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Original Article

# A new distributional record of flying barb, *Esomus metallicus* (Actinopterygii: Cyprinidae), from Kapalo Banda River, West Sumatra, Indonesia

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**Abstract:** A new locality was reported for *Esomus metallicus* in Kapalo Banda River, 50 Kota Regency, West Sumatra Province, Indonesia, about 74 km from Maninjau Lake. Identification of species was made by morphological and molecular methods using the COI gene. We also applied the Neighbor-Joining method to construct the phylogenetic tree. Based on morphological analysis, the specimens from Kapalo Banda River were *E. metalicus* with the unique feature of having long maxillary barbels. Based on the DNA sequence, specimens of West Sumatra were identical to *E. metallicus* sequence of Thailand specimens with a similarity of 99-100%, suggesting the probable origination of the Kapalo Banda River from Thailand.

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## Introduction

The striped flying barb, *Esomus metallicus*, is a freshwater fish from Southeast Asia (Kottelat, 2013), including Thailand (Siriwan et al., 2018; Fu et al., 2021), Laos (Kottelat, 2015), Vietnam (Dao et al., 2017), and Malaysia (Ng et al., 2019; Jamaluddin et al., 2022). It is an introduced species in Indonesia that have recently been discovered (Arbsuwan et al., 2012; Hasan et al., 2020). In Indonesia, *E. metallicus* was accidentally released during the shipping of fry by local fish farmers (Hasan et al., 2020). In many cases, aquaculture is the cause of the exotic fish introduction (De Silva et al., 2009). The exotic fishes change the pattern of food and niche competition in inland waters (Albornoz-Garzón and Villa-Navarro, 2017), though so far, there are no reports of the negative impact of *E. metallicus* on aquatic communities (Arthur et al., 2010; Pulungan et al., 2011; Arbsuwan et al., 2012). However, more research is needed to ascertain the impact of *E. metalicus* on aquatic communities, especially native fish.

Striped flying barb is found in three localities, including the Siak River, Sumatra (Pulungan et al., 2011), Reteh River, Riau Province (Arbsuwan et al., 2012), and Maninjau Lake, West Sumatra Province (Hasan et al., 2020). In this study, we report a new locality for *E. metallicus* in Kapalo Banda River, 50 Kota Regency, West Sumatra Province, Indonesia, about 74 km far from Maninjau Lake, 281 km from Siak River, and 503 km from Reteh River. Kapalo Banda River is a tourist spot and conservation area under customary law and societal rules. There is no punishment for fishing violations in this area; it is just a belief that has been passed down from generation to generation about the bad things that will happen if we damage the aquatic ecosystem, and catching fish is done only for personal use in that place. In this work, we also provide morphological and molecular data of the collected specimens to identify them. For molecular identification, we used DNA barcoding based on Cytochrome C Oxidase

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Figure 1. Kapalo Banda River, the location where *Esomus metallicus* found in 50 Kota District, West Sumatera, Indonesia.

subunit I (COI), a standard method for species identification with a high level of accuracy (Hubert et al., 2008; Luo et al., 2011; Valen et al. 2019a; Lutfiatunnisa et al., 2021).

## Material and Methods

Specimens were collected from an irrigation canal in Kapalo Banda Taram (Fig. 1), 50 Kota Regency, West Sumatra Province, Indonesia. The fish are protected by local customary law and societal rules called "Ikan Larangan". In this case, research specimens need permission from the local customary head. We also use friendly fishing gear to protect the aquatic environment and the natural habitat. The study was conducted from 26-28 January 2022, and 15 specimens were captured using a cast net and fish trap (Serdiati et al., 2021). Ten specimens were fixed in 10% formalin (Serdiati et al., 2020) and deposited at the Laboratory of Universitas Bangka Belitung, Indonesia. For subsequent DNA analysis, five specimens were fixed in 95% ethanol (Valen et al., 2019b). Morphological identification of specimens was made based on Rainboth (1996), Arbsuwan et al. (2012), and Hasan et al. (2020).

