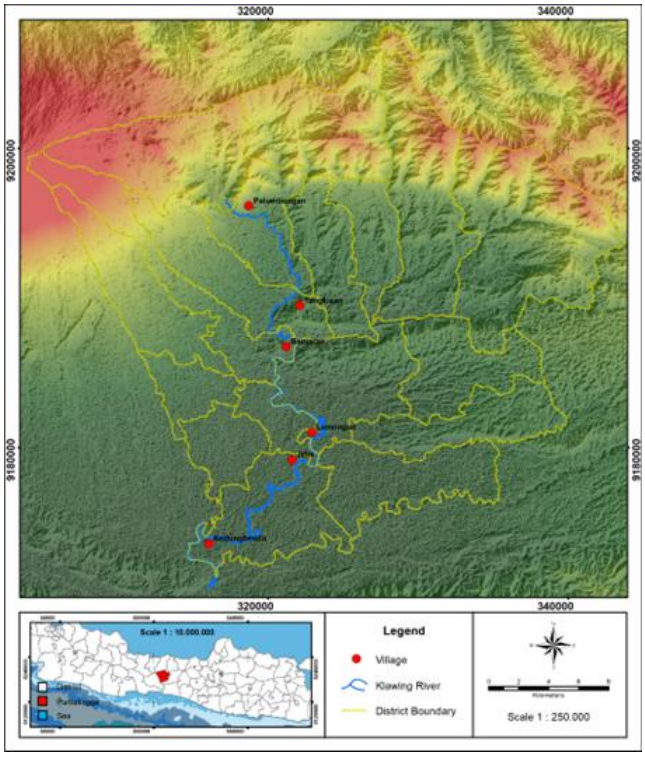


Figure 1. A map showing the sampling sites along Klawing River Central Java, Indonesia (modified by Saprudin et al 2022).

***Sample collections and identification***. Field trips were conducted four times in June 2023, weekly. Fish samples were collected at each sampling site using spread nets targeting adult individuals with a mesh size of approximately 2.5 cm and 3.8 cm. This sampling method ensured that the collected samples had stable morphological characteristics important for morphological identification. Samples collection was performed with the help of local fishermen living close to each sampling site. The obtained fish specimens were placed in a tank with a volume of 25 L, filled with 70% alcohol, and given a label containing information about location data and sampling station (Table 1). Fixation was carried out while the fish was alive or immediately after death. During the fixation process, each fish was straightened. Larger fish samples (over 15 cm) were injected into the stomach using 70% alcohol. In the laboratory, the fixed fish specimens were washed using running water to remove residual alcohol. Next, the fish specimens were transferred into a collection bottle containing new 70% alcohol for permanent storage. Selected barb specimens were identified by comparing the general morphology between samples and figures available in Froese & Pauly (2023), and the validity of the scientific names was checked in Fricke et al (2023).

Table 1

Sample codes and remarks

| *Code* | *Remark* | *Code* | *Remark* | *Code* | *Remarks* |
|---|---|---|---|---|---|
| HC01 | Hilir, Cyprinid 01 | TB02 | Tengah, Brek 02 | TM02 | Tengah, Maracoca 02 |
| HL01 | Hilir, Lukas 01 | TB03 | Tengah, Brek 03 | TM03 | Tengah, Maracoca 03 |
| HL02 | Hilir, Lukas 02 | TB04 | Tengah, Brek 04 | TM04 | Tengah, Maracoca 04 |
| HL03 | Hilir, Lukas 03 | TB05 | Tengah, Brek 05 | TM05 | Tengah, Maracoca 05 |
| HL04 | Hilir, Lukas 04 | TB06 | Tengah, Brek 06 | TM06 | Tengah, Maracoca 06 |
| HL05 | Hilir, Lukas 05 | TB07 | Tengah, Brek 07 | TM07 | Tengah, Maracoca 07 |
| HL06 | Hilir, Lukas 06 | TB08 | Tengah, Brek 08 | TM08 | Tengah, Maracoca 08 |
| TB01 | Tengah, Brek 01 | TM01 | Tengah, Maracoca 01 | | |

Note: Hilir - downstream; Tengah - middle stream; lukas, brek, maracoca - local names; 01 to 08 - sample numbers.

***DNA extraction, marker amplification and sequencing***. Pectoral fin clip samples were shipped for barcoding analysis. Total genomic DNA isolation and amplification of the targeted barcode have been done at PT. Genetika Science Indonesia, in Tangerang, Banten Province, Indonesia. The COI fragment sequencing was done using a bi-directional big dye terminator sequencing technique from the Sanger method at 1st BASE ASIA Malaysia. The protocol used for all steps followed existing procedures at both companies.

