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Molecular identification of peacock bass (*Cichla ocellaris*) from the Bedog River, Yogyakarta, Indonesia

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Abstract. *Andriyono S, Akhmad H, Alam MJ, Dewi NN, Lutfiyah L, Suciyono. 2025. Molecular identification of peacock bass* (Cichla ocellaris) *from the Bedog River, Yogyakarta, Indonesia. Biodiversitas 26: 1565-1573.* The introduction of non-native fish species can lead to the establishment of invasive species groups that pose environmental threats and contribute to biodiversity loss. The primary aim of this study was the precise molecular identification of specific *Cichla* spp. from the Bedog River in Yogyakarta. Seven *Cichla* fish samples were collected from the Bedog River (upstream and downstream area) from August to October 2020 using a fish net. The cytochrome C Oxidase subunit I (COI) gene region serves as the basis for species identification, with comparisons made against the National Center for Biotechnology Information (NCBI) database. In this study, the primers used in PCR were Universal Fish BCL-BCH primers. The PCR results were followed by sequencing using the Sanger platform. Of the seven samples procured from the Bedog River, all unequivocally indicated the presence of the *Cichla* species, identified explicitly as *Cichla ocellaris* (99.68-100% similarity to reference NC030272). Furthermore, upon phylogenetic tree construction, these sequences consistently clustered with *C. ocellaris* and *Cichla temensis* sequences. Nevertheless, discerning characteristics of the color pattern align more closely with *C. ocellaris*. The identification of non-native species in the Bedog River underscores the imperative for vigilant monitoring of fish introductions to avert potential harm to indigenous fish populations. The predatory nature of *C. ocellaris* raises concerns about its capacity to exert pressure on local fish populations, potentially culminating in its invasive establishment—a phenomenon with deleterious implications for tropical fish biodiversity in Indonesia. DNA Barcode applications are essential in supporting the provision of accurate data and in preventing the illegal spread of non-native fish.

Keywords: Aquatic, diversity, DNA, molecular, nan-native species

INTRODUCTION

The introduction of non-native fish species can lead to the establishment of invasive species groups that pose environmental threats and contribute to biodiversity loss. Recent assessments have highlighted a concerning risk, with 39% of global fish species projected to face extinction within the next 400 years because of the impact of invasive alien species (Kiruba-Sankar et al. 2018). Previous research has underscored the complex effects of non-native aquatic species on indigenous freshwater fish communities, noting both positive and negative effects (Tarkan et al. 2017). However, efforts to introduce non-native fish into new habitats often result in a gradual decline in local fish populations, which, over time, may contribute to the extinction of certain species (Harper and Bunbury 2015).

Despite the critical implications of invasive species, studies on this phenomenon in Indonesia remain limited and require more comprehensive coordination. A notable example comes from research conducted in Lake Poso, Sulawesi, revealing the presence of introduced species like striped snakehead *Channa striata* (Bloch, 1793) and Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) (Herder et al. 2022). Some species exhibit remarkable adaptability to introduced environments, particularly ornamental fish imported from tropical waters, raising concerns about their impact on endemic fish populations (Herder et al. 2012). Hatchery and artificial propagation activities further compound the issue, allowing fish seeds to survive and establish themselves in novel environments outside their natural habitats. In the Bedog River area in Yogyakarta, the presence of *Cichla* sp. fish constitutes a novel report, prompting monitoring efforts by the Yogyakarta fish quarantine and fishery quality control station from 2021 to 2022. There is no definite information about the presence of *Cichla* fish in this river area. The survey results were conducted in 2021 based on preliminary information from traditional fishing enthusiasts. The identifying juvenile *Cichla* fish presents particular challenges because of the lack of distinctive color patterns, necessitating molecular approaches to improve accuracy and reduce errors in identification in public waters (Mourão et al. 2017). This underscores the value of advanced scientific methods in addressing the complexities of studying invasive species and their potential impacts on aquatic ecosystems.

The aquatic ecosystem in Yogyakarta is very supportive of ornamental fish development. As evidence, ornamental fish hubs are scattered throughout urban areas in Yogyakarta, showcasing a diverse array of species, notably the cichlid (Ika et al. 2021). Among these, the genus *Cichla*, an introduced species, captivates enthusiasts with its vibrant color patterns, making it a sought-after commodity in fishing circles. Initially introduced in Indonesia for ornamental purposes, this African genus has garnered attention across Asia, including Indonesia (Rachmatika and Wahyudewantoro 2006). The primary concern is their invasive potential in public waters, where the predatory nature of *Cichla* species threatens to dominate aquatic habitats, posing a serious risk to native fish populations that may be unable to compete effectively, ultimately leading to their decline (Umar and Sulaiman 2013). The Quarantine and Quality Control Station for Fishery Products further clarified this issue, beginning with assessments in September 2021. However, significant challenges arise in the morphological identification of juvenile fish, complicating the overall evaluation process. This difficulty is not unique to this case. It also applies to other species with distinctive color patterns, such as trout cod (*Maccullochella macquariensis*) (Cuvier, 1829) and Murray cod (*Maccullochella peelii* (Mitchell, 1838)) instances (Lyon et al. 2018).

Previous studies investigating the invasive potential and DNA barcoding of *Cichla* species have been conducted in Malaysia (Khaleel et al. 2020, 2021). DNA barcoding, a molecular identification technique utilizing mitochondrial DNA in the cytochrome C Oxidase subunit I (COI) region, has become the global standard for molecular identification (Hebert et al. 2003). This technique is broadly applicable and has been successfully applied to various organisms, including fish (Lakra et al. 2011). Although DNA barcoding has been widely adopted in numerous fields, its application in invasive fish research in Indonesia remains limited. The *Cichla* species examined in this study are considered invasive because of their strong dominance within their habitat, a trait evidenced by their widespread distribution, including in Indonesia (Sastraprawira et al. 2020a). In line with this, the purpose of this study is molecular-based identification of specific *Cichla* spp accurately. This study provides essential insights that can inform policy measures to manage the spread of invasive fish species in Indonesia's native ecosystems, with a particular focus on the Yogyakarta Province, Indonesia.

MATERIALS AND METHODS

Samples collection

Seven *Cichla* fish samples were procured from the Bedog River in Sleman District, Yogyakarta Province, Indonesia during August and October 2020 and stored in a refrigerator (-10°C) for molecular-based identification. The collection methodology involved using gillnets and traditional fishing gear to mitigate unintended catches of non-target species. The survey took advantage of the optimal river conditions prevalent during these months, characterized by reduced water discharge typical of the dry season in Indonesia. Two distinct locations along the Bedog River, namely the upstream area (7°44'51.462" S - 110°20'14.326" E) and downstream (7°45'17.036" S - 110°20'02.585" E), were investigated with spatial separation of 5 km (Figures 1 and 2).

