



Morphometric and genetic diversity of *Rasbora* several species from farmed and wild stocks

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Abstract

The morphology and genetic identification of *Rasbora lateristriata* and *Rasbora argyrotaenia* between cultivated and wild populations has never been reported. This study compares morphology and cytochrome c oxidase (COI) genes between farmed and wild stock *Rasbora* spp. in Java and Sumatra island, Indonesia. We analyzed the truss network measurement (TNM) characters of 80 fish using discriminant function analysis statistical tests. DNA was extracted from muscle tissue of 24 fish specimens, which was then followed by polymerase chain reaction, sequencing, phylogenetic analysis, fixation index analysis, and statistical analysis of haplotype networks. Basic Local Alignment Search Tool analysis validated the following species: *R. lateristriata* and *R. argyrotaenia* from farming (Jogjakarta); *Rasbora argyrotaenia* (Purworejo), *R. lateristriata* (Purworejo and Malang), *Rasbora dusonensis* (Palembang), and *Rasbora einthovenii* (Riau) from natural resources. Based on TNM characters, *Rasbora* spp. were divided into four groups, referring to four distinct characters in the middle of the body. The phylogenetic tree is divided into five clades. The genetic distance between *R. argyrotaenia* (Jogjakarta) and *R. lateristriata* (Malang) populations (0.66) was significantly different ($p < 0.05$). *R. lateristriata* (Purworejo) has the highest nucleotide diversity (0.43). *R. argyrotaenia* from Jogjakarta and Purworejo shared the same haplotype. The pattern of gene flow among them results from the two populations' close geographic proximity and environmental effects. *R. argyrotaenia* had low genetic diversity, therefore, increasing heterozygosity in cultivated populations is necessary to avoid inbreeding. Otherwise, *R. lateristriata* (Purworejo) had a greater gene variety that could be used to develop breeding. In conclusion, the middle body parts are a distinguishing morphometric character of *Rasbora* spp., and the COI gene is more heterozygous in the wild population than in farmed fish, therefore, enrichment of genetic variation is required for sustainable *Rasbora* fish farming.

Keywords: Cytochrome c oxidase (COI), Truss network morphometrics, Haplotype, Genetic structure

Introduction

Rasbora spp. is found in Southeast Asia, including Indonesia,

especially in the islands of Sumatra, Java, Kalimantan, Bali, and Lombok (Hubert et al., 2019; Kusuma et al., 2016). Geographically, Indonesia's mainland is separated from

Received: Oct 27, 2022 Revised: Jul 10, 2023 Accepted: Jul 12, 2023

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mountains and inland waters, which serve as a repository for germplasm, including rasbora fish diversity. *Rasbora arundinata*, *Rasbora haru*, *Rasbora maninjau*, and *Rasbora bindumatoga* are four new species discovered in North Sumatra, Indonesia (Lumbantobing, 2014). However, this diversity is at risk of extinction as a result of overfishing, and fish farming is one of sustaining methods for native fish stocks (Domingues et al., 2022; Pinsky & Palumbi, 2014).

Rasbora lateristriata, also known as wader pari, is a popular fish in Java. This fish has a delicious flavor and is high in protein (15.75%–47.54%), fat (12.36%), ferrum (2.9%), and zinc (3.46%) (Erhana & Retnoaji, 2019; Munthe et al., 2016; Sogandi et al., 2020). Wader pari cultivation is a way to meet market demands. *R. lateristriata* has been successfully cultivated in the laboratory, hatchery, and on a large scale (Manganang et al., 2020; Prasetyo & Retnoaji, 2019; Retnoaji et al., 2017; Zulfadhli et al., 2016). However, no attempt has been made to trace and compare the morphometric and genetic characters of *Rasbora* spp. cultivation and natural populations from several regions of Indonesia.

The truss network measurement (TNM) character, one of the morphometric measurement instruments is used to characterize the *Rasbora* spp. morphological diversity (Muchlisin, 2013). The identification of cultured and wild fish using morphometrics has been studied in the species *Sparus aurata* (Doğdu & Turan, 2021), *Dormitator latifrons* (Gonzalez-Martinez et al., 2020), and *Clarias macrocephalus* (Whan-Air et al., 2018). The mitochondrial cytochrome c oxidase (COI) gene is widely used as a marker gene for the taxonomic identification of fish. It is inherited maternally and is used to of compose fish kinship groups, including *Rasbora* spp. (Kusuma et al., 2016). *R. lateristriata* populations discovered in West Java, Central Java, and East Java populations may represent a cryptic species and species complex (Kusuma et al., 2016, 2017). To further understand the relationships between these populations, this study analyzed morphometric data and COI genes to distinguish *Rasbora* spp. from cultivated and wild stock, which will be useful for future cultivation development.

Materials and Methods

Times and places

The research was conducted from September 2020 to December 2021. This research focuses on *Rasbora* spp. from five distinct populations: the cultivated population (Jogjakarta), the wild population on the island of Java (Purworejo and Malang), and the

Sumatra population (Riau and Palembang) (Fig. 1). Cultivated fish is the fourth generation (G4), the progeny of the first generation (G0) that have been domesticated and bred through inbreeding. G4 is the result of the selection of phenotypes with great body size, rapid growth, and adaptability. The polymerase chain reaction (PCR) analysis was carried out in the genetic laboratory of the Research Institute for Inland Fisheries and Extension, Palembang. Sanger sequencing was performed at PT. Genetics Science.

The sample for measuring TNM and gene specimens was collected in a single survey method. In total, 80 *Rasbora* spp. fish were collected and analyzed for TNM characters. The fish were collected from the populations of Jogjakarta (20), Malang (18), Purworejo (18), Riau (12), and Palembang (12). *Rasbora* spp. caudal fin tissue was collected as genetic material from the populations of Jogjakarta (6), Malang (6), Purworejo (5), Riau (4), and Palembang (3). The grouping refers to morphological characteristics, particularly along the fish's lateral line (Hubert et al., 2019; Kusuma et al., 2016). *Rasbora argyrotaenia* is characterized by a distinct black lateral line that extends from the upper pectoral fins to the anterior caudal fins (Hubert et al., 2019). *Rasbora dusonensis* is distinguished by a black caudal fin posterior.

Truss network measurement (TNM)

The manual caliper was used to determine the length of fifteen TNM characters and the total length (TL). The TNM landmark (Fig. 2) referred to Muchlisin (2013) and TNM components details are available in Table 1.