***Data analysis***. Fish morphology data were analyzed descriptively by comparing the picture of the samples with the figure available in Froese & Pauly (2023). This identification technique was carried out to identify specimens into a family category (Fricke et al 2023; Froese & Pauly 2023). The nucleotide base sequence of the COI gene fragment was aligned and edited manually with the help of the Bioedit program (Hall 2005). All obtained sequences were aligned using the ClustalW program as implemented in Bioedit (Hall 2005). Species status was determined by comparing the nucleotide base sequence obtained with the barcoding database in GenBank (Benson et al 2017) using a basic local alignment search tool (BLAST) based on a standard database search set and program selection optimized for highly similar sequences. The sequence of the samples was also tested for similarity to sequence data in the BOLD system using species-level barcode records search for twenty-one specimens and all barcode record searches (for TB04 and TB05). The genetic identity for species boundaries was determined based on the highest percentage in GenBank and BOLD system. In the case of sequence homology to the reference species in GenBank, query cover of 100% and e-value of 0.00 were also utilized for species delineation. These species boundaries were used by considering the geographic localities and the ecology of the species (Jeffery et al 2011; Higashi et al 2011; Kusbiyanto et al 2020). This study also provides information about the monophyly between samples and two top-hits reference species from both databases. The monophyly is presented in the phylogenetic tree, which was reconstructed using the neighbour-joining algorithm and Kimura 2 parameter (K2P) model with the help of the MEGA11 program (Tamura et al 2021). The polarity of the branching pattern was obtained from 1000 bootstrap replicates.

## Results

***Genetic identity***. 25 specimens were identified as members of Cyrpinidae. 23 samples were shipped to a company for the barcoding process, resulting in a reliable sequence for barcoding analysis. Homology test using basic local search alignment tool (BLAST) to DNA sequence data in GenBank resulted in genetic identity of 94.5% between 8 samples and *Systomus orphoides* MK628369. Genetic identity of 100% was obtained between 7 samples and *L. fasciatus* KU692576. The test also considered a query cover of 100% and an e-value of 0.00 (Table 2).

Table 2

Query cover, e-value, and Homology identity (GenBank) of selected cyprinid to their reference species in global databases

| *Sample code* | *Query cover %* | *e-value* | *Genetic identity (%)* | *Reference species* |
|---|---|---|---|---|
| HC01 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL01 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL02 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL03 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL04 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL05 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| HL06 | 100 | 0.00 | 100 | *Labiobarbus fasciatus* KU692576 |
| | 99 | 0.00 | 98.03 | *Labiobarbus leptocheilus* MN342618 |
| TB01 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.97 | *Rhodeus ocellatus* KJ415114 |
| TB02 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.97 | *Rhodeus ocellatus* KJ415114 |
| TB03 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.97 | *Rhodeus ocellatus* KJ415114 |
| TB04 | 100 | 0.00 | 96.81 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.81 | *Rhodeus ocellatus* KJ415114 |
| TB05 | 100 | 0.00 | 96.81 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.81 | *Rhodeus ocellatus* KJ415114 |
| TB06 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.97 | *Barbonymus gonionotus* KU692333 |
| TB07 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.78 | *Rhodeus ocellatus* KJ415114 |
| TB08 | 100 | 0.00 | 96.97 | *Barbonymus schwanefeldii* MK049360 |
| | 100 | 0.00 | 96.97 | *Rhodeus ocellatus* KJ415114 |
| TM01 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM02 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM03 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM04 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM05 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.64 | *Systomus orphoides* MK628369 |
| TM06 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM07 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.50 | *Systomus orphoides* MK628369 |
| TM08 | 100 | 0.00 | 99.83 | *Leptobarbus melanopterus* KU692595 |
| | 100 | 0.00 | 94.55 | *Systomus orphoides* MK628369 |

***Genetic similarity test***. The sequences of three barb species were also subjected to genetic similarity tests to the sequences deposited in the BOLD system. The genetic similarity values ranged from 94.39% (between 8 samples and *S. orphoides* BIN ID BOLD:AU2017) to 100% (7 samples and *Labiobarbus fasciatus* BIN ID BOLD:AAX0792) (Table 3).

Table 3

Genetic similarity between barb samples and their reference species deposited in BOLD system

| *Sample code* | *Genetic similarity (%)* | *Reference species* | *BIN ID* |
|---|---|---|---|
| HC01 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL01 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL02 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL03 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL04 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL05 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| HL06 | 100<br>97.83 | *Labiobarbus fasciatus* KU692576<br>*Labiobarbus leptocheilus* MN342618 | BOLD:AAX0792 |
| TB01 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB02 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB03 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB04 | 96.86<br>96.79 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB05 | 96.86<br>96.79 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB06 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB07 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TB08 | 97.03<br>96.96 | *Barbonymus schwanefeldii* MK049360<br>*Rhodeus ocelatus* KJ415114 | BOLD:AAU0688 |
| TM01 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM02 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM03 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM04 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM05 | 99.82<br>94.53 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM06 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM07 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |
| TM08 | 99.83<br>94.39 | *Leptobarbus melanopterus* KU692595<br>*Systomus orphoides* JF915642 | BOLD:ACT1925<br>BOLD:AAU2017 |

***Monophyly***. All selected cyprinid samples formed a monophyletic group with each reference's species with high bootstrap support (100). Monophyly between TB samples (blue box) and *B. schwanefeldii*, between TM samples and *Leptobarbus melanopterus* (green box), and between HC01, HL samples and *L. fasciatus* (yellow box) had short branch lengths (less than 0.02) (Figure 2).