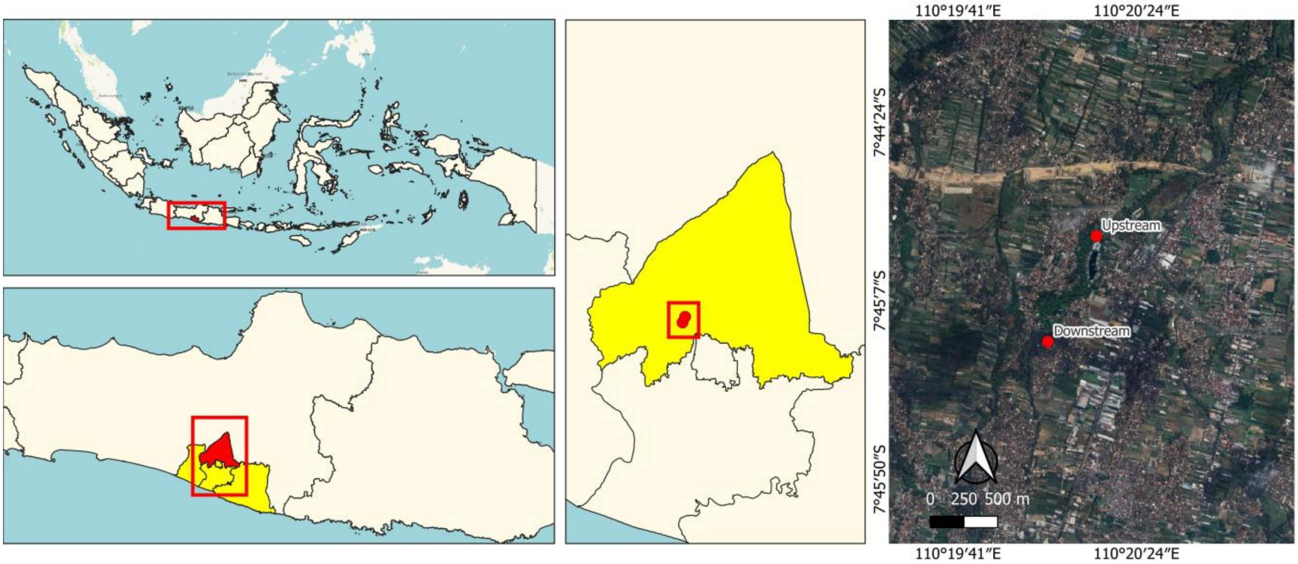

Figure 1. Sample sites of peacock bass (*Cichla ocellaris*) in the Bedog River, Sleman District, Yogyakarta Province, Indonesia. A. Upstream; B. Downstream

Figure 2. Location of: A. Upstream; B. Downstream of the Bedog River, Yogyakarta, Indonesia where *Cichla ocellaris* fish were found

Morphological identification adhered to Food and Agriculture Organisation (FAO) guidelines (Heemstra 1993), with species confirmation achieved through molecular identification utilizing the Cytochrome Oxidase I (COI) gene region. The study did not necessitate specific permits, as the samples were collected from the river in collaboration with local traditional fishermen. All collection operations and experiments are conducted in strict accordance with local laws and relevant laboratory regulations to protect natural resources. Each specimen underwent photographic documentation using a digital camera. The survey for *Cichla* employed a species-specific net as the primary focus. In addition to the fish samples constituting the core of this research, complementary data encompassed measurements related to water quality within the fish habitat. Parameters such as temperature and dissolved oxygen (oxygen meter Lutron DO-5510) and water acidity (hand pH meter accuracy ±0.05 range 0.00-14.00) were assessed to provide comprehensive insights into the ecological context. Water quality measurement followed equipment manuals.

DNA extraction and PCR condition

All the collected specimens were preserved in 90% ethanol to maintain their structural integrity for subsequent laboratory analyses. At the initial stage of genomic DNA extraction, 0.5-g tissue samples from each specimen were aseptically dissected and rinsed with distilled water to remove residual ethanol. The cleaned tissue samples were transferred into microtubes containing 6× lysis buffer and mechanically disrupted using a TissueLyser II instrument (Qiagen, Hilden, Germany). Genomic DNA was extracted using the Accuprep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea), strictly following the manufacturer’s protocol. The DNA concentration was measured using a NanoDrop spectrophotometer (D1000; Thermo Fisher Scientific, Wilmington, DE, USA) and either immediately used for Polymerase Chain Reaction (PCR) applications or stored at -70°C for future analyses.

The COI gene was amplified from DNA samples using a universal primer set previously validated for fish identification: FISH-BCL (5′-`TCA ACY AAT CAY AAA GAT ATY GGC AC`-3′) and FISH-BCH (5′-`TAA ACT TCA GGG TGA CCA AAA AAT CA`-3′) (Baldwin et al. 2011). These primers target the COI region, which is approximately 600 bp long. The PCR reaction mixture (20 µL) contained 11.2 µL of ultra-pure water, 1 µL each of the forward and reverse primers (0.5 µM), 0.2 µL of Ex Taq DNA polymerase (TaKaRa, Kusatsu, Japan), 2 µL of 10× ExTaq Buffer, 2 µL of dNTPs (1 µM, TaKaRa), 0.6 µL of dimethyl sulfoxide, and 2 µL of genomic DNA as the template. The PCR conditions were as follows: initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s; and final extension at 72°C for 5 min. After verifying the desired bands on a 1.5% agarose gel, the PCR products were purified using an AccuPrep Gel Purification Kit (Bioneer).

Data analysis and phylogenetic reconstruction

Sequence alignment was performed using MEGAX software (Kumar et al. 2018), incorporating sequences from the GenBank database for comparative analysis. Raw DNA sequences were trimmed using Chromas software (http://technelysium.com.au/wp/chromas/), and low-quality regions (quality value <20) were removed to ensure sequence integrity. Trimming was applied to both the forward and reverse sequences, and the reverse sequence was reversed and complemented using an online tool (https://www.bioinformatics.org/sms/rev_comp.html) before alignment. Forward and reverse sequences were manually merged to generate an extended sequence, facilitating the accurate identification of the COI region. The combined sequences were aligned using ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalo/) and compared with sequences in the National Center for Biotechnology Information (NCBI) BLASTN database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the species identity. Following species assignment, a phylogenetic tree was constructed using MEGAX to examine the evolutionary relationships. Pairwise evolutionary distances were calculated using the Kimura 2-Parameter method, and a neighbor-joining tree was generated with 1,000 bootstrap replicates to ensure reliability (Kumar et al. 2018). This approach is standard for DNA barcoding and has been previously validated by Collins and Cruickshank (2013). For the outgroup in the phylogenic tree, the DNA sequence of Mozambique tilapia (*Oreochromis mossambicus*

(Peters, 1852)) (GenBank Accession No. MG407408) was included as an outgroup in the phylogenetic analysis (https://www.ncbi.nlm.nih.gov/nuccore/1511211100). This species was chosen due to its belonging to the family Cichlidae and its prevalence in common Indonesian waters.