**DNA Extraction:** DNA extraction was done using a Genomic DNeasy Blood and Tissue Kit (Qiagen). A total of 25 mg of tissue samples were taken using sterile tweezers and put into a 1.5 ml tube, then 180 µl of ATL buffer and 20 µl of proteinase K were added. Afterward, the samples were vortexed and centrifuged for 20s and heated in a heating block at 56°C overnight. 200 µl buffer AL was added, vortexed, and incubated at 56°C for 10 min. Then 200 µl 96% ethanol was added and vortexed. The sample and reagent mixture was transferred to a DNeasy Mini spin column and placed in a 2 ml collection tube. Samples were centrifuged at 8000 rpm for 1 min. Then, the liquid was drained into the collection tube, and placed in the spin column in a new 2 ml collection tube, 500 µl of buffer AW1 was added and centrifuged at 8000 rpm for 1 min. The spin column was placed into a new 2 ml collection tube, and added 500 µl of buffer AW2, and centrifuged at 14,000 rpm for 3 min. The spin column was transferred to a new 1.5 ml tube. DNA was eluted by adding 100 ul of $ddH_2O$ to the center of the membrane spin column, then was incubated at room temperature of 15-25°C for 1 min. Then centrifuge was done at 8,000 rpm for 1 min. The final step was repeated by adding 100 µl of $ddH_2O$ to get a final volume of 200 µl.

The extracted DNA was amplified using the

**Table 1.** DNA sequense of Genus Mystacoleuscus from Genbank.

| Species | Location | Accession Number | Authors |
|---|---|---|---|
| *Esomus metallicus* | Thailand | JF915604.1 | Collins et al. (2016) |
| *Esomus metallicus* | Thailand | FJ753495.1 | Britz, et al. (2016) |
| *Esomus metallicus* | Thailand | KC456399.1 | Panprommin et al. (2013) |
| *Esomus metallicus* | Thailand | MK049431.1 | Panprommin et al. (2019) |
| *Esomus metallicus* | Thailand | KF805365.1 | Phuttawongk et al. (2015) |
| *Esomus Longimanus* | Thailand | HM224169.1 | Tang et al. (2015) |
| *Esomus Longimanus* | Vietnam | MK777274.1 | Thu et al. (2020) |
| *Esomus thermoicos* | Sri Lanka | MK214872.1 | Sudasinghe et al. (2018) |
| *Esomus thermoicos* | Sri Lanka | MK214871.1 | Sudasinghe et al. (2018) |
| *Esomus cf ahli,* | - | EF452888.1 | Mayden et al. (2016) |
| *Esomus danricus* | India | KF511504.1 | Dhar et al. (2015) |
| *Esomus danricus* | India | KF511505.1 | Dhar et al. (2015) |
| *Esomus danricus* | India | KF511506.1 | Dhar et al. (2015) |
| *Castostomus commersonii* | America | HQ557394.1 | April et al. (2011) |

Hotstart method. The parameters used in this method are as follows: denaturation at 94°C for 3 min, denaturing at 94°C for 30s, annealing at 48°C for 30s, and extension at 72°C for 45s (Valen et al., 2021; Valen et al., 2022a), and the PCR process was repeated for 38 cycles. The two primers of FISH F1 TCA ACC AAC CAC AAA GAC ATT GGC AC and FISH R1 TAG ACT TCT GGG TGG CCA AAG AAT CA were used (Ward et al., 2005). The PCR product was visualized in 1% agarose gel via electrophoresis by staining Nucleic Acid Gel Stain (GelRed®) (Hasan et al., 2021). The samples were sequenced using the Sanger sequencing method at a sequencing service facility (Sanger and Nicklen, 1977).

**Data Analysis:** Molecular identification of species was done by comparing the sequences to the BOLD SYSTEM (https://www.boldsystems.org) and BLAST at NCBI (https://blast.ncbi.nlm.nih.gov) to analyze the homology and similarity between sequences. To reconstruct the phylogenetic tree, we downloaded the sequences of *Esomus* species from the Genbank and sequences of *Castostomus commersonii* as an outgroup (Table 1). All sequences were initially aligned using the Clustal W in MEGA6 software (Tamura et al., 2013). The phylogenetic tree was reconstructed using the Neighbor-Joining method (Saitou and Nei, 1987) with a bootstrap test of 10000 replications (Felsentein, 1985). The evolutionary distance was calculated using the uncorrected p-distance method (Nei and Kumar, 2000) in MEGA6 software (Tamura et al., 2013).

## Results and Discussion

A new record of *E. metallicus* was done from an irrigation canal of Kapalo Banda River, 50 Kota District, West Sumatera Province, Sumatera, Indonesia, during 26-28 January 2021 by collecting 15 specimens (470-590 mm) (Fig. 2).

