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Research Article

Morphometric and Genetic Characterization of Dominant Fish Species in Progo River, Yogyakarta, Indonesia

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A study on the morphology and genetics of the three species of dominant wader that live in the Progo River has been conducted. This study aimed to identify the three kinds of dominant waders in the Progo River, their morphology, and their genetics to ensure their right identity. Fish characterization was done by morphological analysis using the *truss-morphometric* method and genetic analysis using molecular markers. Random amplified polymorphic DNA (RAPD) based on PCR was used for diversity identification for interspecies and between species. Several analyses of water quality parameters (temperature, nitrate, phosphate, and ammonia) were also carried out to determine the habitat's suitability for the fish's survival. Based on the results of discriminant function analysis, all wader fish from the Progo River used in this study were classified into three species with the highest differentiation character D4 (distance between the starting point of upper tail fin and the starting point of lower tail fin). Results also show that *wader abang* fish have similar morphometric characteristics and DNA closer to *wader kepe* compared to *wader pari*. The length-weight relationship of wader fish from the Progo River shows that the growth pattern of *wader abang* and *wader kepe* is allometric positive, while that of *wader pari* is allometric negative. The value of the condition factor for wader fish from the Progo River indicates that the environmental habitat of wader fish is good and stable so that it can support the fish growth.

1. Introduction

Wader, a local term for fish belonging to the family of Cyprinidae, is a dominant freshwater fish species living in the Progo River (the longest river in Yogyakarta) [1]. It has become a special raw material for favorite culinary foods in Yogyakarta. Fish is typically prepared in various ways, from simply fried in vegetable oil to cooking with many types of spices [2]. People are eager to consume it due to its delicious and savory taste. Many restaurants in Yogyakarta feature wader dishes as their main menu. Wader is caught directly from its natural habitat [3]. This exploitation has led to a continuous decline in the fish population in the river [4].

To protect these species from extinction and explore possibilities for development (such as domestication and breeding), it is imperative to conduct a study to gather data, with a particular focus on morphological and genetic information.

Preliminary studies have revealed that the wader population in the Progo River comprises several species known locally as *wader pari*, *kepe*, *melem*, *abang*, *bader*, *palung*, and *cakul*. But the three dominant species of *wader* are *wader abang*, *wader pari*, and *wader kepe*. Although the three species have the same local name as wader, they have different morphological appearances. *Wader abang* is characterized by red fins, *wader pari* with blue-yellow stripes along their body, and *wader kepe* with yellowish fins and black stripes at the end of their fins. *Wader abang* is the biggest size among the three and *wader pari* is the smallest. Up to now, only those species mentioned above are known

by the people even though there might be some more species of *wader* that live in the Progo River. Studies on taxonomical and species identification are still limited. Therefore, a study on the morphology and genetics of the three species of *wader is* necessary to confirm their correct classification.

Fish can be characterized by analyzing their morphology through the truss-morphometric method and their genetics using molecular markers such as random amplified polymorphic DNA (RAPD) based on PCR [5, 6]. This study also analyzed condition factors and growth patterns (length-weight relationships) to determine the characteristics of wader fish based on their environment.

2. Materials and Methods

2.1. Sample Collection of Fish in Progo River. Samples used in this study were various wader fish species found in the middle area of the Progo River located around Nanggulan District, Kulon Progo, Special Region of Yogyakarta (7°45′14.1″S 110°13′13.4″E) (Figure 1). Fish were caught using fish nets by the local fishermen. Thirty fish of various sizes were randomly sampled in five locations, and their morphology was measured on the site. The total and standard lengths of the fish were measured using a ruler, and the weight was measured using a digital balance SF400C. The fish fins were taken and kept in 90% alcohol prior to DNA analysis. Water quality (temperature, pH, dissolved oxygen/DO, phosphate, nitrate, and ammonia) was measured or analyzed at the same time as the sample collected.

2.2. Morphological Analysis. Morphological analysis of wader fish was conducted using truss-morphometric analysis [7]. Each wader species with a complete body was selected as the fish sample. Then, truss points were selected based on [7]. The distance between specific points on the fish's body was measured by connecting certain points. Thirty fish of each type were randomly selected from small to large sizes, marked with a needle, and measured using the truss-morphometric method. Distance measurements between points on the fish's body were taken using a ruler. Each truss-morphometric character was divided by the fish's standard length. The fish body was divided into 4 parts (A: head, B: front body, C: rear body, and D: tail) with 10 truss points ((1) pectoral fin, (2) mouth, (3) pelvic fin, (4) gills, (5) base of the anal fin, (6) base of a dorsal fin, (7) end of the anal fin, (8) end of the pectoral fin, (9) lower base of the anal fin, and (10) upper base of the anal fin). The truss is connected, resulting in 21 morphometric truss characters that describe fish diversity (Figure 2). Measurement results of the truss-morphometric were analyzed using discriminant function analysis (DFA) in SPSS version 25.