RESULTS AND DISCUSSION

The laboratory molecular experiment was conducted on May 13, 2022, concurrently with in-situ water quality measurements situated along the Bedog River in Sleman, Yogyakarta. The dataset gathered encompassed water quality metrics from the two designated research locations, as well as peacock bass tissues well preserved by the personnel at the Fish Quarantine and Quality Control Station in Yogyakarta (Figure 3).

Water quality parameters

Based on several water quality parameters, on average, the condition of Bedog River waters is quite supportive of aquatic biota such as fish (Table 1). Despite prior categorizations assigning it to a river exhibiting moderate pollution and low water fertility (Fauzia et al. 2016), previous research, considering the abundance of existing plankton (Anggraini et al. 2016). Insights gleaned from interviews with the local community indicate the continued presence of indigenous fish species such as cyprinid fish (*Rasbora* spp.), Nile tilapia (*O. niloticus* (Linnaeus, 1758)), Mozambique tilapia (*O. mossambicus*), and other varieties in the Bedog River. Meanwhile, the habitat condition of the *Cichla* spp. fish in the same river remains notably favorable, characterized by normal water quality parameters (Figure 2).

Molecular identification

After achieving a uniform length for phylogenetic reconstruction, all collected samples underwent successful amplification, resulting in DNA sequences within the COI region, averaging 600 bp. Gel electrophoresis of the PCR products revealed that the target DNA fragment ranged from 500 to 600 bp, and was subsequently sent for sequencing (Figure 4). Using the open-source software ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalo/), we aligned the DNA sequences of the COI region with the COI reference sequence NC030272, obtaining lengths between 616 and 680 bp. Following alignment with other DNA sequences belonging to the genus *Cichla*, phylogenetic trees were constructed, including calculations of genetic distances, as detailed in Table 3, using MEGAX (Kumar et al. 2018). The identified species for the fish sample was confirmed as *C. ocellaris* after alignment to the reference using BLAST on the NCBI Genbank database and exhibited close to 100% identity for all sequences (Table 2).

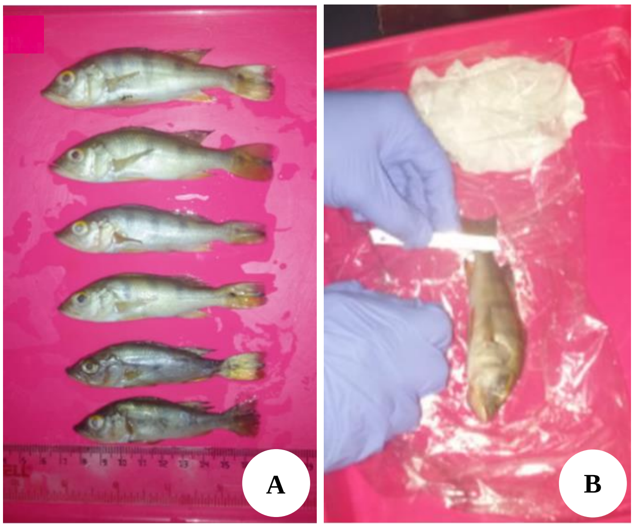

Figure 3. Specimens of this investigation: A. Six out of seven samples of peacock bass (*Cichla ocellaris*) collected from the Bedog River, Sleman Yogyakarta; B. Laboratory activities for morphological measurement

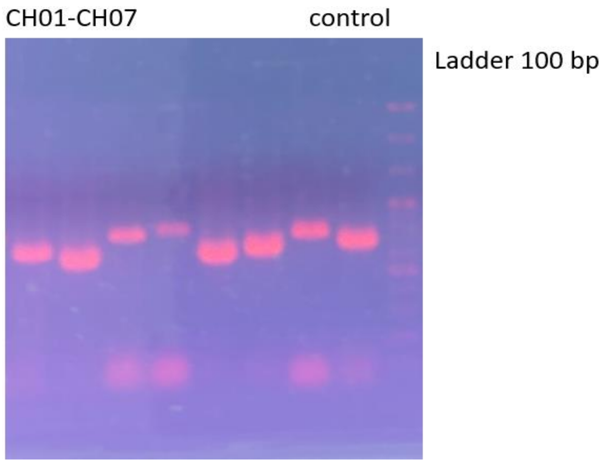

Figure 4. Gel electrophoresis results on PCR product for all samples of *Cichla ocellaris*

Table 1. Water quality data at the two survey locations of *Cichla ocellaris* on the upstream and gentle Bedog River, Yogyakarta, Indonesia

Coordinate location	Average of temperature (°C)	Average of DO (mg/L)	Average of pH value
Upstream; 7°45'17.036" S, 110°20'02.585" E	$28.76^a \pm 0.47$	$7.80^a \pm 0.20$	$6.74^a \pm 0.11$
Downstream; 7°44'51.462" S, 110°20'14.326" E	$28.33^a \pm 0.15$	$8.00^a \pm 0.00$	$7.00^a \pm 0.01$

Table 2. BLASTN DNA sequence results online on the NCBI database of *Cichla ocellaris*

Sample ID	Species name	% Identity	Reference GenBank Acc No.	Sequence length (bp)	GenBank Acc No.
CH01	*C. ocellaris*	100	NC030272*	668	PP999305
CH02	*C. ocellaris*	100	NC030272*	616	PP999306
CH03	*C. ocellaris*	100	NC030272*	680	PP999307
CH04	*C. ocellaris*	99.68	NC030272*	620	PP999308
CH05	*C. ocellaris*	100	NC030272*	668	PP999309
CH06	*C. ocellaris*	100	NC030272*	609	PP999310
CH07	*C. ocellaris*	100	NC030272*	635	PP999311

Note: *: Lin et al. (2017)