Polymerase chain reaction (PCR)

Caudal fin tissue was homogenized using a genomic GB100 DNA Kit, 70% alcohol, Genomic GB100 DNA Kit, loading dye, PCR mix-DNA ladder Bioline brand, methylene blue, primer Forward-Reverse COI gene, and Phosphate Buffer Saline were used for COI gene analysis. Digital scales, an aquarium, a 1.5 mL microtube, centrifugation, digital scales, a vortex, a hot air oven, a thermal cycler T100, an electroporator, a dissecting set, and a UV transilluminator were used as tools.

Sampling was conducted using a random sampling technique from nine distinct populations. Fish from each location are transported to the laboratory for anesthesia by lowering the temperature of the water in the fish rearing container to 4°C for ± 3 min. The water temperature is lowered by administering ice gel. DNA tissue was taken from the caudal



Fig. 1. Five sampling locations and latitude and longitude for *Rasbora* spp. fish were identified on the Sumatra (Riau and Palembang) and Java islands (Jogjakarta, Purworejo, and Malang).

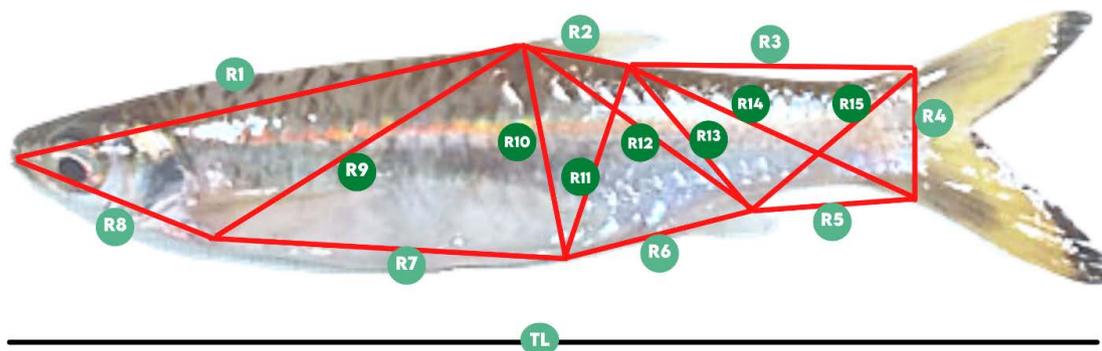


Fig. 2. A 15-part TNM character landmark. The characters R1–R15 and TL are described in detail in Table 1. TNM, truss network measurement; TL, total length.

Table 1. TNM characters of *Rasbora* spp.

Characters	Information
R1	Mouth's base to the anterior of the pinna dorsalis
R2	Anterior to posterior of pinna dorsal
R3	End pinna dorsalis to top of the pinna caudal
R4	The base of the top to the bottom pinna caudal
R5	Lower pinna caudal to tip of pinna anal
R6	End of pinna anal to posterior of pinna pelvic
R7	Posterior of pinna pelvic to lower pinna pectoral
R8	Lower pinna pectoral to the lower mouth's base
R9	Lower pinna pectoral to anterior of pinna dorsalis
R10	Anterior of pinna dorsalis to posterior of pinna pelvic
R11	Posterior of pinna pelvic to posterior of pinna dorsalis
R12	Anterior of pinna dorsalis to posterior of pinna anal
R13	Posterior of pinna dorsalis to posterior of pinna caudalis
R14	Posterior of pinna dorsalis to lower pinna caudalis
R15	Lower pinna caudalis to posterior of pinna analis
TL	Total length

TNM, truss network measurement.

fin using a dissecting set and stored in a microtube containing 70% alcohol. DNA isolation was carried using a Genomic GB100 DNA kit to obtain DNA material purification: sample preparation, lysis, binding, washing, and elution following the previous method (Muslimin et al., 2020a). The DNA isolate products were prepared for PCR amplification by adding 12.5 µL of PCR mix ingredients. These included 6 µL of 2× MyTaq HS Red Mix (Bioline, Little Clacton, UK), 5 µL of Nuclease-Free Water, 0.5 µL of forward and reverse primers, respectively, and 0.5 µL of total DNA. The COI primer used were FishF1-5'TCAACCAACCACAAAGACATTGGCAC'3 (forward) and FishR1-TAGACTTCTGGGTGGCCAAAGAATCA'3 (reverse) (Ward et al., 2005). The PCR amplification temperature was programmed in five stages: initial denaturation for 3 min, continued denaturation for 3 s at the same temperature (94 °C) with 30 cycles, annealing (55 °C) for 30 s, initial extension for 90 s, and final extension for 8 min at the same temperature (72 °C). The base length amount of the PCR outcomes were visualized on 1% agarose under UV light. Finally, the DNA was purified, and the nucleotide base sequences were identified by Sanger sequencing.

Data analysis

Discriminant function analysis (DFA)

Analysis of variance (ANOVA) was used to determine which

characters are significantly different among populations. Furthermore, 15 'TNM characters' data were processed using multivariate statistical analysis via the discriminant function analysis (DFA) method. These characters were adjusted using Turan (1999) data transformation formula to determine the strength of variation between 15 fish morphometric characters ($p < 0.05$). The formula is as follows:

$$\text{Transformation} = \frac{\text{Character measurement (cm)}}{\text{Total length (cm)}}$$

The DFA test eliminates insignificant characters based on their eigenvalues. ANOVA and DFA data analysis were performed using the IBM SPSS version 23.0 program (Armonk, NY, USA).

Phylogenetic tree

Data analysis for phylogenetic tests was performed using the Bioedit application, DNASP version 5.0, and MEGA version X. The National Center for Biotechnology Information (NCBI) platform was used to identify the species using the Basic Local Alignment Search Tool (BLAST). The maximum likelihood method, Kimura2 parameters, and the preparation of bootstraps with 10,000 repetitions were used in the phylogenetic analysis (Kimura, 1980).

Genetic structure and haplotype network

The analysis of nucleotide diversity was conducted using analysis of molecular variance with DNASP version 5.0 and Arlequin 3.5.2.2 program (Excoffier & Lischer, 2010). The Network NETW 10.20 program was used to analyze the network haplotype reconstruction.

Results

Discriminant function analysis

The *Rasbora* spp. in this study were classified as *R. argyrotaenia* (Jogjakarta and Purworejo), *R. lateristriata* (Jogjakarta, Malang, and Purworejo), *Rasbora einthovenii* (Riau), and *R. dusonensis* (Palembang). The COI gene was used to confirm the species identity of all specimens. Fig. 3 illustrates the morphology of the five species examined in this study.