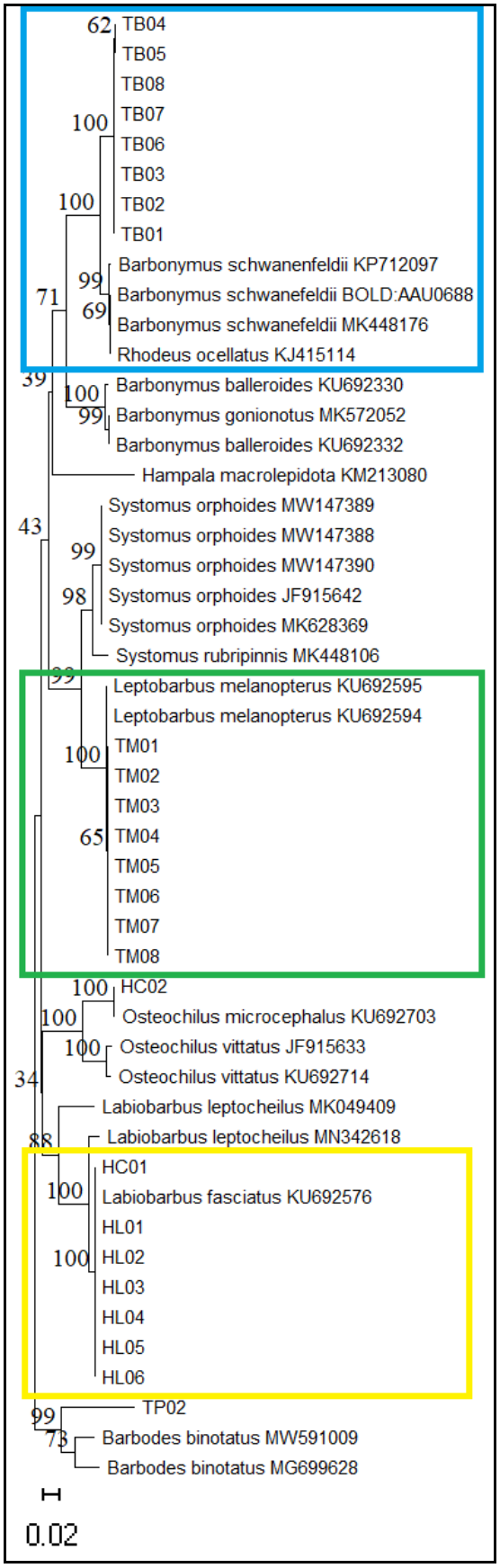


Figure 2. The K2P neighbour-joining tree showing monophyly between samples and their reference species.

**Discussion**. According to genetic identity, as presented in Table 2, 7 specimens (HC01, HL01-HL06) have values over 97% for two different GenBank species. The reference species are *L. fasciatus* and *L. leptocheilus*. Consistent values were also obtained from the similarity test to sequence data in BOLD system (Table 3). Nevertheless, after a more detailed observation of the data in Tables 2 and 3, it was found that genetic identities of 100% were observed between samples and *L. fasciatus* KU692576. In contrast, the genetic identities of those 7 samples were only 98.03% and 97.83% to *L. leptocheilus* MN342618 in GenBank and BOLD system, respectively. Additionally, query cover to *L. fasciatus* KU692576 was 100%, while to *L. leptocheilus* MN342618 was 99%. Based on the genetic identity values of specimens in GenBank and BOLD system and the query cover, 7 samples (HC01, HL01-HL06) were genetically barcoded into species category, i.e., *L. fasciatus*. Instead of validating *L. leptocheilus* as the scientific name for locally known lukas fish, this study genetically identified lukas as *L. fasciatus*.