2.3. Molecular Analysis of Wader Fish Using the Method of Random Amplified Polymorphic DNA (RAPD). Molecular characterization of wader fish was conducted using random amplified polymorphic DNA (RAPD) analysis. DNA was extracted using the method of TIANamp Marines Animal DNA Kit 180123. RAPD analysis was conducted using primers OPA-01 and OPA-02. The amplification process was performed using the method of polymerase chain reaction (PCR) RAPD with the composition of 3 μl DNA, 1.5 μl primer, 12.5 μl Taq DNA polymerase, and 8 μl distilled water. It was then put into a *thermocycler* with 1 denaturation cycle at 94°C for 2 min, 40 doubling cycles consisting of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2.5 min, and final extension at 72°C for 7 min. The PCR results were analyzed through the process of electrophoresis, which involved using a 1.5% agarose gel in Tris-Boric EDTA (TBE) buffer at a voltage of 100 V for 30 minutes. The marker used had a size range of 100–3000 bp and was made by Thermo Scientific. The electrophoresis results were visualized under UV light after staining the agarose gel with FluoroVue nucleic acid gel stain and were documented using gel doc.

The data analysis process was started by examining the presence of DNA fragments in the electrophoresis results of RAPD primers. Each fragment was considered a single locus and translated into binary data, where "1" indicated the presence of the fragment and "2" indicated its absence. TFPGA software was then used to analyze the binary data, generating a genetic distance value (D) [9]. A smaller genetic distance value indicates a greater number of shared fragments between individuals, whereas a larger genetic distance value implies a greater difference in fragments.

2.4. Length-Weight Relationship Analysis and Condition Factors of Wader Fish. Thirty fish of various sizes from small to large were randomly sampled in five locations within the Progo River, and their morphology, length, and weight were measured on the site. The fish's total length was measured from the mouth to tail using a ruler accurate to 0.5 mm in cm, and its weight was measured using a digital balance accurate to 0.01 g. The length-weight relationship was calculated following the [10] method.

$$W = aL^b, \tag{1}$$

where W = total weight (g), L = total length (cm), a = intercept, and b = slope.

The growth pattern of fish was determined from constant b (slope) calculated from length and weight through a hypothesis as follows: H0: if $b = 3$, the growth pattern is isometric (length growth equal to weight growth). H1: if $b \neq 3$, the growth pattern is allometric, namely, (a) if $b > 3$, allometric positive (weight through more dominant). (b) If $b < 3$, allometric negative (length growth more dominant). Furthermore, the condition factors of fish types were calculated using the condition factor equation of Fulton and relative weight. The Fulton condition factor (K) was calculated using the Okgerman [11] equation as follows:

$$K_f = \frac{W}{L_3} \times 100, \tag{2}$$

where K_f = Fulton condition factor, W = fish weight (g), and L = fish length (cm).

The relative weight condition factor of fish was calculated using the equation of [12] as follows:

Figure 1: Location of sample collection (circled).

