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Haplotype Diversity in the Mitochondrial COI Gene of Barred Rainbowfish (*Chilaterina fasciata*) from Mamberamo River of Western New Guinea, Indonesia

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ABSTRACT

A Barred rainbowfish (*Chilatherina fasciata*) is one of the native fish species found in Western New Guinea of Indonesia. This study aimed to observe the levels of haplotype diversity in the partial Cytochrome-c oxidase subunit I (COI) gene of Barred rainbowfish. For the DNA analysis, thirty (30) Barred rainbowfishes were caught from the Mamberamo River. Three (3) molecular packages of BioEdit, MEGA, and DNAsp were used to analyze twenty (30) forward sequences of the COI gene (502 bp). The research showed four (4) haplotypes for the examined population, a total of seven (7) mutations, and low genetic diversity detected in the partial COI gene with the haplotype diversity (Hd) = 0.405 and nucleotide diversity (pi) = 0.003. Meanwhile, the Fu's and Tajima's tests were 1.21 and -0.69, respectively. The UPGMA tree with 1,000 × bootstrap replications revealed that Barred rainbowfishes are grouped into similar clusters with *Melanotaenia vanheurni*, *Chilatherina alleni*, and *Chilatherina bleheri*. In conclusion, haplotype 3 (77%) was detected as the common haplotype for Barred rainbowfishes at the Mamberamo River of Western New Guinea.

1. Introduction

The Barred rainbowfish (*Chilatherina fasciata*) is a Melanotaeniidae fish family with the IUCN conservation status of Least Concern (Allen *et al.* 2020). Generally, rainbowfishes have a high economic value because people keep them as exotic fishes (Elfidasari *et al.* 2015). However, farmers reported many problems when raising Melanotaeniidae fish, such as decreased production, a higher proportion of females per spawning, loss of coloration, lower growth rate, and fecundity (Nugraha *et al.* 2015). Generally, the Melanotaeniidae fish are spawning in wet and dry seasons (McGuigan *et al.* 2005). Barred rainbowfishes are restricted in New Guinea, belonging to the Mamberamo River (Allen 1981). *Chilatherina fasciata* are relatively small fishes; males may reach a maximum size of 12 cm, but females are usually less than 10 cm. Sexual maturity occurs in most species at a relatively small size, usually about 3.5- 4.0 cm in females and 4.5-5.5 cm in males. Diet includes filamentous algae, small crustaceans, terrestrial insects (particularly ants and tiny beetles), and aquatic insect larvae (Allen 1981).

Mamberamo River has a high abundance of avifauna (Pattiselano 2005), herpetofauna (Krey 2008), ichthyofauna (Binur and Ohee 2010), and insects (Rizal *et al.* 2019). Presently, there are eleven (11) fish species of *Chilatherina* sp., such as *C. alleni, C. axelrodi, C. bleheri, C. bulolo, C. campsi, C. crassispinosa; C. fasciata; C. lorentzii; C. pagwiensis; C. pricei* and *C. sentaniensis* (Unmack *et al.* 2013). In addition, there are three (3) main lineages of rainbowfish based on mitochondrial DNA sequence (5,696 bp), i.e., Northern, Southern, and Western New Guinea lineages (Unmack *et al.* 2013). Cytochrome-c oxidase subunit I (COI) gene is a part of the mitochondrial region that can be used for genetic characterization in the Melanotaeniidae fish family (Kadarusman *et al.* 2012; Nugraha *et al.* 2022). Therefore, the North New Guinea rainbowfishes (*Melanotaenia*

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affinis) were detected as a close kinship for Barred rainbowfishes based on the COI gene (Wibowo *et al.* 2020). Moreover, Melanotaeniidae fish was classified into a similar cluster with *Hypoatherina tsurugae* and *Chirostoma humboldtianum* based on mitogenome DNA (Zhao *et al.* 2016).