Table 2 also shows that 8 samples (TB01 to TB08) have similar genetic identities to two different species in GenBank. Genetic identities of 96.81% to 96.97% with query cover of 100% were found between those samples (TB01 to TB08) and *Barbonymus schwanefeldii* MK049360 and also with *Rhodeus ocellatus* KJ415114. Considering genetic identities and query covers only the data in GenBank, we could not delineate the samples into species categories because the genetic identities and query covers are 100% equal to both species. *B. schwanefeldii* is distributed in the freshwater of Asia, including Indonesia. In contrast, *R. ocellatus* is distributed in brackish water or freshwater of subtropic regions, such as eastern Asia, Taiwan, and Russia (Froese & Pauly 2023; Fricke et al 2023). Considering that *B. schwanefeldii* and *R. ocellatus* have different geographic ranges and ecology, this study determined that the samples are genetically identified as *B. schwanefeldii*. Again, instead of identifying brek as *B. balleroides*, this study barcoded brek from the Klawing River as *B. schwanefeldii*. The placement of brek into *B. schwanefeldii*, instead of *B. balleroides*, was supported by a similarity test to the reference species available in the BOLD system (Table 2). 6 specimens showed similarity values over 97% as BIN ID utilized in BOLD system (Ratnasingham & Hebert 2013), and two specimens have higher similarity values to *B. schwanefeldii* than to *R. ocellatus*. Therefore, the placement of TB samples into *B. schwanefeldii* was convincing, especially if supplemented with both species' distribution and ecological information. Moreover, we could utilize a genetic threshold of over 97% down to a minimum of 95% if we consider geographic locality (Higashi et al 2011) and ecological information (Jeffery et al 2011). Even lower intraspecific genetic identity was observed in fish from Brazil (Pereira et al 2013).

Genetic identities of the remaining 8 samples (TM01 to TM08), as presented in Table 2, are 99.83% similar to *L. melanopterus* KU692595 in GenBank. Conversely, genetic identities ranging from 94.50% to 94.64% were observed between TM samples and *S. orphoides* MK628369 in Genbank (Table 2). The tests resulted in a similar 100% query cover value for all samples and both reference species. The data (99.83% identities and 100% query cover) indicated that we obtained reliable values for species delineation. Therefore, this study identified TM samples, locally known as maracoca, as *L. melanopterus*, instead of *S. orphoides* or *S. rubripinnis*.

Species delineation of brek, lukas, and maracoca samples into *B. schwanefeldii*, *L. fasciatus*, and *Leptobarbus melanopterus*, respectively, as determined based on genetic identity (Table 2) and genetic similarity (Table 3) was supported by the monophyly of the samples with their reference species (Figure 2). According to Xu et al (2015) and Kusbiyanto et al (2020), specimens might be referred to as a single species with their reference species if they form a monophyletic group with short branch lengths.

Fish species shows variable intraspecific genetic difference (Diaz et al 2016; Ali et al 2020; Limmon et al 2020; Sholihah et al 2020). Therefore, genetic identity and similarity tests might result in different intraspecific genetic identities among species (Cote et al 2013; Pereira et al 2013; Aguilar et al 2017). These phenomena lead to various genetic similarities or sequences of homology values utilized as species borders during fish barcoding (Ko et al 2013; Mohammed-Geba et al 2021). Some studies used a strict threshold value of 98% to 99%, but it is usually used in closely related or newly

divergent species (Ha et al 2019; Abdalwahhab et al 2020; Salem et al 2021). Some studies utilized a genetic identity of 97% as a species border (Landi et al 2014; Nuryanto et al 2017, 2021, 2023). At the same time, other studies used 97-95% genetic identity as the threshold value for species delineation in animals (Amatya 2019; Karanovic 2015; Candek & Kuntner 2015; Abdalwahhab et al 2020). The use of genetic identity below 97% is allowed but with particular consideration, such as ecological attributes and geographic sites (Jeffery et al 2011; Higashi et al 2011). Therefore, the use of different genetic threshold for each species group in this study is acceptable because it has been reported that intraspecies genetic difference and similarity are different among species groups (Pereira et al 2013).

*L. fasciatus* is distributed in Indonesia and Malaysia (Froese & Pauly 2023). In Indonesia, it was reported to live in Sumatra and Kalimantan (Fricke et al 2023). *L. leptocheilus* was reported in Sumatera, Java, and Kalimantan (Froese & Pauly 2023; Fricke et al 2023). A previous study said that lukas fish from Klawing River belong to *L. leptocheilus* (Pramono et al 2018), while Susatyo & Sugiharto (2014) identified lukas fish as *Puntius bramoides* as a synonym of *B. balleroides* (Fricke et al 2023). Nevertheless, a previous study stated that *L. fasciatus* also lives in the freshwater ecosystem in Java, especially in Ci Hapitan (river) in Ciamis Regency, West Java (Dahruddin et al 2016). Therefore, the placement of lukas fish samples from Klawing River, Central Java, into *L. fasciatus*, instead of *L. leptocheilus*, is reasonable and convincing because it is supported by high genetic identity and query cover and also supported by a study in other locations in Java Island.