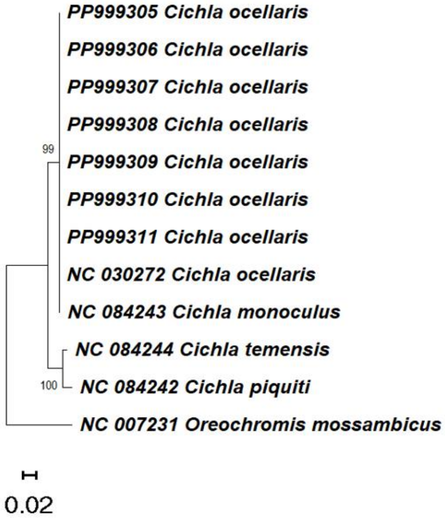

Figure 5. The phylogenetic tree reconstruction of *Cichla ocellaris* from the Bedog River, Yogyakarta. Genbank Accessing number PP99305-PP99311 is a sample of this research

All the obtained the DNA sequence of the cytochrome c oxidase subunit I region with a length of 616-680 bp by aligning with the COI reference sequence NC030272, which has undergone comprehensive mapping of its mitochondrial DNA characteristics (Lin et al. 2017). The BLASTN analysis conducted using the GenBank NCBI database revealed similarities to another species, specifically *Cichla temensis* (Humboldt, 1821), found in the Amazon waters (Willis et al. 2015; Coffill-Rivera et al. 2022) identified by the GenBank Accession Number FJ440622 (Ardura et al. 2010). However, considering additional parameters, such as query coverage and scores, *C. ocellaris* (Bloch & Schneider, 1801) emerged as a more valid sequence identity in our research context. Phylogenetic tree analysis further underscored the similarity between *C. ocellaris* and *C. temensis*, as evidenced by clustering all samples within the same clade. Additionally, the phylogenetic tree illustrates the kinship of *C. ocellaris* with both *C. temensis* and *C. monoculus* (Agassiz, 1831). The COI region, often called the "barcode" region, has become the global standard for molecular identification. This designation is based on extensive records of the COI database in GenBank and its various advantages for DNA barcoding (Hebert et al. 2003; Hubert and Hanner 2015; Antil et al. 2023).

The genetic distance results determined using MEGAX software underscored the distinctiveness of *C. ocellaris* from other *Cichla* species. All samples in this study exhibited a genetic distance of zero compared to samples of the same species from China, as identified by GenBank numbers KU878410 and NC030272 (Table 3). However, *C. temensis* and *C. monoculus* also manifested relatively close genetic proximity to *C. ocellaris*, registering values of 0.0000 and 0.0019, respectively. In tandem with reinforcing the disparities in their morphological characteristics, a detailed exploration of their genetic traits was also undertaken through comparative analysis. This exploration emphasizes the necessity of subjecting these two species to testing across other gene regions that can differentiate them genetically.

Discussion

Since 2016, the Technical Implementation Units of the Fish Quarantine Center under the Ministry of Maritime Affairs and Fisheries have diligently mapped potentially hazardous invasive fish species across Indonesia. This undertaking reflects a concerted government effort to regulate the proliferation of prohibited invasive foreign fish that, despite restrictions, persistently find their way into widespread trade, cultivation, and maintenance for various community purposes. This regulatory initiative is crucial for curtailing the rampant introduction of invasive fish into public waters and ensuring the sustainable management of fishery resources. The control of dangerous and harmful invasive fish dates back to 1982, initially through the Minister of Agriculture's Decree and later updated by PERMEN KP No. 19/2020. However, over more than four decades, an increasing number of prohibited invasive fish species have circulated in the community (Haryono et al. 2016), primarily facilitated by the ornamental fish industry and recreational fishing (Achmad et al. 2018, 2020). This was the first stage of obtaining results on the presence of invasive fish in river waters. The results of this study may serve as a basis for regulating the spread of Cichla fish species in Indonesia.

Introducing invasive fish into typical aquatic environments in Yogyakarta harms local ecosystems. A compelling case is the proliferation of the red devil fish (*Amphilophus labiatus* (Günther, 1864) and *Amphilophus citrinellus* (Günther, 1864)) in the Sermo Kulonprogo Reservoir. The dominance of this fish suppresses native fish populations, with the red devil fish comprising more than 70% of every fishing effort (Djumanto et al. 2017).

Another example involves the frequent release of tilapia, which are suspected to have contributed to the outbreak of the tilapia lake virus. This virus can mutate and infect native fish, including gurami (*Osphronemus gouramy* (Lacepède, 1801)) (DKP Kab Gunungkidul 2019). Invasive fish species, such as the flower horn (*Cichlasoma trimaculatum* (Günther, 1867)), alligator gar (*Atractosteus* spp.), jaguar cichlid (*Parachromis managuensis* (Günther, 1867)), and *Polypterus senegalus* (Cuvier, 1829) have been introduced to various common aquatic environments in Yogyakarta (Akmal et al. 2022). Notably, the damage inflicted by louhan fish has been recorded. It has expanded its invasion area by 6 km/year in Lake Matano, South Sulawesi (Sentosa and Hedianto 2020) with concerns that a similar scenario might be unfolding in various common waters in Yogyakarta. Moreover, the outbreak of a peacock bass population in Malaysia, with its potential to hybridize between species, poses a threat to native fish populations in common aquatic environments. These omnivorous fish tend to prey on smaller fish, including their own species (Sastraprawira et al. 2020a). This evidence challenges the prevailing myth among hobbyists, dispelling the notion that the invasiveness of fish species diminishes after domestication, a misconception often conveyed by users of quarantine services seeking certification for the trafficking of invasive fish.

Community activities play a pivotal role in driving widespread circulation and introduction of invasive fish into common aquatic environments. An annual study conducted by BKIPM Yogyakarta demonstrated that community perceptions regarding the release of invasive fish are intricately linked to levels of community knowledge, awareness, and responsibility (Achmad et al. 2020). The efficacy of legal instruments to prevent invasive fish's release is a critical determinant. The absence of local regulations addressing this matter poses challenges for law enforcement officers in ascertaining the legal status of community actions. Moreover, the increased economic value associated with various invasive fish species, particularly those prominent in the ornamental fish industry, significantly influences public awareness. Economic interests frequently supersede concerns regarding the potential harm posed by the maintenance of invasive fish. Additionally, the prevalence of various cultural and religious rituals contributes significantly to the elevated instances of invasive fish release, particularly when adequate support and outreach efforts are not directed toward these communities (Maixenchs et al. 2019).