**Diagnosis:** Meristic characters of *E. metallicus* are shown in Table 2. Body compressed and elongated; eye moderately large; head small; mouth superior, upper jaw shorter than lower jaw; snout moderately long; maxillary barbels long reaching to ventral body, rostral barbels quite long; lateral line incomplete.

**The color of fresh specimen:** Yellow body; a black band running at dorsal midline of body from posterior end of opercle to caudal fin base; two light yellow bands present at lateral side of body, running parallel to black lateral band just above it and attaching to it at around caudal peduncle. All fin membranes colorless or transparent.

The discovery of *E. metallicus* in the Kapalo Banda River is a new record in addition to its previous records (Fig. 3). New records of fish contribute to understanding species biogeography (Valen et al., 2022b, c) and the distribution range of species (Ihwan et al., 2020; Valen et al., 2020).

**Figure 2.** Specimen of *Esomus metallicus* found in Kapalo Banda River in 50 Kota District West Sumatera Province.

**Table 2.** Meristic characteristics of *Esomus metallicus* collected from the Kapalo Banda River in 50 Kota District, West Sumatera Province, Indonesia (n=15).

| Meristics (counts) | Present study | Hasan et al. (2020) | Rainboth (1996) |
|---|---|---|---|
| Pored Lateral line scales | 13-14 | - | 10-14 |
| Dorsal fin rays | 8 | 8 | 8 |
| Anal fin rays | 8 | 8 | 8 |
| Pectoral fin rays | 14 | 14 | - |
| Ventral fin rays | 7 | 7 | - |
| Caudal fin forked | Forked | Forked | forked |

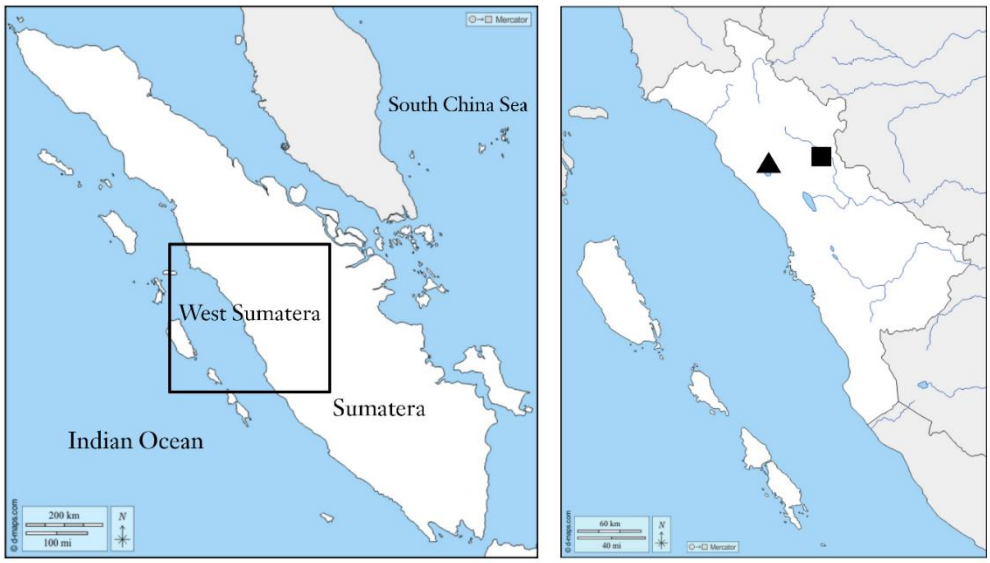


**Figure 3.** Distribution of an invasive *Esomus mettalicus* in Sumatera. The triangle circles are the previous record. The square is the recent record from Kapalo Banda River, 50 Kota District, West Sumatera, Indonesia.

Kapalo Banda River is not connected to Maninjau Lake, and their distance is about 74 km; therefore, *E. metallicus* are not naturally moved to this new record from that previously recorded site. Therefore, *E. metallicus* was probably released into Kapalo Banda River accidentally by local farmers, although this area is not an aquaculture area. However, several streams connected to the Kapalo Banda River have some fish farming activities, so it is possible that fish have been introduced into Kapalo Banda River. *Esomus metallicus* is an exotic species with 2 long beards in the mouth and used as an ornamental fish.