Table 2 summarizes the mean values for fifteen TNM characters and the rasbora fish's length. Significant difference is followed-up using Tukey analysis, with significantly different

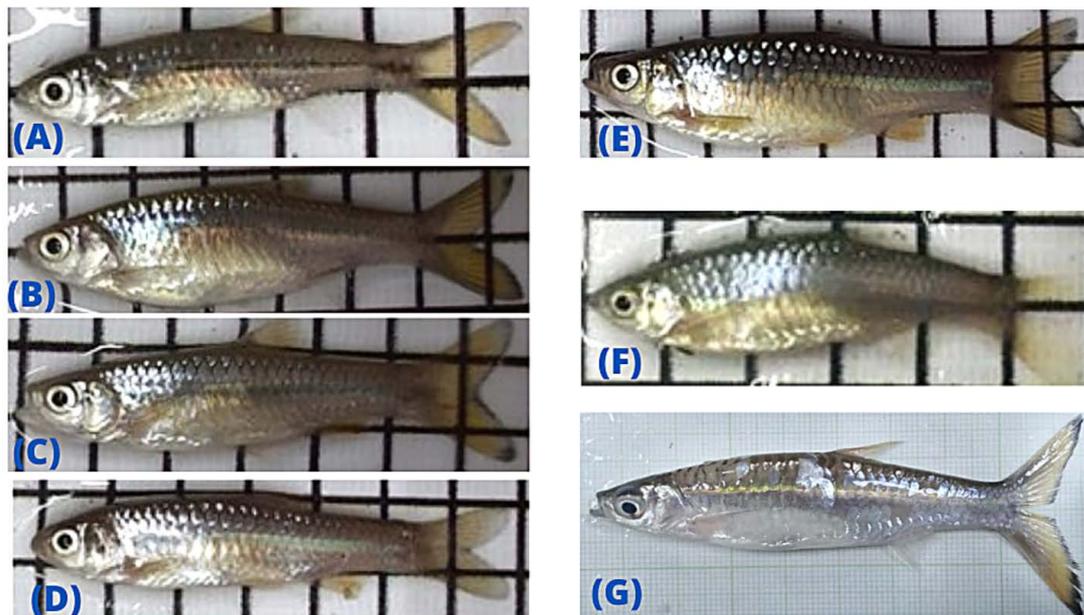


Fig. 3. *Rasbora* spp. from farmed stock (A and B) and wild stock (C–H). (A) *Rasbora argyrotaenia* (Jogjakarta), (B) *Rasbora lateristriata* (Jogjakarta), (C) *R. argyrotaenia* (Purworejo), (D) *R. lateristriata* (Purworejo), (E) *R. lateristriata* (Malang), (F) *Rasbora einthovenii* (Riau), and (G) *Rasbora dusonensis* (Palembang).

characters ($p < 0.05$) denoted by superscripts. *R. dusonensis* from Palembang had the highest mean that was significantly different for all characters. *R. lateristriata* in the Malang population vary significantly according to R2, R10–R15, and TL characters.

Based on the value of the Wilks lambda, the DFA test divides fifteen characters into six functional groups (Table 3). The discriminatory character in the function discriminant group is determined based on the eigenvalue, percentage of variation, and canonical correlation. The greater the value of the three, the stronger the variant character of each group (Muslimin et al., 2020b). The highest eigenvalues were discriminant function 1 (df1; 2.43) and df2 (1.58). The df group with the highest percentage of variation is df1 (43.9%), followed by df2 (28.6%). The highest canonical correlation values (> 0.65) are df1 (0.84) and df2 (0.78). The distinguishing character is determined using df1–df3, as this group contains the significant discriminant. The significant characters are denoted by superscripts (R12, R11, R2, and R10). According to the percentage of variance, eigenvalues, and canonical correlation values, df1 and df2 are significantly different discriminant characters ($p < 0.05$). These characters, R2, R10, R11, and R12 are denoted by a superscript.

The canonical coefficient values of discriminant functions are plotted and summarized to represent the centroid points

of populations and species, respectively. Fig. 4 illustrates the distribution of centroid points. The four quadrants classify the population according to the discriminant characteristics listed in Table 3. In Quadrant I, the *R. dusonensis* (Palembang) and *R. argyrotaenia* (Jogjakarta). Quadrant II contains the *R. argyrotaenia* (Purworejo) and *R. lateristriata* (Purworejo) populations from Purworejo, as well as the *R. einthovenii* (Riau) population from Riau. *R. lateristriata* are the species found in Quadrant III (Jogjakarta). *R. lateristriata* from Malang is found in quadrant IV (Malang).

Barcoding DNA

Confirmation of species was carried out using the COI gene BLAST results and is shown in Table 4. *R. lateristriata* and *R. argyrotaenia* species constitute the cultivated population in Jogjakarta. *R. lateristriata* and *R. argyrotaenia* are found in the Purworejo population. *R. lateristriata* is the native species in Malang, *R. dusonensis* is the endemic species in Palembang, and *R. einthovenii* is the local species in Riau. The sequencing results have been submitted to the NCBI database containing Table 5.

Phylogenetic tree

The *Rasbora* spp. nucleotide sequence was compared to

Table 2. The length average of sixteen TNM characters from *Rasbora* spp.