Unfortunately, the genetic diversity of the mitochondrial COI gene in Barred rainbowfishes has not been reported before. Previous studies reported that a mitochondrial COI gene can be used to obtain the haplotype diversity in many Indonesian fish species, such as Green catfish (*Hemibagrus nemurus*), Kissing Gourami (*Helostoma temminckii*), Striped Snakehead (*Channa striata*), Tilapia (*Oreochromis* sp.), Cyprinid (*Barbonymus* sp.) and Selais (*Ompok hypophthalmus*) fishes (Nuryanto *et al.* 2019; Arisuryani *et al.* 2019; Arisuryani *et al.* 2020; Dailami *et al.* 2021; Batubara *et al.* 2021; Kasayev and Arisuryani 2022). This study aimed to observe the haplotype diversity in Barred rainbowfish from the Mamberamo River based on partial COI gene sequences. The results of this study are important to characterize Barred rainbowfishes based on their molecular genetics information. Additionally, molecular studies in endangered animals have an important role in presenting genetic database information about this species that can be used as a conservation guideline in the future (Pekkala *et al.* 2014).

2. Materials and Methods

2.1. Sample and Research Site

Thirty (30) samples of adult Barred rainbowfish were used in this study for DNA analysis. These fishes were collected in 2016 from the Mamberamo River of Western New Guinea, Indonesia. Four research sites at Mamberamo river i.e. Sungai Putus (03842086.100S, 140816079.900E), Telaga (03843098.400S, 140818019.200E), Kerumi (03844038.900S, 140817088.200E) and Kali Merah (03844037.800S, 140818055.500E) as illustrated in Figure 1. The fish samples were caught with six experimental gill nets (stretched size 12.7, 25.4, 38.1, 50.8, 76.2, and 101.6 mm). All nets were randomly placed in the water of each research site 1.5 m deep and 15 m long. The nets were placed in the morning along 1,700 h. Thus, the fish were collected from the experimental nets every evening every 700 h.

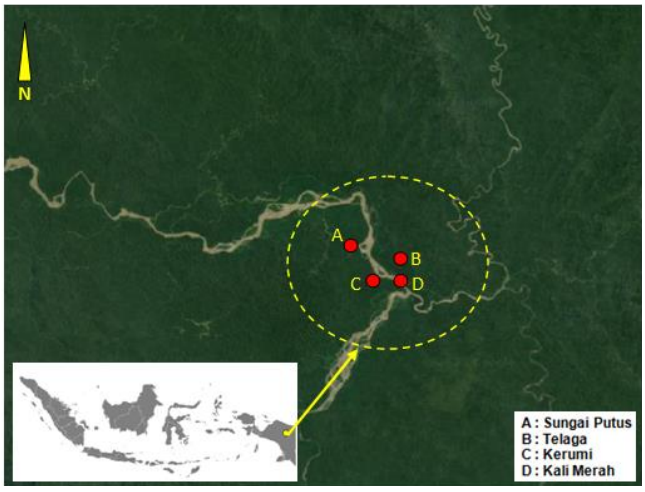

Figure 1. Four research sites at the Mamberamo River of Western New Guinea, Indonesia, i.e., Sungai Putus (A), Telaga (B), Kerumi (C) and Kali Merah (D)

2.2. Post-collected Fish Handling

The caught fish were signed by a labeling machine (Dymo, USA) and captured by digital camera. Therefore, the DNA samples were collected from the fish muscle tissue (1 cm × 1 cm) with a sterilized scalpel or scissors and deposited into 1.5 ml microtubes with 96% ethanol (EtOH) for DNA extraction. The species identification was assessed by observing the morphological characteristics of Allen (1991).

2.3. DNA Extraction, Amplification and Sequencing

The DNA extraction was performed with the muscle tissue of fish using a Genomic DNA extraction kit (Geneaid, Taiwan) following the manufacturer's protocol. The PCR reaction was performed in a total volume of 50 µL consisting of 5 µL of DNA template, 25 µL of PCR mastermix (KAPA Biosystems, USA), 2 µL of each primer (1mM), and 16 µL of free-nuclease water. A universal primer pair of fish (Ivanova *et al.* 2007) was used in this study to amplify fish COI gene along 501 bp as follows: Forward: 5'- TAA TAC GAC TCA CTA TAG GGT TCT CCA CCA ACC ACA ARG AYA TYGG -3' and Reverse: 5'- ATT AAC CCT CAC TAA AGG GCA CCT CAG GGT GTC CGA ARA AYC ARAA -3'. The PCR program was performed using a master cycler machine with pre-denaturation at 95°C for 15 min followed by 35 cycles of denaturation at 94°C for

30 s, annealing at 55°C at 90 s; extension at 72°C for 30 s and final extension at 72°C for 5 min. The DNA visualization was performed in 1% agarose gel and captured by UV-transilluminator. Therefore, the forward sequence analysis was performed through a commercial laboratory service (Macrogen, South Korea).