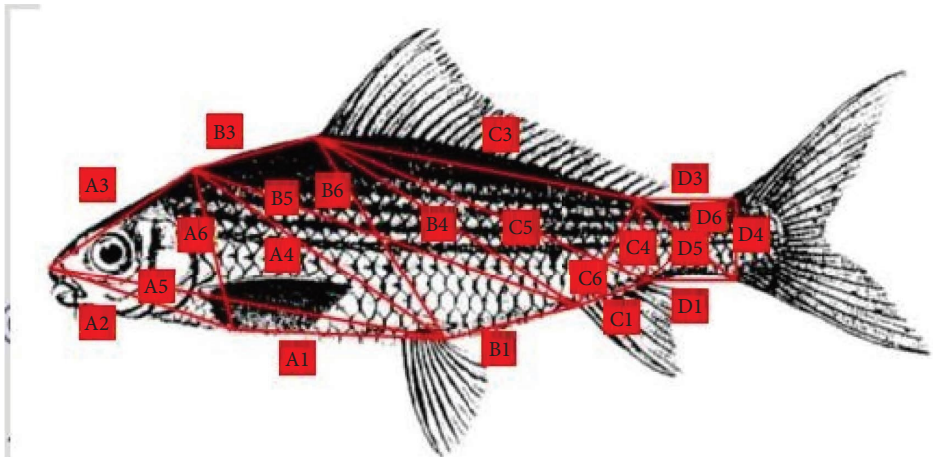

Figure 2: Determination of *truss-morphometric* points on fish [8]. Notes: A1: distance between the lower point of the pectoral fin and the endpoint of the pelvic fin. A2: distance between the lower point of the pectoral fin and the end of the mouth. A3: distance between end of the mouth and midpoint of the head and pectoral fin. A4: distance between the midpoint of the head and pectoral fin and end of the pelvic fin. A5: distance between the endpoint of the pelvic fin and the end of the mouth. A6: distance between the lower point of the pectoral fin and the midpoint of the head and pectoral fin. B1: distance between the endpoint of the pelvic fin and starting point of the anal fin. B3: distance between the midpoint of the head and pectoral fin and starting point of the pectoral fin. B4: distance between the starting point of the pectoral fin and starting point of the anal fin. B5: distance between the starting point of the anal fin and the midpoint of the head and pectoral fin. B6: distance between the starting point of the pectoral fin and the end of the pelvic fin. C1: distance between the starting point of the anal fin and the endpoint of the anal fin. C3: distance between the starting point of the pectoral fin and the end of the pectoral fin. C4: distance between the endpoint of the pectoral fin and the endpoint of the anal fin. C5: distance between the endpoint of the anal fin and starting point of the pectoral fin. C6: distance between the starting point of the anal fin and the end of the pectoral fin. D1: distance between the endpoint of the anal fin and the lower part of the anal fin. D3: distance between the end of the pectoral fin and starting point of the anal fin. D4: distance between the upper starting point of the tail fin and the lower starting point of the tail fin. D5: distance between the lower starting point of the tail fin and the end of the pectoral fin. D6: distance between the endpoint of the anal fin and the upper starting point of the tail fin.

$$\mathrm{Wr} = \left(\frac{W}{\mathrm{Ws}}\right) \times 100, \tag{3}$$

where Wr = relative weight condition factor, W = fish weight (g), and Ws = predicted fish weight based on the linear allometric model (LAM).

The results can then be compared with the results of other studies on the same fish species or results from different habitats.

2.5. Water Quality Measurement. As supporting data, condition factors of fish and water quality (temperature, pH, dissolved oxygen, nitrate, ammonia (NH_3), and phosphate) of the sampling location were analyzed. Before analysis, the river water was taken using a bottle sampler. The water quality was analyzed on-site, using a test kit. Ammonia was analyzed using Tetra Ammonia NH3.NH4 Test Kit, nitrate was analyzed using API Nitrate (NO_3) Test Kit, and phosphate was measured using a Compact Laboratory Water

Tester. All three test kits use reagents in the process, which create color in proportion to the compound amount or concentration measured. Dissolved oxygen (DO) was measured using a Lutron D5509 DO meter, while pH was measured using a Kedida CT 6022 pH meter, and the temperature was measured using a mercury thermometer. Both DO and pH meters were calibrated before use. The DO meter was calibrated following the calibration instruction manual, while the pH meter was calibrated using a pH buffer solution from pH 4.01, pH 6.86, and pH 9.18. The water quality results were compared to government regulation no. 22 of 2021 (water quality standards) in Appendix VI [13].

3. Results

3.1. The Morphological Character of Wader Fish. *Truss-morphometric* analysis was performed covering 21 characteristics of three dominant *wader* fish, i.e., *wader abang*, *wader pari,* and *wader kepe*. The mean, standard deviation, and coefficient of variation of the tested parameters are presented in Table 1.

The coefficient of variation of 21 morphometric characters of *wader* fish ranges from 12.03 to 56.79%. The character with the highest CV is C1 (distance from the base to the end of the anal fin), while the lowest is A4 (distance from the center between the head and the dorsal fin to the end of the pelvic fin) (Table 1).

The analysis of morphometric data uses discriminant function resulting in 2 functions, i.e., functions 1 and 2 (Table 2). Discriminant function analysis was conducted to determine the contributing function of grouping morphometric data into different groups. Function 1 with an *Eigenvalue* of 9.272 explains 83.5% of the *total variance*, and function 2 with an *Eigenvalue* of 1.832 explains the rest of 16.5% of the *total variance*. All characters contributed to function 1, and character D4 (distance between the upper base and lower base of the anal fin) was the highest contributor (Table 2).