Characters	Population						
	<i>Rasbora Argyrotaenia</i>		<i>Rasbora lateristriata</i>			<i>Rasbora dusonensis</i>	<i>Rasbora einthovenii</i>
	Jogjakarta (cm)	Purworejo (cm)	Jogjakarta (cm)	Malang (cm)	Purworejo (cm)	Palembang (cm)	Riau (cm)
R1	3.16 ± 0.45	2.42 ± 0.93	3.33 ± 0.49	3.56 ± 0.35	2.57 ± 0.31	5.19 ± 0.92 [†]	2.46 ± 0.51
R2	0.84 ± 0.19	0.55 ± 0.15	0.86 ± 0.17	1.04 ± 0.17 [†]	0.63 ± 0.10	1.05 ± 0.22 [†]	0.63 ± 0.11
R3	1.77 ± 0.36	1.26 ± 0.10	1.93 ± 0.38	1.98 ± 0.27	1.28 ± 0.20	2.79 ± 0.59 [†]	1.36 ± 0.30
R4	0.78 ± 0.17	0.57 ± 0.10	0.79 ± 0.13	0.91 ± 0.13	0.55 ± 0.75	1.14 ± 0.25 [†]	0.60 ± 0.13
R5	1.09 ± 0.21	1.00 ± 0.90	1.20 ± 0.27	1.35 ± 0.16	0.92 ± 0.12	1.64 ± 0.35 [†]	0.91 ± 0.15
R6	1.31 ± 0.28	0.80 ± 0.20	1.26 ± 0.23	1.42 ± 0.23	0.80 ± 0.10	1.84 ± 0.36 [†]	0.79 ± 0.21
R7	1.88 ± 0.47	1.16 ± 0.14	1.87 ± 0.28	2.11 ± 0.24	1.55 ± 0.27	3.23 ± 0.74 [†]	1.49 ± 0.38
R8	1.55 ± 0.20	1.19 ± 0.11	1.64 ± 0.27	1.70 ± 0.15	1.21 ± 0.14	2.30 ± 0.33 [†]	1.22 ± 0.26
R9	2.06 ± 0.43	1.53 ± 0.13	2.08 ± 0.29	2.28 ± 0.32	1.66 ± 0.24	3.36 ± 0.80 [†]	1.60 ± 0.33
R10	1.38 ± 0.29	0.98 ± 0.78	1.49 ± 0.24	1.71 ± 0.17 [†]	1.12 ± 0.20	2.13 ± 0.51 [†]	1.10 ± 0.21
R11	1.30 ± 0.22	0.93 ± 0.11	1.41 ± 0.19	1.64 ± 0.19 [†]	1.05 ± 0.20	2.00 ± 0.48 [†]	1.00 ± 0.18
R12	1.72 ± 0.34	1.05 ± 0.10	1.85 ± 0.31	2.09 ± 0.20 [†]	1.25 ± 0.20	2.75 ± 0.59 [†]	1.31 ± 0.27
R13	1.20 ± 0.25	0.73 ± 0.51	1.23 ± 0.18	1.35 ± 0.16 [†]	0.82 ± 0.14	1.88 ± 0.44 [†]	0.90 ± 0.20
R14	2.01 ± 0.39	1.44 ± 0.85	2.19 ± 0.46	2.42 ± 0.25 [†]	1.52 ± 0.19	3.09 ± 0.63 [†]	1.59 ± 0.37
R15	1.35 ± 0.23	1.03 ± 0.12	1.50 ± 0.24	1.71 ± 0.21 [†]	1.15 ± 0.15	2.08 ± 0.49 [†]	1.18 ± 0.23
TL	6.70 ± 0.95 [†]	4.97 ± 0.28	7.20 ± 1.04 [†]	7.75 ± 0.67 [†]	5.37 ± 0.57	10.71 ± 2.1 [†]	5.19 ± 1.12

[†]A statistically significant difference ($p < 0.05$) of TNM characters in seven population, i.e., *R. argyrotaenia* (Jogjakarta and Purworejo) and *R. lateristriata* (Jogjakarta, Malang, and Purworejo), *R. dusonensis* (Palembang), and *R. einthovenii* (Riau).
TNM, truss network measurement; TL, total length.

Table 3. df and the canonical relationship between TNM characters

TNM	df					
	1	2	3	4	5	6
R12	0.21	-0.45 [†]	0.00	0.32	-0.24	0.26
R11	-0.09	-0.37 [†]	-0.08	0.06	0.11	0.06
R2	-0.20	-0.37 [†]	0.06	0.29	0.12	-0.17
R10	-0.15	-0.32 [†]	-0.13	0.28	-0.00	0.20
R6	0.24	-0.37	0.47 [†]	-0.11	0.24	-0.23
R8	-0.17	0.15	0.31 [†]	0.23	-0.19	-0.08
R7	0.19	0.19	-0.44	0.48 [†]	0.28	0.08
R15	-0.30	-0.16	-0.16	0.43 [†]	-0.17	0.32
R13	0.23	-0.23	0.25	0.43 [†]	-0.31	0.25
R3	0.12	-0.07	0.29	0.08	-0.43 [†]	0.16
R9	0.03	0.27	-0.06	0.17	0.27 [†]	0.25
R4	-0.04	-0.25	0.37	0.33	-0.05	0.60 [†]
R5	-0.34	-0.04	0.11	-0.05	0.08	0.56 [†]
R1	0.09	0.38	-0.04	0.00	0.21	0.40 [†]
R14	-0.02	-0.33	0.15	0.21	-0.26	0.33 [†]
Eigenvalue	2.43	1.58	0.68	0.43	0.28	0.11
% of variance	43.9	28.6	12.4	7.9	5.2	2.1
Canonical correlation	0.84	0.78	0.63	0.55	0.47	0.32

df, discriminant function; TNM, truss network measurement.

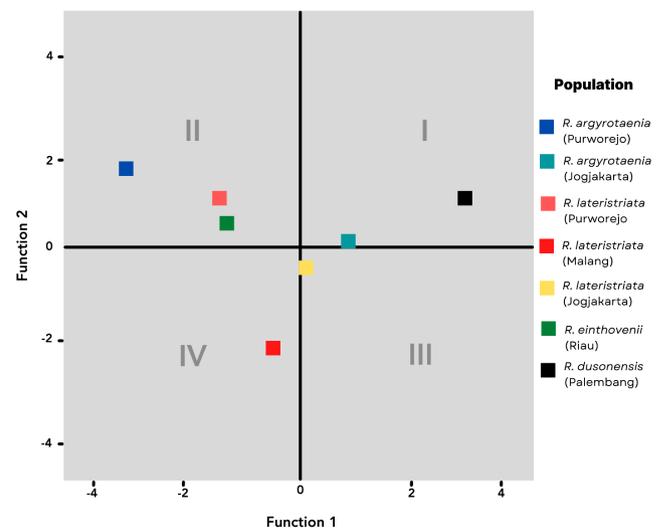


Fig. 4. Discriminant function graph based on canonical centroid point for TNM character of *Rasbora* spp. Population is color-coded and segmented into quadrants. TNM, truss network measurement.

Table 4. Identification of species using BLAST results from DNA sequencing in the NCBI database

Population	n	Base length	Species
MalangRL	6	576–651	<i>Rasbora lateristriata</i>
JogjakartaRA	3	693–702	<i>Rasbora agryotaenia</i>
JogjakartaRL	3	589–654	<i>Rasbora lateristriata</i>
PurworejoRA	2	589–703	<i>Rasbora agryotaenia</i>
PurworejoRL	3	589–624	<i>Rasbora lateristriata</i>
RiauRE	4	615–654	<i>Rasbora einthovenii</i>
PalembangRD	3	589	<i>Rasbora dusonensis</i>

BLAST, Basic Local Alignment Search Tool; NCBI, National Center for Biotechnology Information.

Table 5. An accession number accompanies sequence results recorded at NCBI

Species	Population	Accession No.
<i>Rasbora lateristriata</i>	Malang	ON936278, ON936280, ON936276, ON935718, ON932643, and ON933580
	Jogjakarta	ON936281, ON927242, and ON936279
	Purworejo	ON932568, ON933953, and ON933579
<i>Rasbora agryotaenia</i>	Jogjakarta	ON960224, ON960223, and ON959608
	Purworejo	ON935439 and ON959607
<i>Rasbora dusonensis</i>	Palembang	ON935443, ON935717, and ON935437
<i>Rasbora einthovenii</i>	Riau	ON936277, ON935615, ON935436, and ON935619

NCBI, National Center for Biotechnology Information.