Hence, the prohibition of invasive fish trafficking represents an initial measure envisaged to be efficacious at curtailing the potential for the community's irresponsible ownership and utilization of such species. However, the quarantine identification process, a pivotal component in inspecting fish commodities slated for trafficking, poses an enduring challenge for enforcement officers. This complexity arises from the sheer multitude of fish species—152 initially, as stipulated in PERMEN KP No. 41/2014, later revised to 76 in PERMEN KP No. 19/2020—whose morphological characteristics necessitate a comprehensive understanding. Fish health certificates, essential for trade, rely predominantly on external morphological observations, leading to potential errors, especially for species prone to hybridization. Consequently, certifications may be erroneously obtained for hybrid fish originating from prohibited parental species. Health certificates for fish entering Yogyakarta often list the Latin name of the fish, with only the genus classification, neglecting to specify the species. This oversight allows for the trafficking of fish, such as louhan fish (*C. trimaculatum*), for which prohibited species may exist within the genus. These practices contravene the precautionary principles of the international biosecurity system. Despite international compliance, this circumstance persists because of the absence of clear regulations mandating the genetic examination of fish species. The practice of "legalizing" the transport of fish suspected to carry genes of invasive species prohibited by laws and regulations poses a potential threat to natural ecosystems in destinations such as Yogyakarta. Furthermore, the absence of robust rules and regulations concerning the control and monitoring of invasive fish, coupled with a lack of punitive actions against violations, transforms each invasive fish keeper into a potential propagule, significantly increasing the risk of unintentional release (Achmad et al. 2020).

As a competent authority tasked with controlling and preventing the trafficking of prohibited invasive fish, the Fish Quarantine Department must possess the capability to conduct identification with a maximum success rate. This proficiency is essential to prevent the escape of prohibited commodities and to provide assurance regarding the accuracy of certified products, thereby mitigating potential disputes in trade relations. Consequently, it is imperative to adopt appropriate, accurate, and continually updated identification methods. Incorporating genetics-based identification methods, such as DNA barcoding, immunology, and protein analysis, alongside the conventional routinely used methods is crucial. The precision of the identification results benefits service users by ensuring that fish, especially hybrids intended for certification, are not mistakenly identified as prohibited invasive species, and aligns with the precautionary principle of fish quarantine, which advocates postponing shipments if indications suggest the presence of prohibited fish until conclusive results are obtained. In addition, the accuracy of fish identification provides buyers with assurance by acting as a deterrent against fraudulent practices by sellers.

In the present study, we successfully performed identification using molecular approaches in a noteworthy case involving *Cichla* fish. Specifically, we identified seven *Cichla* fish samples, and the molecular analysis results uniformly affirmed their classification as the species *C. ocelaris* (Table 2). *C. ocelaris* is an ornamental fish traded extensively in Yogyakarta and other regions. Based on our observations in the Bedog River, environmental conditions in river waters are ideal for developing cichlid fish species, including *C. ocellaris* (Table 1). These conditions correspond to the habitat of *Cichla* sp. in its natural habitat. In tropical regions like Indonesia, *Cichla* sp. can breed in temperature conditions of 23-28°C, at neutral pH up to 7.8, and live in aquatic with dissolved oxygen concentrations in the range of 5 mg/L (Espínola et al. 2014; Franco et al. 2018). Noteworthy for its appealing traits, including distinct color patterns and vibrant orange anal fins, this species generates enthusiasm, making it a popular choice for fishing tourism. The presence of *C. ocelaris* in public waters is attributed to its exceptional adaptability and the potential release of fry from *Cichla* fishing areas. However, this raises concerns regarding the possible extinction of indigenous species in these waters. Before it evolves into an invasive species, introducing this fish demands earnest attention from all sectors of society. The results of this identification process serve as a foundation for formulating policies regarding the dissemination of introduced fish and other potentially invasive species. Ambiguities arising from species determination can be effectively resolved by implementing a combined approach involving morphological and molecular analyses, as exemplified by the present findings.

The presence of introduced fish can be an indication that needs significant attention. Introduced fish should not be widely developed in public waters, such as rivers and lakes. This study identified fish species using both morphological and molecular approaches. The identified species, *C. ocellaris*, has successfully adapted to the Bedog River in Yogyakarta, raising concerns about its potential negative impacts on native fish populations. Water quality at this location is ideal for tropical fish, including cichlids, which were the focus of this study. Suitable habitat conditions, such as appropriate temperature, water quality, and depth, enable *C. ocellaris* to become invasive (Espínola et al. 2010). Despite its known potential impacts, the intentional release of this species into the wild for recreational or other purposes is common in tropical regions, including Indonesia, which have similar water conditions to the native habitat of (Willis et al. 2012, 2015). Several cases of fish introduction have been previously reported. In Lake Poso, Sulawesi, the cichlid fish *Melanochromis auratus* (Boulenger, 1897) grew and dominated the freshwater area. The fish was first reported in 2012 and has grown significantly in the waters of Lake Poso (Herder et al. 2022). Another report described a type of cichlid, *Andinoacara rivulatus* (Günther, 1860) found in the waters of the Brantas River (Serdiati et al. 2020). The *C. ocellaris* type was introduced to Malaysia in the 1990s and has even been released almost to spread in Peninsular Malaysia (Rahim et al. 2013). The *C. ocellaris* is a native of the South American fish species, which is a predatory fish and wide variety of prey, it can potentially be dangerous for the native fish in a particular location (Saba et al. 2020). The introduction of foreign fish into Indonesia has been around for quite a long time (Herder et al. 2022). Meanwhile, *C. ocellaris* in Indonesia has been identified as a potential ornamental and sport fish. Developments in fishing hobbyists state that many types of *C. ocellaris* have been found in the Sleman area, Yogyakarta (Achmad and Novianto 2019).

Further research on the DNA sequences of non-native fish in Indonesia is required. Several studies have involved collaboration with foreign researchers who have reported the discovery of non-native fish species in public waters, such as rivers, lakes, and aquaculture commodities. However, releases in the wild cause many species to develop hybrids, which creates bias if identification is based only on color patterns and morphological characteristics. Research on the existence of potential hybrids in the *Cichla* genus has been conducted, and the distribution of these fish species is quite broad. Genetic distance analysis of *C. ocelaris* revealed distinctions from the outgroup *O. mossambicus*, with a recorded distance of 0.1659. Conversely, the closest affinities are observed with species within the same genus, particularly *C. temensis* (0.0000) and *C. monoculus* (0.0019). We posit the presence of a hybridization event between the *C. ocellaris* and *C. temensis* species, resulting in a zero genetic distance. However, those displaying a low genetic distance maintained only slight differences. The occurrence of hybridization in nature is plausible because of the shared genus of the two species, which facilitates natural or unprogrammed breeding. This process may occur during the joint maintenance of *C. ocellaris* and *C. temensis* within a single-rearing container by ornamental fish hobbyists. This is also the case with *Oreochromis* (Bbole et al. 2014; Deines et al. 2014) and *Clarias* (Duong et al. 2017, 2022) species in captivity and the wild.