*B. schwanefeldii* is distributed in Sumatera, Java, and Kalimantan (Fricke et al 2023), but according to Froese & Pauly (2023) summarized that *B. schwanefeldii* is only found in Sumatera and Kalimantan. At the same time, *B. balleroides* is also distributed in Java, Sumatra, and Kalimantan (Fricke et al 2023). Therefore, it was reasonable that a previous study identified brek fish from Klawing River, Central Java, as *B. balleroides* (Haryono et al 2014, 2017; Pramono et al 2018), while other studies identified brek fish as *Puntius orphoides* as a synonym of *S. orphoides* (Suryaningsih et al 2012; Suryaningsih et al 2014; Susatyo et al 2016; Suryaningsih et al 2018; Suryaningsih et al 2020; Susatyo et al 2022) and as *S. rubripinnis* (Suryaningsih et al 2020; Susilo et al 2022). However, we suggest that the placement of brek fish into *P. orphoides* (*S. orphoides*) and *S. rubripinnis* could have been a misidentification. It is because *P. orphoides* (*S. orphoides*) and *S. rubripinnis* are scientific names for other Javaen barb species, locally called maracoca (Pramono et al 2018; Fricke et al 2023; Froese & Pauly 2023). This study proved that brek samples (TB01-TB08) from Klawing River are genetically identified as *B. schwanefeldii* instead of *B. balleroides* (Haryono et al 2014, 2017; Pramono et al 2018). This species determination was supported because none of the specimens had a high genetic identity (over 96%) to *B. balleroides* deposited in GenBank and BOLD system. The geographic distribution of *B. schwanefeldii* also covers Java Island (Fricke et al 2023).

*L. melanopterus* is known only from Kalimantan (Fricke et al 2023; Froese & Pauly 2023). A similar species from Klawing River, Java Island, is identified as *S. rubripinnis*, locally known as maracoca (Pramono et al 2018). Currently, *S. rubripinnis* is referred to as a synonym of *S. orphoides*. *S. orphoides* is also reported from Serayu River, Banyumas and several rivers in Java Island (Fricke et al 2023). Nevertheless, a previous study reported that *L. melanopterus* is also found in Java Island (Mojokerto, East Java) (Dahruddin et al 2016). Therefore, in this study, the placement of maracoca fish from Klawing River, Central Java, as *L. melanopterus* is convincing. In addition, it is supported by high genetic identities (over 99%) and geographic locality information (Dahruddin et al 2016). All maracoca samples have a low genetic identity to *S. orphoides*, below 95%, a minimum genetic identity used in several studies as a species border (Candek & Kuntner 2015; Karanovic 2015; Kusbiyanto et al 2020).

**Conclusions**. Instead of validating *B. balleroides* as a scientific name for brek fish, *L. leptocheilus* for lukas fish, and *S. orphoides* (*S. rubripinnis*) as scientific name for maracoca fish, this study proved that brek fish from Klawing River, Central Java belong

to *B. schwanefeldii*. Lukas fish was genetically barcoded as *L. fasciatus*, while maracoca was genetically identified as *L. melanopterus*. We suggest the placement of brek, lukas, and maracoca from Klawing River as *B. balleroides*, *L. leptocheilus*, and *S. orphoides*, respectively, are the result of misidentification.

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**Conflict of Interest**. The authors declare that there is no conflict of interest.

## References

Abdalwahhab O., Galal-Khallaf A., Saber S. A. E., Osman A. G. M., Mohammed-Geba K., 2020 A case study for application of DNA barcoding in identifying species and genetic diversity of fish from the Suez city market, Egypt. Aquatic Living Resources 33:11.

Aguilar A., Maeda-Martinez A. M., Murugan G., Obregon-Barboza H., Rogers D. C., McClintock K., Krumm J. L., 2017 High intraspecific genetic divergence in the versatile fairy shrimp *Branchinecta lindahli* with a comment on cryptic species in the genus *Branchinecta* (Crustacea: Anostraca). Hydrobiologia 801:59-69.

Ahmed M. S., Datta S. J., Zhilik A. A., 2020 Molecular diversity of freshwater fishes of Bangladesh assessed by DNA barcoding. Bangladesh Journal of Zoology 48(1):1-19.

Ali F. S., Ismail M., Aly W., 2020 DNA barcoding to characterize biodiversity of freshwater fishes of Egypt. Molecular Biology Reports 47:5865-5877.

Amatya B., 2019 DNA barcoding of cyprinid fish *Chagunius chagunio* Hamilton, 1822 from Phewa Lake, Nepal. International Journal of Biology 11(4):88-100.

Anita S., Haryono, Wahyudewantoro G., 2019 Nutritional component of *Barbonymus balleroides*: a wild freshwater fish from Indonesia. Biodiversitas 20(2):581-588.

Arifin O. Z., Subagja J., Prakoso V. A., Suhud E. H., 2017 Effect of stocking density on growth performance of domesticated barb (*Barbonymus balleroides*). Indonesian Aquaculture Journal 12(1):1-6.

Becker R. A., Sales N. G., Santos G. M., Santos G. B., Carvalho D. C., 2015 DNA barcoding and morphological identification of neotropical ichthyoplankton from the Upper Paraná and São Francisco. Journal of Fish Biology 87(1):159-168.

Benson D. A., Cavanaugh M., Clark K., Karsch-Mizrachi I., Lipman D. J., Ostell J., Sayers W. E., 2017 GenBank. Nucleic Acid Research 45:D37-D42.