NCBI BLAST data for the COI gene of *R. lateristriata* of the Pasuruan population (Accession No. LC130692.1) to construct a phylogenetic tree. The *Rasbora* spp. kinship tree, constructed using MEGA X and 10,000 repetitions, revealed five distinct clades. Fig. 5 illustrates the relationship between cultured fish (Jogjakarta) and wild stock populations (Malang, Purworejo, Palembang, and Riau). Clade I (*R. lateristriata* from Purworejo, Jogjakarta, and Malang), Clade II (*Rasbora agryotaenia* form Jogjakarta and Purworejo), Clade III (*R. dusonensis* form Palembang), Clade IV (*R. einthovenii* from Riau), and Clade V (*R. lateristriata* from Purworejo).

Fixation index (FST) and genetic structure

Table 6 displays the results of calculating genetic distance using Kimura 2 parameters with 10,000 replications and nucleotide diversity coefficients among populations using Nei's (1972) analysis. The *R. lateristriata* (Jogjakarta and Malang) had the

lowest fixation coefficient (0.00), while the *R. argyrotaenia* (Purworejo) and *R. dusonensis* (Palembang) had the highest fixation coefficient (0.96), not significantly different, respectively. The *R. argyrotaenia* (Purworejo) and *R. dusonensis* (Palembang) had the lowest nucleotide diversity (0.00), significantly different ($p < 0.05$), respectively. Meanwhile, *R. lateristriata* (Purworejo) exhibited the greatest diversity of nucleotides (0.43) with the statistically insignificant difference.

Haplotype network

All specimens' haplotype relationships were analyzed using the SP DNA program and Network 10.2.0.0, as shown in Fig. 6. The total number of haplotypes produced was 23 color-coded according to the population. The genetic structure of H1 to H23 demonstrates a mutation indicated by a number on the connecting line between the haplotypes. H1 comprised of *R. argyrotaenia* from Jogjakarta and Purworejo. Except for H15 (*R. lateristriata* from Purworejo), intraspecific mutations exhibit a low mutation rate and higher variation interspecies between H6 (*R. lateristriata* from Jogjakarta) and H15 (*R. lateristriata* from Purworejo).

Discussion

Discriminant function analysis

R2, R10, R11, and R12 are the TNM characteristics that distinguish cultivated fish (Jogjakarta) from wild type (Purworejo, Malang, Palembang, and Riau) (Table 3). Palembang's population is larger and significantly more diverse in all dimensions (Table 2). R2, R10, R11, and R12 of *R. lateristriata* wild type (Malang) were significantly different in length ($p < 0.05$). Pinna dorsalis (R2) *R. lateristriata* (Malang) is longer than in other populations. Pinna dorsal length variation can be influenced by geographical conditions, fish stocking density, water quality, and sexual dimorphism (Brown et al., 2016; Klima et al., 2013). R10, R11, and R12 denote the average height of fish in each population. *R. lateristriata* (Malang) is significantly taller in the wild than in the cultivated population. Similar findings were made in the *S. aurata* (Šegvić-Bubić et al., 2014) and *R. lateristriata* (Harris et al., 2021). This character difference is thought due to morphological characteristics inherited from previous generations, the season, food availability, and age (Doğdu & Turan, 2021).

Otherwise, R2, R10, R11, and R12 of farmed *R. argyrotaenia* (Jogjakarta) were the longest than the wild type (Purworejo). A similar discovery on the cultivated fish of *D. latifrons* (Gonzalez-

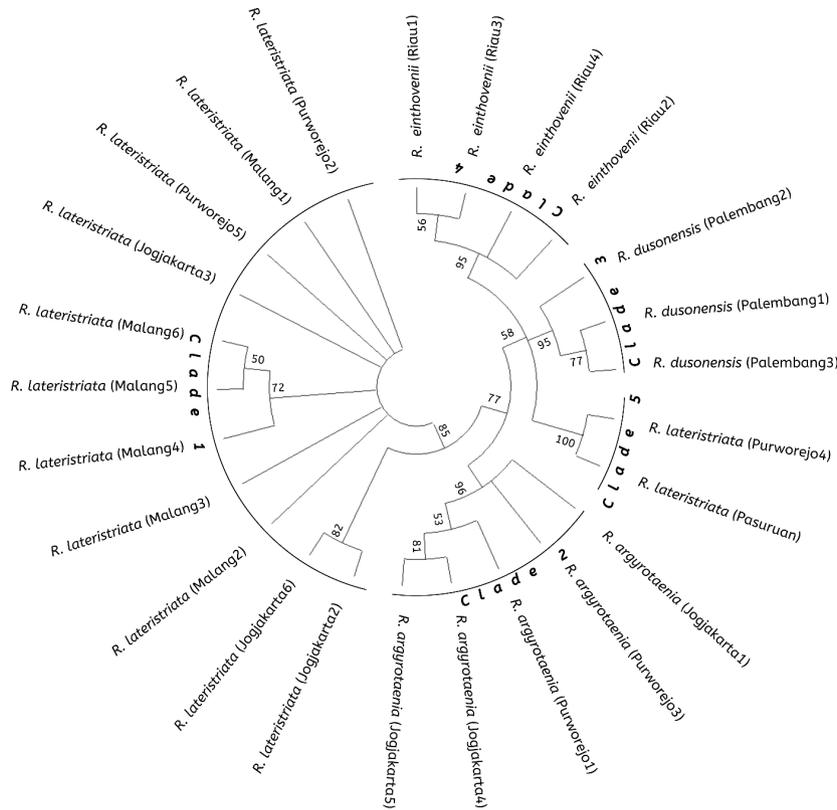


Fig. 5. The phylogenetic tree of *Rasbora* spp. in comparison to NCBI gene bank data with accession no. LC130692.1 (*Rasbora lateristriata* form Pasuruan). NCBI, National Center for Biotechnology Information.