2.4. Data Analysis

The observed sequences in this study were analyzed using three (3) molecular software BioEdit (Hall 2011), MEGA (Hall 2013), and DNAsp (Librado and Rozas 2009). BioEdit was used for sequencing alignment and detecting the mutation sites in the observed sequences. MEGA was used to create a phylogenetic tree of Barred rainbowfish with the UPGMA 1,000 × bootstrap replicates method. DNAsp was used to obtain the genetic diversity parameters such as haplotype diversity (Hd), nucleotide diversity (pi), Fu's Fs statistics, and Tajima's test. Therefore, a similar sequence of the Melanotaeniidae family of fishes was collected from the BOLDSYSTEMS database (https://www.boldsystems.org) to create a phylogenetic tree of Barred rainbowfish

3. Results

3.1. Genetic Diversity

Along 501 bp of COI gene in Barred rainbowfish was amplified successfully based on universal COI primer as shown in Figure 2. Interestingly, seven (7) mutation sites were detected in the 1st, 17th, 43rd, 52nd, 178th, 187th, and 346th nucleotides based on sequencing analysis as shown in Figure 3. According to these mutation sites, four (4) haplotypes of H1 (1 fish), H2 (3 fishes), H3 (23 fishes), and H4 (3 fishes) were observed in the Barred rainbowfishes from the Mamberamo River. Commonly, Barred rainbowfish (H3) has a nucleotide composition of 24.30% for A, 28.88% for C, 17.13% for G, and 29.68% for T. Hence, the GC and AT contents of H3 fish were 46.01% and 53.99%, respectively. Therefore, the haplotype diversity (Hd) and nucleotide diversity (pi) in the COI gene of fish under study were 0.405 and 0.003, respectively (Table 1). Thus, the Neutrality test of Fu and Tajima values in fish under study were 1.21 and -0.69, respectively.

3.2. Phylogenetic Tree

According to the UPGMA tree (1,000 × bootstrap) of partial COI gene, Melanotaeniidae family fishes can be grouped into four (4) clusters based on partial COI gene, i.e., cluster 1 (*M. mairasi*), cluster 2 (*M. susii, M. longispina, M. klasioensis, M. fasinensis, M. sembrae, M. boesemani, M. ajamaruensis, M. arfakensis, M. manibuii, M. irianjaya, M. sikuensis, M. angfa, M. rumberponensis, M. naramasae, M. veoliae, M. arguni, M. ammeri, M. synergos, M.catherinae*), cluster 3 (*M. vanheurni, M. rubripinnis, M. affinis, M. praecox, C. campsi, C. alleni, C. bleheri, C. pricei, C. fasciata*) and cluster 4 (*M. duboulayi, M. fluviatilis, M. parkinsoni, M. eachamensis, M. australis, M. gracilis, M. maccullochi, M. papuae, M. sexlineata, M. splendida, M. herbetaxelrodi, M. goldiei, M. oktediensis, M. lacustris, M. trifasciata*)