Bilgin R., Utkan M. A., Kalkan E., Karhan S.U., Bekbolet M., 2015 DNA barcoding of twelve shrimp (Crustacea: Decapoda) from Turkish sea reveals cryptic diversity. Mediterranean Marine Sciences 16(1):36-45.

Candek K., Kuntner M., 2015 DNA barcoding gap: Reliable species identification over morphological and geographical scales. Molecular Ecology Resources 15(2):268-277.

Caputi L., Osca D., Ceruso M., Venuti I., Sepe R. M., Anastasio A., D'Aniello S., Crocetta F., Pepe T., Sordino P., 2021 The complete mitochondrial genome of the white seabream *Diplodus sargus* (Perciformes: Sparidae) from the Tyrrhenian Sea. Mitochondrial DNA Part B 6(9):2581-2583.

Ceruso M., Mascolo C., De Luca P., Venuti I., Biffali E., Ambrosio R. L., Smaldone G., Sordino P., Pepe T., 2021 *Dentex dentex* frauds: establishment of a new DNA barcoding marker. Foods 10(3):580.

Ceruso M., Mascolo C., Palma G., Anastasio A., Pepe T., Sordino P., 2018 The complete mitochondrial genome of the common dentex, *Dentex dentex* (Perciformes: Sparidae). Mitochondrial DNA Part B, Resources 3(1):391-392.

Cote I. M., Green S. J., Morris Jr. J. A., Akins J. L., Steinke D., 2013 Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. Marine Ecology Progress Series 472:249-256.
Dahruddin H., Hutama A., Busson F., Sauri S., Hanner R., Keith P., Hadiaty R., Hubert N., 2016 Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: Taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Molecular Ecology Resources 17(2):288-299. DOI: 10.1111/1755-0998.12528.
Diaz J., Villanova G. V., Brancolini F., del Pazo F., Posner V. M., Grimberg A., Arranz S. E., 2016 First DNA barcode reference library for the identification of South American freshwater fish from the Lower Paraná River. PLoS ONE 11(7):e0157419.
Eissa S. A., Fiteha Y. G., Elhifnawy H. T., Magdy M., Mamoon A., Hussien N., Rashed M. A. S., 2021 DNA barcoding for identification of some fish species (Sparidae) in Mediterranean Sea Area. Journal of Scientific Research in Science 38(2):168-182.
Fotedar S., Lukehurst S., Jackson G., Snow M., 2019 Molecular tools for identification of shark species involved in depredation incidents in Western Australian fisheries. PLoS ONE 14(1):e0210500.
Ha T. T. T., Huong N. T., Hung N. P., Guiguen Y., 2019 Species identification using DNA barcoding on processed panga catfish products in Viet Nam Revealed Important Mislabeling. Turkish Journal of Fisheries and Aquatic Sciences 18:457-462.
Hall T. A., 2005 BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.
Haryono, Rahardjo M. F., Affandi R., Mulyadi, 2017 [Morphological character and habitat of barb fish (*Barbonymus balleroides* Val.1842) in Serayu River of Central Java]. Jurnal Biologi Indonesia 13(2):223-232. [In Indonesian].
Haryono, Rahardjo M. F., Mulyandi, Affandi R., 2014 [Growth pattern and sex ratio of barbs fish, *Barbonymus balleroides* Vall. 1842 in fragmented habitat of Serayu River in Central Java]. Jurnal Biologi Indonesia 10(2):297-305. [In Indonesian].
Hayati A., Rasyad M. F. M., Putra I. D. S., Nurhariyati T., Amin M. H. M., Putranto T. W. C., Wanguyun A. P., Sugiharto, Affandi M., 2019 Morphometric variations of fish from Brantas River, East Java, Indonesia. Ecology, Environment and Conservation 25:S57-S61.
Hayati A., Tiantono N., Rasyad M. F. M., Putra I. D. S., Abdizen M. M., Seta A. R., Solikha B. M., Fu'adil M. H., Putranto T. W. C., Rosmida A. M., 2017 Water quality and fish diversity in the Brantas River, East Java, Indonesia. Journal of Biological Researches 22(2):43-49.
Higashi R., Tsukagoshi A., Kimura H., Kato K., 2011 Male dimorphism in a new interstitial species of the genus *Microloxoconcha* (Podocopida: Ostracoda). Journal of Crustacean Biology 31(1):142-152.
Jeffery N. W., Elias-Guttierrez M., Adamowicz S. J., 2011 Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. PLoS ONE 6(5):e18364.
Jutagate T., Rattanachai A., Udduang S., Leak-Ang S., Lek S., 2016 Spatio-temporal variations in abundance and assemblage patterns of fish larvae and their relationships to environmental variables in Sirindhron Reservoir of the Lower Mekong Basin, Thailand. Indian Journal of Fisheries 63(3):11-23.
Karanovic I., 2015 Barcoding of ancient lake Ostracods (Crustacea) reveals cryptic speciation with extremely low distances. PLoS ONE 10(3):e0121133.
Ko H. L., Wang Y. T., Chiu T. S., Lee M. A., Leu M. Y., Chang K. Z., Chen W. Y., Shao K. T., 2013 Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. PLoS ONE 8(1):e53451.
Kusbiyanto, Bhagawati D., Nuryanto A., 2020 DNA barcoding of crustacean larvae in Segara Anakan, Cilacap, Central Java, Indonesia using cytochrome c oxidase gene. Biodiversitas 21(10):4878-4887.
Landi M., Dimech M., Arculeo M., Biondo G., Martins R., Carniero M., Carvalho G. R., Brutto S. l., Costa F. O., 2014 DNA barcoding for species assignment: The case of Mediterranean marine fishes. PLoS ONE 9(9):e106135.