*Esomus metallicus* is exotic to Indonesia and inhabits with natural and endemic fishes of Sumatra e.g. *Rasbora* spp. can be a competitor to those small fishes (Hasan et al., 2020; Albornoz-Garzón and

**Table 3.** Sequence of Striped flying Barb, *Esomus metallicus* from the Kapalo Banda River in 50 Kota District, West Sumatera Province, Indonesia.

| DNA Barcoding |
|---|
| AAGACATTGGCACCCTTTATTTAGTATTTGGTGCCTGAGCTGGAATAGTGGGAACCGCCCTGAGCCTTCTTATCCGAGCTGAA |
| CTAAGCCAACCCGGATCACTTCTAGGCGATGACCAAATTTACAATGTTATTGTTACTGCCCACGCTTTTGTAATAATCTTTTTT |
| ATAGTTATACCAATTCTTATTGGAGGATTTGGAAACTGACTTGTACCATTAATGATTGGAGCACCAGACATAGCATTTCCACG |
| AATAAATAATATGAGTTTCTGACTCCTGCCCCCATCATTTCTTTTACTACTAGCCTCATCTGGGGTTGAGGCTGGAGCAGGAA |
| CAGGGTGAACAGTATATCCACCTCTTGCTGGAAACCTTGCCCATGCGGGAGCATCAGTAGATCTAACTATCTTTTCGTTACAC |
| TTAGCAGGTGTTTCATCAATTTTAGGAGCAATTAATTTTATTACTACCATTATTAATATGAAGCCCCCTGCCATTTCTCAATAT |
| CAAACACCACTGTTTGTTTGAGCCGTTTTAGTAACAGCCGTTCTTCTCCTACTATCACTACCAGTTTTGGCGGCTGGAATTACA |
| ATGCTTCTTACAGACCGTAACCTCAATACCACCTTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTTTATCAACACTTATT |
| CTGATTCTTTGGC |

**Table 4.** Species identification and similarity.

| Specimen | Similarity | Species identified | Accession Number | Family | Genus |
|---|---|---|---|---|---|
| Striped flying Barb | 100% | *Esomus metallicus* | FJ753495 | Cyprinidae | *Esomus* |
| Striped flying Barb | 100% | *Esomus metallicus* | MK049431 | Cyprinidae | *Esomus* |

Villa-Navarro, 2017). Alien or invasive fishes enter new areas either intentionally or not and can potentially disrupt endemic fishes by adapting to new environments and competing with natural fishes (Radkhah et al., 2016; Insani et al., 2020; Bariyyah et al., 2021). Hence, there is a need for an in-depth study of the impact of *E. metallicus* in waterbodies on endemic fish and their habitats for making policies to control invasive fishes. The establishment of non-native species has a severe impact on ecosystem functioning by affecting community levels of native species (Sato et al., 2010; Mousavi-Sabet and Eagderi, 2016). Ecological risks of alien fishes are difficult to be estimated in the initial stages, but their harm is irreversible. Moreover, the Kapalo Banda River is protected by customary law to maintain native and endemic fish, an ancestral heritage that must be preserved.

DNA-Barcoding of striped flying Barb from Kapalo Banda River successfully sequenced with a base-pair length of 680 bp (Table 3). Fragments with more than 658 base pairs of COI genes can be used as a standard for differentiating animals (Hebert et al., 2003). The results revealed that the samples from the Kapalo Banda River are *E. metallicus* with a similarity of about 99-100% (Table 4). To ensure species validity, we also performed species identification by BOLD SYSTEM by specimen identification tools to check the species-level barcode records. The submitted sequence has been matched to *E. metallicus* with a similarity of 100% to the BIN ID: BOLD: AAF2628. According to Hebert et al. (2003), species with 99-100% similarity levels are identical.

In the phylogenetic tree, striped flying Barb from West Sumatra, Indonesia (Red circle) clustered within *E. metallicus* species (Fig. 4) with 100% DNA similarity with those of Thailand. Therefore, specimens of West Sumatra probably originated from Thailand, or they are from the same ancestor. The Kapalo Banda River and Thailand were formerly connected to the Sundaland River; however, this species was not collected before in Sumatra (Kottelat et al., 1993), and since there is no difference between their sequences, as is common for long-distant species, therefore striped flying Barb from West Sumatra has originated from Thailand. According to Hasan et al. (2020), *E. metallicus* in Indonesia was introduced and accidentally released from fish farms.