Table 6. The *Rasbora* spp. were examined for their FST and nucleotide diversity values

Population	1	2	3	4	5	6	7	Nucleotide diversity	p-value
1	0.00	0.73	0.09	0.06	0.00	0.09	0.04	0.02	0.01
2	0.08	0.00	0.09	0.47	0.04	0.09	0.09	0.00	0.00
3	0.74	0.74	0.00	0.58	0.41	0.09	0.18	0.04	0.03
4	0.25	0.10	0.01	0.00	0.30	0.06	0.00	0.43	0.32
5	0.66*	0.64*	0.00	0.11	0.00	0.00	0.00	0.06	0.03
6	0.91	0.96	0.84	0.30	0.73*	0.00	0.03	0.00	0.00
7	0.73	0.71	0.67	0.31	0.64*	0.72*	0.00	0.05	0.03

The bottom diagonal represents the FST value, while the top diagonal represents the p-value. Information for population 1: *Rasbora argyrotaenia* (Jogjakarta), 2: *R. argyrotaenia* (Purworejo), 3: *Rasbora lateristriata* (Jogjakarta), 4: *R. lateristriata* (Purworejo), 5: *R. lateristriata* (Malang), 6: *Rasbora dusionensis* (Palembang), and 7: *Rasbora einthovenii* (Riau).

*The significance level (0.05).

FST, fixation index.

Martinez et al., 2020). This is because cultured fish are selectively bred to produce offspring with larger body types (Doğdu & Turan, 2021). The fin size of cultured fish can be influenced by aquaculture water settings such as water discharge, stocking density, volume, and feeding (Doğdu & Turan, 2021). The

difference in character results between *R. lateristriata* and *R. argyrotaenia* in cultivated and natural populations are believed due to genetic and environmental factors (Whan-Air et al., 2018).

Rasbora spp. has been classified into four groups based on the four distinct TNM characters (Fig. 4). In quadrant

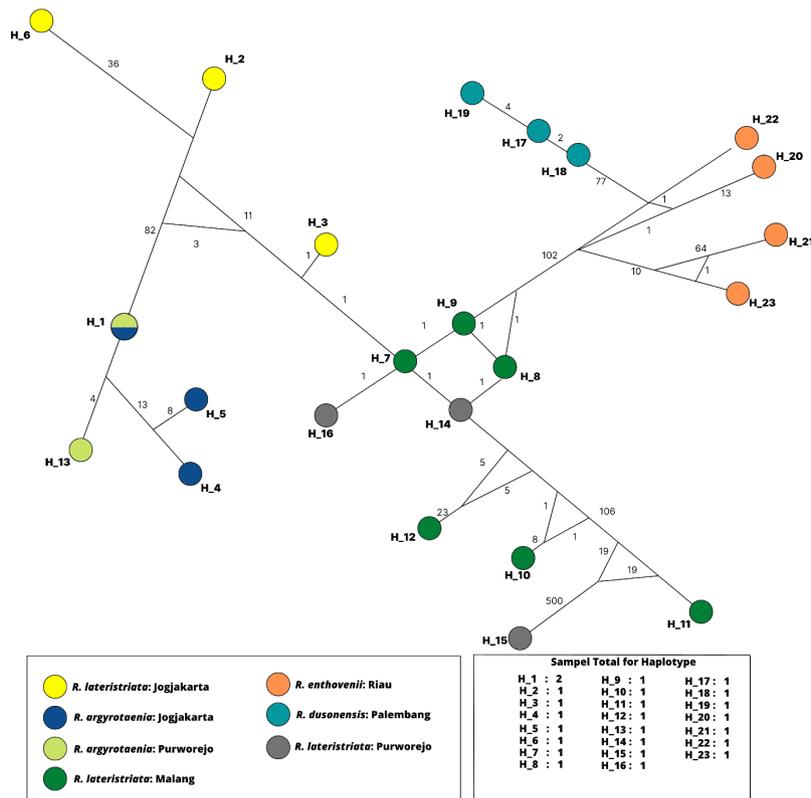


Fig. 6. Haplotype network of *Rasbora* spp. from five populations. Cultivated fish (Jogjakarta) comprised of *Rasbora lateristriata* (H2, H3, and H6) and *Rasbora argyrotaenia* (H1, H4, and H5). Wild stock from Malang: *R. lateristriata* (H7 s.d. H12), Purworejo: *R. lateristriata* (H14 s.d. H16) and *R. argyrotaenia* (H1 and H13), Palembang: *R. dusonensis* (H17 s.d. H19), and Riau: *R. enthovenii* (H20–H23).

III, cultured fish (*R. lateristriata*) on Jogjakarta into a single group and separated them from wild type group, however, *R. argyrotaenia* (Jogjakarta) shared morphological characteristics with the wild type (*R. dusonensis*). This can be a result of environmental factors influencing morphological variation. These environmental influences can be classified as internal (genetic flow, inherited features, and disease) or external (environmental factors such as climate change) (predators, temperature, water level, and geography) (Aminan et al., 2020; Whan-Air et al., 2018).

Phylogenetic tree

The fish group *Rasbora* spp. was classified into five clades (Fig. 5). *R. argyrotaenia* was found to be in a distinct clade from *R. dusonensis* and *R. eitnhovenii*. Aminan et al. (2020) found different results, placing *R. argyrotaenia* and *R. dusonensis* in the same clade and a separate clade from *R. enthovenii*. In contrast,

R. lateristriata Purworejo, which is separate from other clades, was identified in this study (Clade 5). *R. lateristriata* is a species with a high degree of gene variety on the Indonesian island of Java (Kusuma et al., 2016). This is demonstrated by Clade 1 (Fig. 5) and its sub-clades. One of the subclades has a bootstrap value of 77%, indicating that the populations (East Java and Central Java) and some cultivated populations (Jogjakarta) are relatively distantly connected. Indeed, Clade 5 places the population of Purworejo (Central Java) in the same group as the Pasuruan (outgroup) population in East Java.

Sholihah et al. (2020) classify *R. lateristriata* of Central and East Java as a single species. Otherwise, Kusuma et al. (2016) declared that Central and East Java populations are distinct. These group distinctions can be attributed to variances in subpopulations or domestication influences on fish genetic diversity. Similar discoveries have been made for Atlantic salmon and sturgeon species, which exhibit variation between cultured

and wild fish, and it is hypothesized that genetic change happens both naturally (genetic pool, genetic drift, and gene flow) and as a result of cultivation (cross-breeding and generational differences) (Besnier et al., 2015; Zhang et al., 2013). Therefore, additional research is necessary to ascertain the genetic diversity of the next-generation cultivars of *R. lateristriata*.

Fixation index (FST) and genetic structure

Fixation index (FST) indexation represents gene differentiation (Willing et al., 2012). Pairwise FST scores between the cultured and wild type of *C. macrocephalus* are 0.013–0.039 (Duong & Scribner, 2018) and *Piaractus mesopotamicus* is 0.0015–0.055 in range (Pazo et al., 2021). In this study, the lowest gene differentiation was observed between *R. lateristriata* (cultivated population from Jogjakarta) and natural resources (Malang) (Table 6). The low FST value observed in this study could be explained by the two populations sharing the same parental origin (Doğdu & Turan, 2021). Additionally, connected waters, restocking in the wild from cultured fish, and mass recruitment of fish from nature could be affected by gene differentiation (Duong & Scribner, 2018).