Limmon G., Delrieu-Trottin E., Patikawa J., Rijoly F., Dahruddin H., Busson F., Steinke D., Hubert N., 2020 Assessing species diversity of Coral Triangle artisanal fisheries: A DNA barcode reference library for the shore fishes retailed at Ambon harbor (Indonesia). Ecology and Evolution 10(7):3356-3366.
Mogalekar H. S., Canciyal J., 2018 Freshwater fish fauna of Tamil Nadu, India. Proceedings of the International Academy of Ecology and Environmental Sciences 8 (4): 213-230.
Mohammad M. S., Fadzil N. F. M., Sah A. S. R. M. D., Zakeyuddin M. S., Darwin E. D., Hashim Z. H., 2018 A freshwater fish biodiversity and distribution at Bukit Merah Reservoir River feeders, Perak, Peninsular Malaysia. Malayan Nature Journal 70(4):463-470.
Mohammed-Geba K., Yousif A., Younis A. M., Harby-Rashed A., 2021 DNA barcoding and molecular phylogeny confirm the presence of the cryptic *Penaeus japonicus* form II (*P. pulchricaudatus*) in the Egyptian demersal fisheries of the Gulf of Suez and the Bitter Lakes. Egyptian Journal Aquatic Biology and Fisheries 25(4):615-627.
Mote N., Affandi R., Haryono, 2014 [Reproductive biology of barb (*Barbonymus balleroides* Cuvier & Val. 1842) in the zone above and below Panglima Besar Soedirman Reservoir in Sungai Serayu, Jawa Tengah]. Jurnal Iktiologi Indonesia 14(2):111-122. [In Indonesian].
Muchlisin Z. A., Batubara A. S., Fadli N., Muhammadar A. A., Utami A. I., Farhana N., Siti-Azizah M. N., 2017 Assessing the species composition of tropical eels (Anguillidae) in Aceh Water, Indonesia, with DNA barcoding gene cox1. F1000Research 6:258.
Ng C. K. C., Abdulla F., Biun H., Ibrahim M. K., Mustapha S., Sade A., 2017 A working checklist of the freshwater fish diversity for habitat management and conservation work in Sabah, Malaysia, North Borneo. Biodiversitas 18(2):560-574.
Nuryanto A., Bhagawati D., Rofiqoh A. A., 2023 Barcoding of Sparidae collected during east monsoon season in the eastern Indian Ocean south of Java, Indonesia. AACL Bioflux 16(2):805-817.
Nuryanto A., Komalawati N., Sugiharto, 2019 Genetic diversity assessment of *Hemibagrus nemurus* from rivers in Java Island, Indonesia using COI gene. Biodiversitas 20(9):2707-2717.
Nuryanto A., Kusbiyanto, Bhagawati D., 2021 Molecular barcoding of marine ornamental fish from the southern coast of West Java validates conventional identification. E3S Web of Conferences 322:01004.
Nuryanto A., Pramono H., Kusbiyanto, Ghifari M. I., Andareswari N., 2018 Barcoding of fin clip samples revealed high exploitation of *Plectropomus leopardus* in Spermonde Archipelago. Biosaintifika 10(3):629-635.
Nuryanto A., Pramono H., Sastranegara M. H., 2017 Molecular identification of fish larvae from East Plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. Biosaintifika 9(1):33-40.
Pereira L. H. G., Hanner R., Foresti F., Oliverira C., 2013 Can DNA barcoding accurately discriminate megadiverse neotropical freshwater fish fauna? BMC Genetics 14:20.
Pin K., Nut S., Hogan Z. S., Chandra S., Saray S., Touch B., Chheng P., Ngor P. B., 2020 Cambodian freshwater fish assemblage structure and distribution patterns: using a large-scale monitoring network to understand the dynamics and management implications of species clusters in a global biodiversity hotspot. Water 12:2506.
Pramono T. B., Arifiati D., Widodo M. S., Yanuhar U., 2018 [Ichthyofauna in the downstream of Klawing River, Purbalingga District, Central Java]. Samaika: Jurnal Ilmu Perikanan 9(2):65-69. [In Indonesian].
Rasyad M. F. M., Widyanti A., Pebriani N., Fadliyah S., Ainiyah R. K., Pratama W. N., Putra A. J., Wahyunindita V., Wahyuni H. I., Dewangga E. P., Hayati A., 2020 Fish diversity as bioindicator of downstream pollution in the Surabaya River and Jagir River. Ecology, Environment and Conservation 26:S69-S76.
Ratnasingham S., Hebert P. D. N., 2013 A DNA-based registry for all animal species: The barcode index number (BIN) system. PLoS ONE 8(8):e66213.