There are nine *Esomus* species, including *E. danrica* (Shangningam and Kosygin, 2022; Abujam et al., 2021), *E. nimasowi* (Abujam et al., 2021), *E. metallicus* (Jamaluddin et al., 2022), *E. malayensis* (Kottelat et al., 2013), *E.thermoicos* (Sudasinghe et al., 2019), *E. longimanu*s (Kottelat, 2001), *E. caudiocellatus* (Kottelat, 2013), *E. bengalensis* (Bhakat and Sinha, 2020), *E, altus*

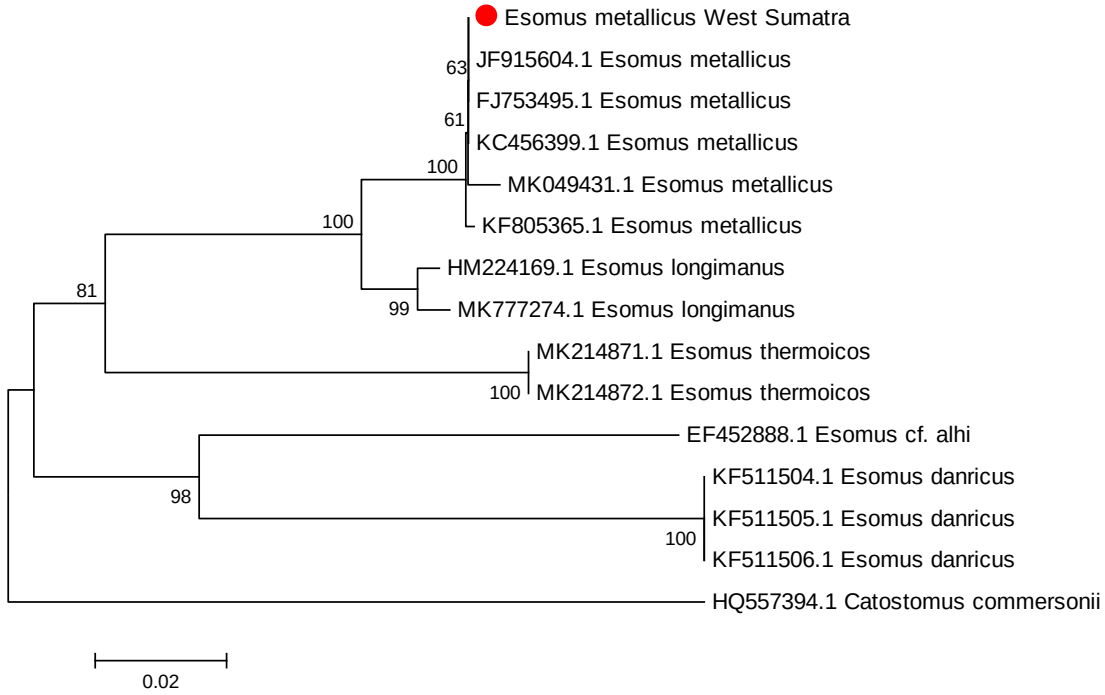


**Figure 4.** Phylogenetic tree of *Esomus metallicus* based on COI Gene.

**Table 5.** The genetic distance of *Esomus metallicus*.

| | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1 | *Esomus metallicus* West Sumatera | | | | | |
| 2 | *Esomus metallicus* | 0.000 | | | | |
| 3 | *Esomus longimanus* | 0.028 | 0.028 | | | |
| 4 | *Esomus thermoicos* | 0.120 | 0.120 | 0.115 | | |
| 5 | *Esomus danricus* | 0.169 | 0.169 | 0.164 | 0.168 | |
| 6 | *Esomus cf. alhi* | 0.163 | 0.163 | 0.164 | 0.174 | 0.150 |

(Kottelat, 2013) and *E. ahli* (Kottelat, 2013). In the GenBank, there are only five *Esomus* species' COI genes, including *E. metallicus* (FJ753495) from Thailand, *E. danricus* (KF511504) from India, *Esomus* cf. *alhi* (EF452888), *E. thermoicos* (MK214871) from Sri Lanka and *E. longimanus* (HM224169) from Vietnam. The genetic distance of *E. metallicus* from Kapalo Banda River was 0.00 to that of Thailand. *Esomus metallicus* is the closest species to *E. longimanus*, with a genetic distance of 0.028 (Table 5). *Esomus metallicus* is native to Thailand (Beamish et al., 2006) and Southeast Asia, and *E. longimanus* is native to Asia (Doi, 1997) and is also found in Thailand, showing a close ancestor with *E. metallicus*.

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