In the Asia-Pacific region, Cichla species are found in Malaysia, Indonesia, and Guam, with the dominant species being *C. ocellaris* (Sastraprawira et al. 2020a). In this study, morphological identification was performed by observing the fundamental pattern similarity on the lateral side, even though this species is very close to *C. temensis* based on the phylogenetic tree (Figure 5). In addition, the anal and ventral fins have a bright orange color that is quite striking, and can be used to differentiate from other fish species in this genus (De Campos et al. 2015). The findings for the introduced fish *C. ocellaris* in public waters should be applied to different types of introduced fish in Indonesia, which also pose a threat to local habitats. Our molecular identification results showed high similarity, based on genetic distance (Table 3), between *C. ocellaris*, *C. temensis*, and *C. monoculus*; therefore, morphological characteristics are also needed to identify the species. It is likely that *C. ocellaris* was introduced into Indonesia before the new regulations were implemented. However, *C. monoculus* are included on the list of fish that have been banned from entering Indonesia since 2014 based on the Indonesian Ministry of Marine Affairs and Fisheries Regulation Number 41/PERMEN-KP/2014 regarding the prohibition of the entry of dangerous fish species from abroad into the territory of the Republic of Indonesia.

The documented presence of *Arapaima gigas* (Schinz, 1822) (Fadjar et al. 2019) and *Atractosteus spatula* (Lacepède, 1803) (Hasan et al. 2020) in river waters in East Java, underscores the vulnerabilities inherent in the invasive fish contract system in Indonesia. These species, originally intended as ornamental fish, have been identified in various regions across Indonesia and pose significant threats to native fish populations. Consequently, they have been included in the lists of freshwater fish prohibited from entering Indonesia (Andriyono and Fitrani 2021). Through concerted efforts from all stakeholders, we aspire to increase the attention paid to research on invasive fish, leading to robust law enforcement mechanisms that effectively prohibit the entry of potentially invasive fish species. Such measures are imperative for preserving biodiversity in Indonesia. The presence of *C. ocellaris* in the Bedog River of Yogyakarta provides evidence that non-native fish have been introduced into Indonesian waters. The negative impacts of *C. ocellaris* as an invasive species have been documented by several researchers (Pelicice and Agostinho 2009; Vitule et al. 2012), and this issue warrants continued attention beyond the Yogyakarta area. This fish, which is widely distributed throughout Indonesia (Sastraprawira et al. 2020), is popular as an ornamental fish and a target for recreational fishing because of its attractive coloration in adulthood. However, in the juvenile stage, its color pattern does not differ significantly from other cichlid species, making morphological identification challenging. The molecular identification results from this study can supplement traditional identification methods. Additionally, the advantages of DNA barcoding can be leveraged to collect genetic information on genetic resources across various regions not only in the COI gene region, including Cytochrom-b (Cyt-b), control region and so on (Breman et al. 2016; Tadmor-Levi et al. 2022). DNA barcoding plays a crucial role, especially as traditional taxonomy becomes less popular among the current generation (Hubert and Hanner 2015).

In conclusion, this study successfully identified seven samples of *C. ocellaris* from the Bedog River in Yogyakarta, confirming the presence of non-native fish with the potential to become invasive species. All the DNA sequences obtained in this study were submitted to GenBank. The need for regular monitoring of public waters in Indonesia is underscored by the distribution of introduced fish, particularly those with invasive potential. It is crucial to promote comprehensive studies on invasive fish in Indonesia, raise awareness to prevent their release into public waters, and mitigate their adverse impacts on society and Indigenous fish biodiversity in the country.

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REFERENCES

Achmad H, Martini L, Wibowo SA, Nugroho L. 2018. Sebaran, potensi, introduksi, dan pengendalian ikan berbahaya/invasif dan berpotensi invasif di Yogyakarta dan sekitarnya. Jurnal Penelitian Perikanan Indonesia 24: 61-72. DOI: 10.15578/jppi.1.1.2018.61-72. [Indonesian]

Achmad H, Novianto H. 2019. Sebaran dan potensi pelepasliaran ikan asing melalui rekreasi memancing dan upaya pengendaliannya di Yogyakarta. Prosiding Seminar Nasional III Pascasarjana UNS. [Indonesian]

Achmad H, Wibowo SA, Rahman H. 2020. Peningkatan persepsi positif publik dalam pencegahan pelepasliaran ikan asing invasif di Yogyakarta dan sekitarnya. Quarantamina 2 (1): 13-32. [Indonesian]

Akmal SG, Yonvitner, Jerikho R, Yulianda F, Wardiatno Y, Novák J, Kalous L, Slavík O, Patoka J. 2022. Culture, trade and establishment of *Polypterus senegalus* in Indonesia with first record of wild populations. Aquac Environ Interact 14: 127-133. DOI: 10.3354/aei00433.

Andriyono S, Fitrani M. 2021. Non-native species existence and its potency to be invasive species on freshwater ecosystem in East Java province, Indonesia. Egypt J Aquat Biol Fish 25: 1013-1024. DOI: 10.21608/ejabf.2021.170621.

Anggraini A, Sudarsono S, Sukiya S. 2016. Kelimpahan dan tingkat kesuburan plankton di Perairan Sungai Bedog. Kingdom J Biol Stud 5 (6): 1-9. DOI: 10.21831/kingdom.v5i6.5923. [Indonesian]

Antil S, Abraham JS, Sripoorna S, Maurya S, Dagar J, Makhija S, Bhagat P, Gupta R, Sood U, Lal R, Toteja R. 2023. DNA barcoding, an effective tool for species identification: A review. Mol Biol Rep 50 (1): 761-775. DOI: 10.1007/s11033-022-08015-7.

Ardura A, Linde AR, Moreira JC, Garcia-Vazquez E. 2010. DNA barcoding for conservation and management of Amazonian commercial fish. Biol Conserv 143: 1438-1443. DOI: 10.1016/j.biocon.2010.03.019.

Baldwin CC, Castillo CI, Weigt LA, Victor BC. 2011. Seven new species within western Atlantic *Starksia atlantica, S. lepicoelia*, and *S. sluiteri* (Teleostei, Labrisomidae), with comments on congruence of DNA barcodes and species. ZooKeys 3: 21-72. DOI: 10.3897/zookeys.79.1045.

Bbole I, Katongo C, Deines AM, Shumba O, Lodge DM. 2014. Hybridization between non-indigenous *Oreochromis niloticus* and native *Oreochromis* species in the lower Kafue River and its potential impacts on fishery. J Ecol Nat Environ 6 (6): 215-225. DOI: 10.5897/jene2014.0444.

Breman FC, Loix S, Jordaens K, Snoeks J, Van Steenberge M. 2016. Testing the potential of DNA barcoding in vertebrate radiations: The case of the littoral cichlids (Pisces, Perciformes, Cichlidae) from Lake Tanganyika. Mol Ecol Resour 16 (6): 1455-1464. DOI: 10.1111/1755-0998.12523.