Figure 3 shows that functions 1 and 2 differentiate wader fish into 2 different groups. Function 1 is grouping wader fish into 2 groups, i.e., *wader pari* on the left of 0 on the *X* axis (negative correlation) and *wader abang* as well as *wader kepe* on the right of 0 on the *X* axis (positive correlation). Function 2 also differentiates wader fish into 2 groups, i.e., one group above 0 on the *Y* axis (positive), i.e., *wader abang*, a small portion of *wader kepe*, and a portion of *wader pari* and one group below 0 on the *Y* axis (negative), i.e., *wader kepe* and part of *wader pari*. Though they are differentiated into different groups, *wader abang* and *wader kepe* still intersect each other.

The classification result of the discriminant canonical function of *wader* fish from the Progo River is presented in Table 3. Predicted group membership in the classification was based on the value of the degree of morphometric character similarity measured in the analysis [10]. The results of classification showed that 96.6% of the morphometric data of wader fish from Progo River were able to be classified. The population of *wader pari* was completely separated (100%) from the other population, and those of *wader abang* and *wader kepe* were 92.6% and 97%, respectively. There was a morphometric character similarity of 7.4% between *wader abang* and *wader kepe*, and on the other hand, there was a morphometric character similarity of 3% between *wader kepe* and *wader abang*.

3.2. The Genetic Character of Wader Fish from the Progo River Is Based on the Method of Random Amplified Polymorphic DNA (RAPD). The analysis result of RAPD is presented in Figure 4. OPA-1 and OPA-2 primers have fragments that can be used as differentiators among the tested wader fish. Some RAPD fragments amplified by OPA-1 and OPA-2 primers indicate that the three types of wader fish are different species. The three types of wader fish have their specific fragments, for example, amplification with OPA-1 primer results in 1200 and 1500 bp fragments which are found only in *wader pari*, 900 and 1100 bp fragments only found in *wader kepe*, or 700 bp fragments in *wader abang*. The same thing is also seen in the RAPD fragment which was simplified using the OPA-2 primer. *Wader abang* has a specific fragment of 1600 and 790 bp, wader pari 800 and 1500 bp, and *wader kepe* 550 and 900 bp.

In general, the RAPD fragment of *wader abang* which was amplified with OPA-1 and OPA-2 primers had many similarities with *wader pari* compared with *wader kepe* (Figure 4). This result can be seen in the genetic distance (Table 4). Based on genetic distance calculation, the three types of wader fish living in the Progo River had a relatively far distance from each other. The closest genetic distance was between wader *abang* and *wader pari,* i.e., 0.6439, followed by the genetic distance between *wader abang* and *wader kepe,* i.e., 0.7809, and the farthest was between *wader pari* and *wader kepe,* i.e., 0.7963.

3.3. Length-Weight Relationship and Condition Factor of Wader Fish. Based on the length-weight relationship analysis of *wader* fish, the *b* values of *wader abang* = 3.4311, *wader kepe* = 3.1618, and *wader pari* = 2.8705, so the equation of the length-weight relationship of the three fish was as follows: *wader abang* was $W = 0.0033L^{3.4311}$, *wader kepe* was $W = 0.0068L^{3.1618}$, and *wader pari* was $W = 0.0102L^{2.8705}$ (Figure 5). Based on a *t*-test with a 95% confidence level, the growth patterns of *wader abang* and *wader kepe* were allometric positive, i.e., the weight growth rate was higher than the length growth rate. In contrast, the growth pattern of *wader pari* was allometric negative meaning that the length growth rate was higher than the weight growth rate (Table 5).

The mean value of the condition factor of Fulton (K) for *wader abang* was 1.07 ± 0.17, while *wader kepe* was 0.02 ± 0.20 and *wader pari* was 0.77 ± 0.14 (Table 6). This result showed that *wader abang* had a round shape, while *wader* kepe and *wader pari* had a very flat shape. *Wader abang* had a relative weight condition factor (Wr) with a mean value of 99.47 ± 10.742, while *wader kepe* was 101.56 ± 19.590 and *wader pari* was 95.437 ± 25.266. This result showed that the surrounding water quality of *wader* fish was relatively good.

Table 1: Morphometric characters of *wader abang*, *wader pari*, and *wader kepe*.