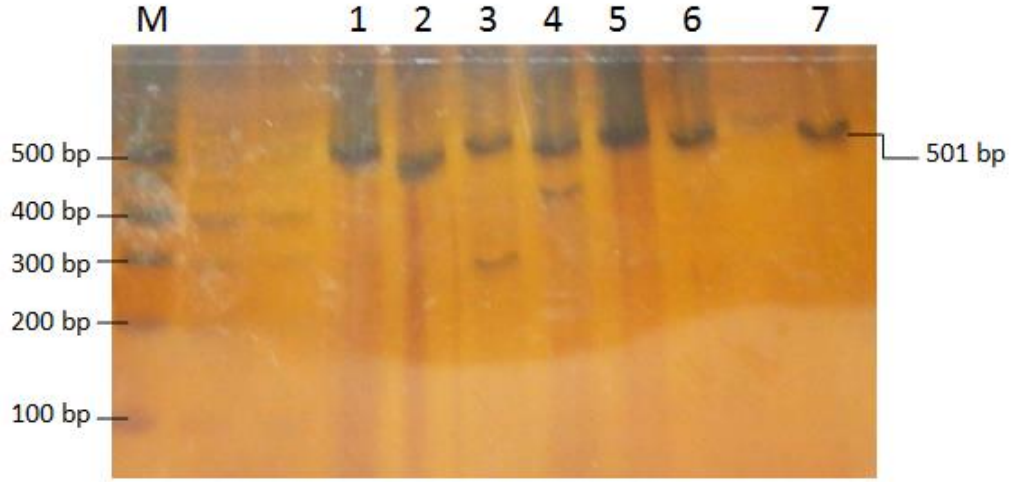

Figure 2. The partial COI gene (501 bp) amplification in Barred rainbowfish (*Chilatherina fasciata*) on 10% acrylic gel. M: DNA marker 100 bp; Line 1-7: DNA sample

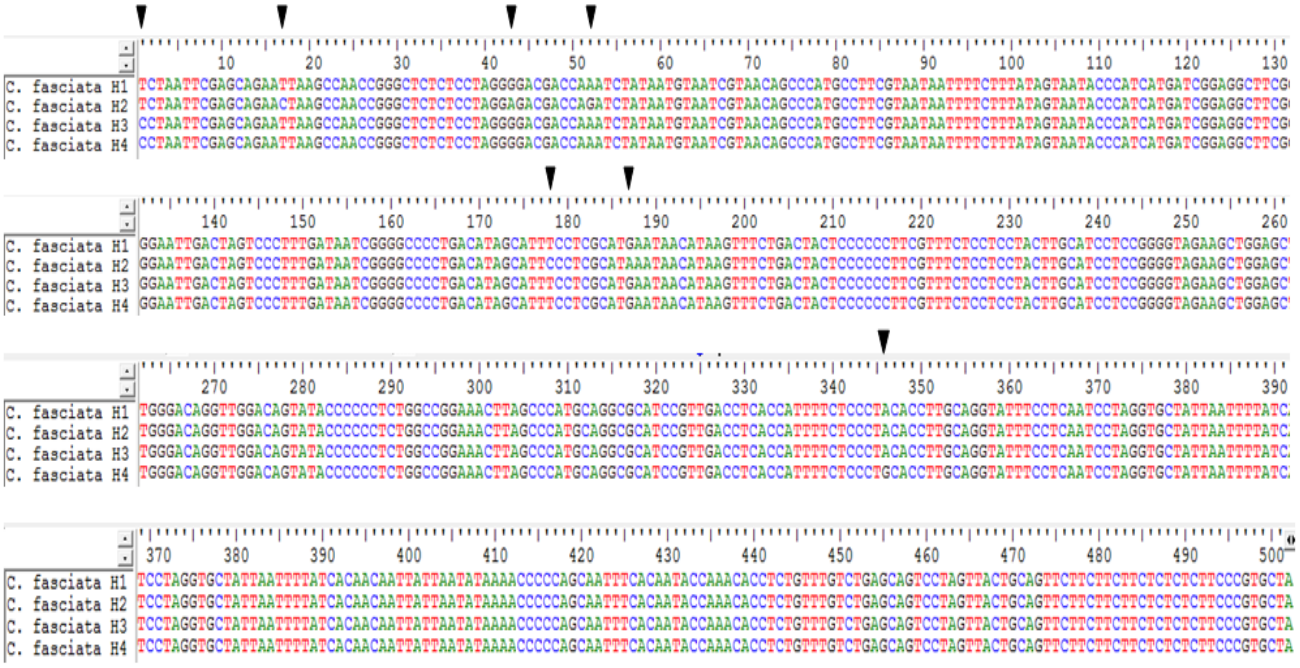

Figure 3. Sequence alignment among haplotype of COI gene (502 bp) in Barred rainbowfish (*Chilaterina fasciata*) reveals seven (7) mutation sites (black arrow) at 1st, 17th, 43th, 52th, 178th, 187th and 346th nucleotides