Coffill-Rivera ME, Neal JW, Allen PJ. 2022. Effects of temperature on growth of juvenile speckled peacock bass *Cichla temensis*. N Am J Aquac 84 (4): 486-492. DOI: 10.1002/naaq.10262.

Collins RA, Cruickshank RH. 2013. The seven deadly sins of DNA barcoding. Mol Ecol Resour 13 (6): 969-975. DOI: 10.1111/1755-0998.12046.

De Campos CP, de Carvalho Freitas CE, Amadio S. 2015. Growth of the *Cichla temensis* Humboldt, 1821 (Perciformes: Cichlidae) from the middle rio Negro, Amazonas, Brazil. Neotrop Ichthyol 13 (2): 413-420. DOI: 10.1590/1982-0224-20140090.

Deines AM, Bbole I, Katongo C, Feder JL, Lodge DM. 2014. Hybridisation between native *Oreochromis* species and introduced Nile tilapia *O. niloticus* in the Kafue River, Zambia. Afr J Aquat Sci 39 (1): 23-34. DOI: 10.2989/16085914.2013.864965.

Djumanto D, Pranoto BE, Diani VS, Setyobudi E. 2017. Makanan dan pertumbuhan ikan Bandeng, *Chanos chanos* (Forsskål, 1775) tebaran di Waduk Sermo, Kulon Progo. J Iktiologi Indonesia 17 (1): 83-100. DOI: 10.32491/jii.v17i1.306. [Indonesian]

DKP Kab Gunungkidul. 2019. Waspadalah Terhadap TILV (Tilapia Lake Virus). https://dkp.gunungkidulkab.go.id/berita-289/waspadalah-terhadap-tilv-tilapia-lake-virus.html). [Indonesian]

Duong T-Y, Nguyen N-TT, Nguyen TT, Huynh LTT, Bui TM, Pham LT, Dunham R. 2022. Growth and survival of crossbreeds between wild and domestic strains of Mekong Delta bighead catfish (*Clarias macrocephalus* Günther, 1864) cultured in tanks. Aquaculture 547: 737507. DOI: 10.1016/j.aquaculture.2021.737507.

Duong T-Y, Nguyen T-T, Pham T-L. 2017. Morphological differentiation among cultured and wild *Clarias macrocephalus, C. macrocephalus* x *C. gariepinus* hybrids, and their parental species in the Mekong delta, Viet Nam. Intl J Fish Aquat Stud 5 (1): 233-240.

Espínola LA, Minte-Vera CV, Júlio Jr HF. 2010. Invasibility of reservoirs in the Paraná Basin, Brazil, to *Cichla kelberi* Kullander and Ferreira, 2006. Biol Invasions 12: 1873-1888. DOI: 10.1007/s10530-009-9598-x.

Espínola LA, Minte-Vera CV, Junior HFJ, Santos LN, Winemiller KO. 2014. Evaluation of factors associated with dynamics of *Cichla ocellaris* invasion of the Upper Paraná River floodplain system, Brazil. Mar Freshw Res 66 (1): 33-40. DOI: 10.1071/MF13105.

Fadjar M, Islamy RA, Herawati EY. 2019. First record of *Arapaima gigas* (Schinz, 1822) (Teleostei: Osteoglossomorpha), in the Brantas River, Sidoarjo, East Java, Indonesia. Biodiversitas 20 (12): 3527-3531. DOI: 10.13057/biodiv/d201209.

Fauzia AZ, Suhartini S, Sudarsono S. 2016. Kualitas perairan di Sungai Bedog, Yogyakarta berdasarkan keanekaragaman plankton. Jurnal Biologi 5 (6): 50-61. [Indonesian]

Franco ACS, dos Santos LN, Petry AC, García-Berthou E. 2018. Abundance of invasive peacock bass increases with water residence time of reservoirs in southeastern Brazil. Hydrobiologia 817: 155-166. DOI: 10.1007/s10750-017-3467-x.

Harper GA, Bunbury N. 2015. Invasive rats on tropical islands: Their population biology and impacts on native species. Glob Ecol Conserv 3: 607-627. DOI: 10.1016/j.gecco.2015.02.010.

Haryono, Wahyudewantoro G, Walidi W, Tani DD, Anggraeni Y, Arta AP, Supardan A. 2016. Jenis Ikan Invasif, Ancaman dan Pengendaliannya. Bidang Keamanan Hayati Ikan, Pusat Karantina dan Keamanan Hayati Ikan, Kementerian Kelautan dan Perikanan, Bekerjasama dengan Pusat Penelitian Biologi-LIPI, Jakarta. [Indonesian]

Hasan V, Widodo MS, Islamy RA, Pebriani DAA. 2020. New records of Alligator gar, *Atractosteus spatula* (Actinopterygii: Lepisosteiformes: Lepisosteidae) from Bali and Java, Indonesia. Acta Ichthyol Piscat 50 (2): 233-236. DOI: 10.3750/AIEP/02954.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc Biol Sci 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.

Heemstra PC. 1993. Groupers of the World (Family Serranidae, Subfamily Epinephelinae). An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper and Lyretail Species Known to Date. FAO Species Catalogue 16, FAO, Rome, Italy.

Herder F, Möhring J, Flury JM, Utama IV, Wantania L, Wowor D, Boneka FB, Stelbrink B, Hilgers L, Schwarzer J, Pfaender J. 2022. More non-native fish species than natives, and an invasion of Malawi cichlids, in ancient Lake Poso, Sulawesi, Indonesia. Aquat Invasions 17 (1): 72-91. DOI: 10.3391/ai.2022.17.1.05.

Herder F, Schliewen UK, Geiger MF, Hadiaty RK, Gray SM, McKinnon JS, Walter RP, Pfaender J. 2012. Alien invasion in Wallace's Dreamponds: Records of the hybridogenic" flowerhorn" cichlid in Lake Matano, with an annotated checklist of fish species introduced to the Malili Lakes system in Sulawesi. Aquat Invasions 7 (4): 521-535. DOI: 10.3391/ai.2012.7.4.009.

Hubert N, Hanner R. 2015. DNA barcoding, species delineation and taxonomy: A historical perspective. DNA Barcodes 3 (1): 44-58. DOI: 10.1515/dna-2015-0006.