According to Mccusker & Bentzen (2010), nucleotide diversity has a low value (0.3) and a high value (> 0.4). In this study, *R. lateristriata* (Jogjakarta) gene heterozygosity was low (0.04), but *R. lateristriata* (Purworejo) gene heterozygosity was high (0.43). Both population's intraspecific morphology exhibits low heterozygosity. That could influence the high proportion of A-T nucleotides, contributing to transversion mutations, frameshift mutations, and non-synonymous amino acids dominating (Muslimin et al., 2020a; Ramadhaniaty et al., 2018). The highest nucleotide diversity resulted in a cultivated population, i.e., *R. lateristriata* (Jogjakarta), with adenine (29.08%), thymine (26.24%), guanine (26%), and cytosine (18.68%). While *R. lateristriata* (Purworejo) has the highest nucleotide diversity in non-cultivated populations, with nucleotide structure consisting of adenine (28.36%), thymine (27.51%), guanine (23.33%), and cytosine (20.81%).

Haplotype network

Haplotype connections are used to characterize the relationship between species from distinct populations based on the genetic distance between gene mutations (Ramadhaniaty et al., 2018). The cultivation of *R. argyrotaenia* in Jogjakarta is interspecifically considered to share genetic material with the Purworejo population. Jogjakarta and Purworejo are geographically adjacent

and share a watershed. Fish from distinct geographical areas with connected waterways could share the same gene source, which involves gene flow and causes a low genetic divergence between populations (Pazo et al., 2021; Ramadhaniaty et al., 2018). Reduced gene diversity can be affected by a bottleneck effect that occurs naturally or as a result of human activity, such as overfishing (Doğdu & Turan, 2021; Pazo et al., 2021). Another influential element is human-caused fish translocation (Muslimin et al., 2020b).

The cultivated *R. lateristriata* (Jogjakarta) haplotype is not genetically related to natural populations (Malang and Purworejo). The results are identical to the farmed fish of *C. macrocephalus* that have been bred with high-intensity wild stock, potentially boosting gene diversity (Duong & Scribner, 2018). There was no evidence of genetic exchange between the Malang and Purworejo populations. *R. lateristriata* and *R. argyrotaenia* are well-known species with a diverse gene pool from East and Central Java, respectively (Kusuma et al., 2016). This is because the physical barrier of the water between geographical areas restricts gene flow (Doğdu & Turan, 2021). High mutation rates can result in changes in codons, amino acid composition, and frameshift mutations and influence fish morphometric characteristics (Muslimin et al., 2020a).

This study found relatively low intraspecific gene diversity in farmed fish. A gene selection program to increase fish gene diversity on an aquaculture scale is essential for further breeding development. Low-diversity genes in a population of the same fish species tend to have the same gene flow. The impact of gene homogeneity within the same fish species can result in harmful mutations, reduce environmental adaptation, and lower survival rates (Duong & Scribner, 2018; Furlan et al., 2012). The genetic differences in cultured fish must be considered to produce heterozygous fish, minimize inbreeding, and enhance genetic drift and genetic flow variation. A sustainable breeding program can be implemented by focusing on gene variation in the selection of traits/characteristics such as environmental adaptability, accelerated fish growth, reproduction, and disease resistance (Carvalho, 1993). According to the findings of this study, the Purworejo population possesses a variety of fish genetic resources that could serve as a source of genetic material for future breeding. The aquaculture development program will advance by recruiting potential parents from this population using genetic selection techniques related to growth and inbreeding traits.

Discriminant function analyses revealed a distinct group of cultivating *R. lateristriata* and *R. argyrotaenia* from a non-

cultivating group. Lower genetic differentiation in farmed *R. argyrotaenia* and *R. lateristriata* than in wild stock, thereby necessitating the development of additional genetic selection programmes for breeding. In conclusion, the characteristics of the COI genes of farmed fish (*R. argyrotaenia* and *R. lateristriata*) tend to be homologous interspecies, except *R. lateristriata* (Purworejo), which can be used as a new genetic source for future aquaculture research.

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Not applicable.

Acknowledgements

The author would like to extend the deepest gratitude to the Faculty of Biology, Universitas Gadjah Mada for the research facilities.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals.

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References

- Aminan AW, Kit LLW, Hui CH, Sulaiman B. Morphometric analysis and genetic relationship of *Rasbora* spp. in Sarawak, Malaysia. *Trop Life Sci Res.* 2020;31:33-49.
- Besnier F, Glover KA, Lien S, Kent M, Hansen MM, Shen X, et al. Identification of quantitative genetic components of fitness variation in farmed, hybrid and native salmon in the wild. *Heredity.* 2015;115:47-55.
- Brown AM, Bejder L, Parra GJ, Cagnazzi D, Hunt T, Smith JL, et al. Sexual dimorphism and geographic variation in dorsal fin features of Australian humpback dolphins, *Sousa sahulensis*. *Adv Mar Biol.* 2016;73:273-314.
- Carvalho GR. Evolutionary aspects of fish distribution: genetic variability and adaptation. *J Fish Biol.* 1993;43:53-73.
- Doğdu SA, Turan C. Genetic and morphological impact of the cultured gilthead sea bream (*Sparus aurata* Linnaeus, 1758) populations on wild stocks. *Egypt J Aquat Biol Fish.* 2021;25:499-511.
- Domingues A, Alexandre CM, Mateus CS, Silva S, Pereira J, Almeida PR. Into the wild: a new approach to the aquaculture production of brown trout (*Salmo trutta* L.) to enhance restocking success. *Biol Life Sci Forum.* 2022;13:115.
- Duong TY, Scribner KT. Regional variation in genetic diversity between wild and cultured populations of bighead catfish (*Clarias macrocephalus*) in the Mekong delta. *Fish Res.* 2018;207:118-25.
- Erhana E, Retnoaji B. Histological structure of intestine, number of goblet cells, and survival rate of wader pari (*Rasbora lateristriata* Bleeker, 1854) due to influence of temperature. In: *Proceedings of the 6th International Conference on Biological Science*; 2019; Yogyakarta, Indonesia.
- Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour.* 2010;10:564-7.
- Furlan E, Stoklosa J, Griffiths J, Gust N, Ellis R, Huggins RM, et al. Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. *Ecol Evol.* 2012;2:844-57.
- Gonzalez-Martinez A, Lopez M, Molero HM, Rodriguez J, González M, Barba C, et al. Morphometric and meristic characterization of native chame fish (*Dormitator latifrons*) in Ecuador using multivariate analysis. *Animals.* 2020;10:1805.
- Harris A, Liliyanti MA, Sumahiradewi LG, Artiningrum NT. Morphometric, meristic characters, and gonad mature level of pepudah fish (*Rasbora lateristriata*) from Sokong rivers, North Lombok district. *Penbios J Pendidik Fis Sains.* 2021;6:28-37.
- Hubert N, Lumbantobing D, Sholihah A, Dahruddin H, Delrieu-Trottin E, Busson F, et al. Revisiting species boundaries and distribution ranges of *Nemacheilus* spp. (Cypriniformes: Nemacheilidae) and *Rasbora* spp. (Cypriniformes: Cyprinidae) in Java, Bali and Lombok through DNA barcodes: implications for conservation in a biodiversity hotspot.