Salem M. A., Abdel-Maogood S. Z., Abdelsalam M., Mahdy O. A., 2021 Comparative morpho-molecular identification of *Clinostomum phalacrocoracis* and *Clinostomum complanatum* metacercaria coinfecting Nile tilapia in Egypt. Egyptian Journal of Aquatic Biology and Fisheries 25(1):461-475.
Saprudin S., Soedibja P. H. T., Hidayati N. V., Pertiwi R. P. C., Sulistyo I., 2022 [Fish diversity in the middle part of Klawing River, Purbalingga Regency, Central Java Province]. Jurnal Maiyah 1(4):166-176. [In Indonesian].
Serdiati N., Safir M., Islamy R. A., 2022 New record of the non-native species of Mayan cichlid (*Cichlasoma urophthalmus* Günther, 1867) in Klawing River, Central Java, Indonesia. Ecology, Environment and Conservation 28(1):29-32.
Setyaningrum N., Lestari W., Krismono, Nuryanto A., 2022 Genetically continuous populations of striped snakehead (*Channa striata*) in the Cingcingguling River fragmented by Sempor Reservoir, Central Java, Indonesia. Biodiversitas 23(1):222-230.
Sholihah A., Delrieu-Trottin E., Sukmono T., Dahruddin H., Risdawati R., Elvyra R., Wibowo A., Kustiati K., Busson F., Sauri S., Nurhaman U., Dounias E., Zein M. S. A., Fitriana Y., Utama I. V., Muchlisin Z. A., Agnèse J. F., Hanner R., Wowor D., Steinke D., Keith P., Rüber L., Hubert N., 2020 Disentangling the taxonomy of the subfamily Rasborinae (cypriniformes, Danionidae) in Sundaland using DNA barcodes. Scientific Reports 10:2818.
Suryaningsih S., Bhagawati D., Sukmaningrum S., Sugiharto, Puspitasari I. G. A. A. R., 2020 Freshwater fish diversity in three tributary streams in Serayu Basin, Central Java, Indonesia. Biodiversitas 21(12):5811-5817.
Suryaningsih S., Sagi M., Kamiso H. N., Hadisusanto S., 2014 [Sexing in the red chick barb *Puntius orphoides* (Valenciennes, 1863) by using truss morphometrics method]. Biosfera 31(1):8-16. [In Indonesian].
Suryaningsih S., Sagi M., Nitimulyo K. H., Hadisusanto S., 2012 [Spawning aspects of Javaen barb *Puntius orphoides* (Valenciennes, 1842) in Klawing River, Purbalingga, Central Java]. Jurnal Iktiologi Indonesia 12(1):35-48. [In Indonesian].
Suryaningsih S., Sukmaningrum S., Simanjuntak S. B. I., Kusbiyanto, 2018 Diversity and longitudinal distribution of freshwater fish in Klawing River, Central Java, Indonesia. Biodiversitas 19(1):85-92.
Susatyo P., Lestari W., Sugiharto, Chasanah T., 2022 Reproductive aspects of Javaen barb fish, *Systomus orphoides* in the initial domestication program. Biodiversitas 23(3):1511-1519.
Susatyo P., Sugiharto, 2014 [A karamba scale test of floating net cages for juvenile F2 lukas (*Puntius bramoides*) predomestication (I): Egg quality, survival rate and spawning capability]. Biosfera 31(3):98-107. [In Indonesian].
Susatyo P., Sugiharto, Hana, Chasanah T., 2016 Effects of some feed supplements types to the growth of Javaen barb/brek fish (*Puntius orphoides*) second filial as domestication product. Biosaintifika 8(3):278-285.
Susilo U., Rachmawati F. N., Wibowo E. S., 2022 Protease and amylase activities of Javaen barb (*Systomus rubripinnis* Val.). Jurnal Biodjati 7(1):45-55.
Tamura K., Stecher G., Kumar S., 2021 MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7):3022-3027.
Xu X., Liu F., Chen J., Li D., Kuntner M., 2015 Integrative taxonomy of the primitively segmented spider genus *Ganthela* (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology. Zoological Journal of the Linnean Society 175(2):288-306.
*** Fricke R., Eschmeyer W. N., van der Laan R., 2023 Eschmeyer's catalog of fishes: Genera, species, references. Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp
*** Froese R., Pauly D., 2023 FishBase. Available at: www.fishbase.org

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