Ika SR, Syamsiro M, Mulyono A. 2021. Penerapan teknologi pembuatan pakan untuk pemberdayaan kelompok peternak ikan hias di kota Yogyakarta. In: Seminar Nasional Karya Pengabdian. [Indonesian]

Khaleel AG, Ismail N, Ahmad-Syazni K. 2021. Introduction of invasive peacock bass (*Cichla* spp.), its rapid distribution and future impact on freshwater ecosystem in Malaysia. Croat J Fish 79 (1): 33-46. DOI: 10.2478/cjf-2021-0004.

Khaleel AG, Nasir SAM, Ismail N, Ahmad-Syazni K. 2020. Origin of invasive fish species, peacock bass *Cichla* species in Lake Telabak Malaysia revealed by mitochondrial DNA barcoding. Egypt J Aquat Biol Fish 24 (3): 311-322. DOI: 10.21608/ejabf.2020.92251.

Kiruba-Sankar R, Raj JP, Saravanan K, Kumar KL, Angel JRJ, Velmurugan A, Roy SD. 2018. Invasive species in freshwater ecosystems-Threats to ecosystem services. In: Sivaperuman C, Velmurugan A, Singh AK, Jaisankar I (eds). Biodiversity and Climate Change Adaptation in Tropical Islands. Academic Press, London, UK. DOI: 10.1016/B978-0-12-813064-3.00009-0.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. J Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.

Lakra WS, Verma MS, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh KV, Ward RD, Hebert P. 2011. DNA barcoding Indian marine fishes. Mol Ecol Resour 11 (1): 60-71. DOI: 10.1111/j.1755-0998.2010.02894.x.

Lin M, Mu X, Yan X. 2017. The complete mitochondrial genome of *Cichla ocellaris*. Mitochondrial DNA B Resour 2 (2): 620-622. DOI: 10.1080/23802359.2017.1372713.

Lyon JP, Tonkin Z, Moloney PD, Todd C, Nicol S. 2018. Conservation implications of angler misidentification of an endangered fish. Aquat Conserv: Mar Freshw Ecosyst 28: 1396-1402. DOI: 10.1002/aqc.2980.

Maixenchs M, Anselmo R, Pérez GM, Oruko K, Agnandji ST, Minsoko PCA, Diarra K, Djiteye M, Bhutta ZA, Zaidi S, Carrilho C, Sanz A, Ordi J, Menendez C, Bassat Q, Munguambe K. 2019. Socio-anthropological methods to study the feasibility and acceptability of the minimally invasive autopsy from the perspective of local communities: Lessons learnt from a large multi-centre study. Glob Health Action 12: 1559496. DOI: 10.1080/16549716.2018.1559496.

Mourão AAF, Freitas-Souza D, Hashimoto DT, Ferreira DC, do Prado FD, Silveira RV, Foresti F, Porto-Foresti F. 2017. Molecular and morphological approaches for species delimitation and hybridization investigations of two *Cichla* species. Iheringia. Sér Zool 107: e2017016. DOI: 10.1590/1678-4766e2017016.

Pelicice FM, Agostinho AA. 2009. Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. Biol Invasions 11: 1789-1801. DOI: 10.1007/s10530-008-9358-3.

Rachmatika I, Wahyudewantoro G. 2006. Jenis-jenis ikan introduksi di perairan tawar Jawa Barat dan Banten: Catatan tentang taksonomi dan distribusinya. Jurnal Iktiologi Indonesia 6 (2): 93-97. DOI: 10.32491/jii.v6i2.225. [Indonesian]

Rahim KAA, Esa Y, Arshad A. 2013. The influence of alien fish species on native fish community structure in Malaysian waters. Kuroshio Sci 7 (1): 81-93.

Saba AO, Ismail A, Zulkifli SZ, Shohaimi S, Jamil NR, Nawi NM, Ghani IFA, Halim MRA, Amal MNA. 2020. Checklists, production trends, and potential ecological and socioeconomic impacts of non-native freshwater fishes in Malaysia: A review. Aquat Invasions 15 (4): 646-670. DOI: 10.3391/ai.2020.15.4.07.

Sastraprawira SM, Razak IHA, Shahimi S, Pati S, Edinur HA, John AB, Ahmad A, Kumaran JV, Martin MB, Chong JL, Chowdhury AJK, Nelson BR. 2020. A review on introduced *Cichla* spp. and emerging concerns. Heliyon 6: e05370. DOI: 10.1016/j.heliyon.2020.e05370.

Sentosa AA, Hedianto DA. 2020. Gillnets selectivity and effectivity for controlling invasive fish species in Lake Matano, South Sulawesi. IOP Conf Ser: Earth Environ Sci 535: 012039. DOI: 10.1088/1755-1315/535/1/012039.

Serdiati N, Yonarta D, Pratama FS, Faqih AR, Valen FS, Tamam MB, Hamzah YIG, Hasan V. 2020. *Andinoacara rivulatus* (Perciformes: Cichlidae), an introduced exotic fish in the upstream of Brantas River, Indonesia. AACL Bioflux 13: 137-141.

Tadmor-Levi R, Cummings D, Borovski T, Shapira R, Marcos-Hadad E, David L. 2022. A method for quick and efficient identification of cichlid species by high resolution DNA melting analysis of minibarcodes. Front Ecol Evol 10: 1010838. DOI: 10.3389/fevo.2022.1010838.

Tarkan AS, Vilizzi L, Top N, Ekmekçi FG, Stebbing PD, Copp GH. 2017. Identification of potentially invasive freshwater fishes, including translocated species, in Turkey using the Aquatic Species Invasiveness Screening Kit (AS-ISK). Intl Rev Hydrobiol 102 (1-2): 47-56. DOI: 10.1002/iroh.201601877.

Umar C, Sulaiman PS. 2013. Status introduksi ikan dan strategi pelaksanaan secara berkelanjutan di perairan umum daratan di Indonesia. Jurnal Kebijakan Perikanan Indonesia 5: 113-120. DOI: 10.15578/jkpi.5.2.2013.113-120. [Indonesian]

Vitule JRS, Skóra F, Abilhoa V. 2012. Homogenization of freshwater fish faunas after the elimination of a natural barrier by a dam in Neotropics. Divers Distrib 18 (2): 111-120. DOI: 10.1111/j.1472-4642.2011.00821.x.

Willis SC, Macrander J, Farias IP, Ortí G. 2012. Simultaneous delimitation of species and quantification of interspecific hybridization in Amazonian peacock cichlids (genus *Cichla*) using multi-locus data. BMC Evol Biol 12: 96. DOI: 10.1186/1471-2148-12-96.

Willis SC, Winemiller KO, Montaña CG, Macrander J, Reiss P, Farias IP, Ortí G. 2015. Population genetics of the speckled peacock bass (*Cichla temensis*), South America's most important inland sport fishery. Conserv Genet 16: 1345-1357. DOI: 10.1007/s10592-015-0744-y.