Table 1. Genetic diversity in the partial COI gene of Barred rainbowfish (*Chilatherina fasciata*) at Mamberamo River of Western New Guinea, Indonesia

Parameter	Value
Number of the observed sequence	30
Number of observed site	502
Number of the mutation site	7
Number of haplotypes	4
Haplotype diversity (Hd)	0.405
Nucleotide diversity (pi)	0.003
Fu's Fs statistics	1.21
Tajima's D test	-0.69

as shown in Figure 4. However, according to the partial COI gene, the Barred rainbowfish (*C. fasciata*) has a close genetic relationship with Van Heurn's rainbowfish (*M. vanheurni*).

4. Discussion

Commonly, H3 is detected as the origin lineage of Barred rainbowfish at Mamberamo River because of its high frequency. Thus, Fatimah *et al.* (2023) reported that the AT content (63.90%) in Asian Horseshoe crabs (T*achypleus tridentatus; Tachypleus gigas; Carcinoscorpius rotundicauda*) was higher than GC content (36.20%) and close to this study. The Hd and pi values in this study are classified into low categories. The Hd value is classified into low (<0.50) and high (>0.50) categories. In contrast, the pi is categorized into low (0.01-0.04), moderate (0.05-0.07), and high (0.08-1.00) categories (Nei and Kumar 2000). Low Hd value in Barred rainbowfishes can be caused by isolated geographical factors and natural barriers that inhibit the genetic flow from another population. A low pi value in Barred rainbowfishes indicates low sequence variation because of no cross-mating with other rainbowfishes. The haplotype diversity is controlled by various processes, including mutation, recombination, marker ascertainment, and demography (Stumpf 2004). A high genetic diversity will follow a high haplotype diversity. Haplotype and nucleotide diversity of mtDNA are two important indicators for assessing population polymorphism and genetic differentiation. Despite this, the species expansion may be shown in Barred rainbow fish, signed by a negative in Tajima's test (-0.69). Furthermore, the negative value of the neutrality test indicated an excessive number of alleles due to population constraints on the selection being too dominant (Nei and Kumar 2000). Interestingly, Melanotaeniidae genera, such as Melanotaenia, Chilatherina, and Glossolepis, can be hybridized when kept in captivity