- Conserv Genet. 2019;20:517-29.
- Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 1980;16:111-20.
- Klima O, Kopp R, Hadašová L, Mareš J. Fin condition of fish kept in aquacultural systems. *Acta Univ Agric Silv Mendel Brun.* 2013;61:1907-16.
- Kusuma WE, Ratmuangkhwang S, Kumazawa Y. Molecular phylogeny and historical biogeography of the Indonesian freshwater fish *Rasbora lateristriata* species complex (Actinopterygii: Cyprinidae): cryptic species and west-to-east divergences. *Mol Phylogenet Evol.* 2016;105: 212-23.
- Kusuma WE, Samuel PD, Wiadnya DGR, Hariati AM, Kumazawa Y. Complete mitogenome sequence of *Rasbora argyrotaenia* (Actinopterygii: Cyprinidae). *Mitochondrial DNA B Resour.* 2017;2:373-4.
- Lumbantobing DN. Four new species of *Rasbora* of the Sumatran group (Teleostei: Cyprinidae) from northern Sumatra, Indonesia. *Zootaxa.* 2014;3764:1-25.
- Manganang YAP, Hananya A, Pujiyati S, Retnoaji B. Bio-fuel algal waste diet effect on growth and histological structure of wader pari (*Rasbora lateristriata* Bleeker, 1854) intestine. *IOP Conf Ser Earth Environ Sci.* 2020;429:012028.
- Mccusker MR, Bentzen P. Positive relationships between genetic diversity and abundance in fishes. *Mol Ecol.* 2010;19:4852-62.
- Muchlisin ZA. Morphometric variations of *Rasbora* group (Pisces: Cyprinidae) in lake Laut Tawar, Aceh Province, Indonesia, based on truss character analysis. *Hayati J Biosci.* 2013;20:138-43.
- Munthe I, Isa M, Winaruddin W, Sulasmi S, Herrialfian H, Rusli R. Protein content analysis of depik (*Rasbora tawarensis*) in Laut Tawar lake Aceh Tengah. *J Med Vet.* 2016;10:67-9.
- Muslimin B, Rustadi, Hardaningsih, Retnoaji B. Morphometric variation of cork fish (*Channa striata* Bloch, 1793) from nine populations in Sumatra island, Indonesia. *Iran J Ichthyol.* 2020b;7:209-21.
- Muslimin B, Rustadi, Hardaningsih, Retnoaji B. Polymorphism in exon 4 of snakehead fish (*Channa striata*) growth hormone gene from Sumatra (Indonesia) and its association with growth traits. *AAFL Bioflux.* 2020a;13:3163-74.
- Nei M. Genetic distance between populations. *Am Nat.* 1972;106:283-92.
- Pazo F, Sánchez S, Posner V, Sciara AA, Arranz SE, Villanova GV. Genetic diversity and structure of the commercially important native fish pacu (*Piaractus mesopotamicus*) from cultured and wild fish populations: relevance for broodstock management. *Aquac Int.* 2021;29:289-305.
- Pinsky ML, Palumbi SR. Meta-analysis reveals lower genetic diversity in overfished populations. *Mol Ecol.* 2014;23:29-39.
- Prasetyo A, Retnoaji B. Migration biomodelling of wader pari fish (*Rasbora lateristriata* Bleeker, 1854) toward varied current direction and substrate type. In: Proceedings of the 6th International Conference on Biological Science; 2019; Yogyakarta, Indonesia.
- Ramadhaniaty M, Setyobudiandi I, Madduppa HH. Morphogenetic and population structure of two species marine bivalve (Ostreidae: *Saccostrea cucullata* and *Crassostrea iredalei*) in Aceh, Indonesia. *Biodiversitas J Biol Divers.* 2018;19:978-88.
- Retnoaji B, Nurhidayat L, Husni A, Suwarman. Cultivation and conservation of Indonesian native fish (*Rasbora lateristriata*) through fish farmer group empowerment in Yogyakarta. In: Proceedings of the 1st International Conference on Tropical Agriculture; 2017; Yogyakarta, Indonesia.
- Šegvić-Bubić T, Taližančić I, Grubišić L, Izquierdo-Gomez D, Katavić I. Morphological and molecular differentiation of wild and farmed gilthead sea bream *Sparus aurata*: implications for management. *Aquac Environ Interact.* 2014;6:43-54.
- Sholihah A, Delrieu-Trottin E, Sukmono T, Dahruddin H, Risdawati R, Elvyra R, et al. Disentangling the taxonomy of the subfamily Rasborinae (Cypriniformes, Danionidae) in Sundaland using DNA barcodes. *Sci Rep.* 2020;10:2818.
- Sogandi S, Sanjaya RE, Baity N, Syahmani S. Identification of nutritional content and profiles of amino acids from selling fish (*Rasbora* sp.). *J Nutr Food Res.* 2020; 42:73-80.
- Turan C. A note on the examination of morphometric differentiation among fish populations: the truss system. *Turk J Zool.* 1999;23:259-64.
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australia's fish species. *Philos Trans R Soc B Biol Sci.* 2005;360:1847-57.
- Whan-Air W, Thongprajukaew K, Salaeharæ T, Yoonram K. Identification of wild and farmed broadhead catfish (*Clarias macrocephalus* Günther, 1864) based on morphometry, digestive indexes and flesh quality. *J Oceanol Limnol.* 2018;36:1788-97.
- Willing EM, Dreyer C, van Oosterhout C. Estimates of genetic differentiation measured by F_{ST} do not necessarily require

large sample sizes when using many SNP markers. PLOS ONE. 2012;7:e42649.

Zhang X, Wu W, Li L, Ma X, Chen J. Genetic variation and relationships of seven sturgeon species and ten interspecific hybrids. Genet Sel Evol. 2013;45:21.

Zulfadhli Z, Wijayanti N, Retnoaji B. The development of ovarian wader pari fish (*Rasbora lateristriata* Bleeker, 1854): histological approach. J Trop Fish. 2016;3:32-9.