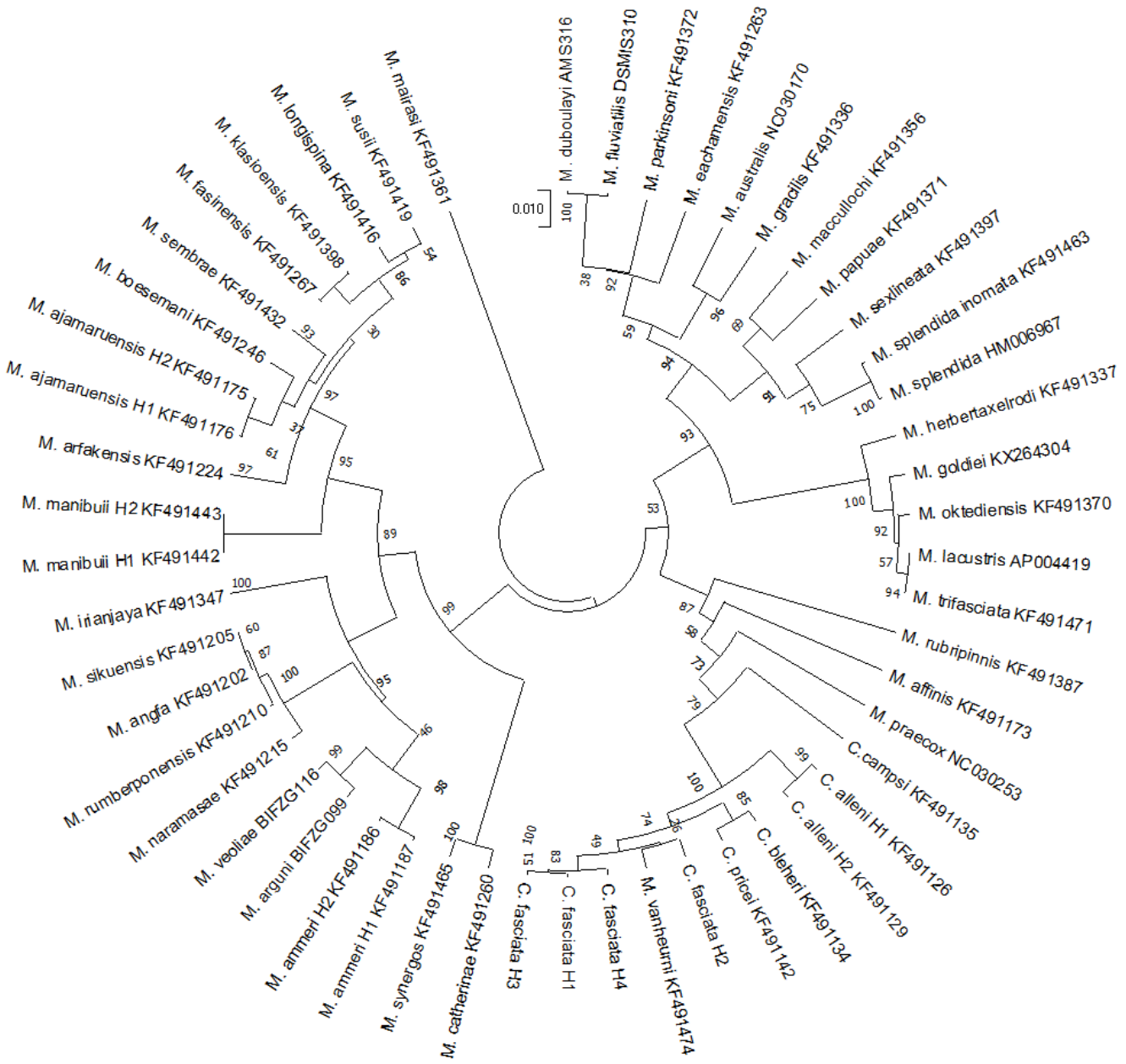

Figure 4. The phylogenetic tree of Barred rainbowfish (*Chilatherina fasciata*) and Melanotaeniidae family fishes based on partial COI gene with UPGMA 1,000 × bootstrap replications

(McGuigan *et al.* 2005). Hence, the hybridization among Melanotaeniidae genera can cause genetic and phenotypic variations in the species population.

In this study, *M. vanheurni* is detected as the closest kinship of *C. fasciata* based on a partial COI gene. In addition, Unmack *et al.* (2013) reported that Chilatherina and Glossolepis fishes have a close genetic relationship with *M. affinis* based on 6,827 bp of partial mitogenome sequence (Unmack *et al.* 2013). Moreover, *C. fasciata* fishes have a close genetic relationship with *M. affinis* based on the partial COI gene (Wibowo *et al.* 2020). Melanotaenidae family fishes are categorized into seven (7) genera: Cairnsichthys, Iriatherina, Pelangia, Rhadinocentrus, Melanotaenia, Chilatherina and Glossolepis (Tappin 2011; Kadarusman *et al.* 2012; Allen and Unmack 2012). In the present study, the family fish of Cyprinidae is the closest kinship for Melanotaeniidae fishes rather than the Claridae and Cichlidae families. Despite this, Bedotidae, Telmatherinidae, and Pseudomugilidae fish families are the sister clusters of Melanotaenidae fish

based on the mitochondrial ND5 gene (Sparks and Smith 2004). In addition, the karyotyping analysis revealed that *Melanotaenia* sp. is close to *Glossolepis* sp. (Majtanova *et al.* 2022).

We concluded that seven mutation sites were detected in the partial COI gene with four (4) haplotypes of H1 (3%), H2 (10%), H3 (77%) and H4 (10%). Hence, H3 is the common haplotype of Barred rainbowfish at Mamberamo River. Therefore, the phylogenetic tree analysis revealed that *M. vanheurni* is the closest kinship of the Barred rainbowfish.

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