Morphometric characters	Wader abang ($n = 27$)				Wader pari ($n = 28$)				Wader kepe ($n = 33$)			
	Min (cm)	Max (cm)	SD	CV (%)	Min (cm)	Max (cm)	SD	CV (%)	Min (cm)	Max (cm)	SD	CV (%)
A1	1.5	3.5	0.37	16.14	0.6	1.7	0.23	22.55	1.3	2.3	0.26	14.82
A2	1.4	3.5	0.46	20.92	0.4	1.3	0.23	23.23	1	2	0.27	16.48
A3	2.8	8.7	1.05	16.99	1.5	3.7	0.22	21.56	3.5	5.3	0.53	12.43
A4	1.5	3.9	0.51	19.11	0.5	1.5	0.27	26.76	1.4	2.2	0.22	12.03
A5	2.4	6.2	0.86	21.24	0.9	2.3	0.24	24.28	1.9	3.6	0.45	17.04
A6	3.1	7.5	1.02	19.68	1	2.9	0.24	24.01	2.7	4.4	0.44	12.72
B1	2.5	6.8	0.95	19.82	1	2.7	0.21	21.05	2	3.9	0.53	18.79
B3	4.2	11.1	1.58	20.52	1.7	4.5	0.26	25.8	3.8	6.5	0.73	14.57
B4	2.6	6.7	0.99	21.37	0.8	2.2	0.27	27.32	2.1	3.7	0.5	16.49
B5	1.7	4.3	0.69	22.69	0.6	1.9	0.32	31.85	1.1	2.9	0.48	23.51
B6	2.7	7.9	1.12	21.89	0.8	2.5	0.38	37.5	2.6	4.4	0.49	14.72
C1	1.2	3.4	0.43	20.52	0.4	1.7	0.57	56.79	0.8	2.3	0.34	23.49
C3	2.9	8.1	1.14	21.69	1	2.9	0.34	34.41	2.6	4.6	0.56	15.56
C4	2	5.7	0.83	22.23	0.7	1.7	0.25	25.01	1.8	3.2	0.35	14.65
C5	0.8	3.4	0.49	29.55	0.3	0.9	0.24	23.71	0.6	1.9	0.24	21.19
C6	1.8	5.4	0.78	22.6	0.7	2	0.27	27.32	1.2	3.7	0.47	19.92
D1	2.3	5.9	0.82	18.86	1.1	2.7	0.26	26.15	2	3.9	0.49	15.99
D3	2.8	6.9	0.91	18.13	1.3	2.9	0.24	23.99	2.4	4.5	0.52	14.94
D4	1.9	4.1	0.49	15.27	0.8	1.8	0.22	22.34	1.5	2.6	0.31	14.78
D5	1.4	2.8	0.37	15.61	0.6	1.6	0.3	29.62	1.2	2.1	0.25	15.55
D6	1.2	3.2	0.46	21.96	0.5	1.1	0.22	22.12	0.8	1.9	0.24	19.74

Table 2: Structure matrix of ordered correlation between measurement characteristics and functions for morphometric characteristics.

Functions	1	2
Eigenvalue	9.272	1.832
% of variance	83.5	16.5
% of cumulative	83.5	100
Canonical correlation	0.950	0.804
D4	0.723*	0.235
A4	0.678*	0.026
A3	0.668*	0.136
C4	0.666*	0.101
B4	0.662*	0.072
D3	0.649*	0.051
A6	0.646*	0.120
D5	0.638*	0.114
B6	0.634*	0.063
A1	0.605*	−0.052
D6	0.600*	0.293
B3	0.598*	0.163
B1	0.598*	0.328
C3	0.594*	0.046
D1	0.587*	0.039
C6	0.557*	0.044
A2	0.552*	−0.037
A5	0.538*	0.199
B5	0.468*	0.098
C1	0.465*	0.043
C5	0.453*	0.075

Notes: *gives a contribution to morphometric character differences of wader fish.

3.4. Water Quality of the Progo River. The water temperature of the Progo River measured during fish sampling was 26.9–27°C, pH was 6–6.5, dissolved oxygen (DO) was 7.1–7.7 mg/L, nitrate was 5 mg/L, phosphate was 0.5 mg/L, and ammonia was 0.25 mg/L (Table 7). Measurement was conducted at 11.30 WIT at the location of 7f 7°45′14.3″S 110°13′13.3″E.

4. Discussion

4.1. The Morphological and Genetic Characteristics of Wader Fish in the Progo River. Wader fish is one of the freshwater fish types found in the Progo River which has a high economic value and is very potential to be developed. This fish is caught or harvested directly from its natural habitat resulting in a continuously decreasing stock in the river. To protect this species from extinction, a study to obtain data and information about its biological condition needs to be conducted. Therefore, the preservation of the existence of wader fish through domestication and breeding programs starts with collecting information and a genetic database of wader fish living in the Progo River through analysis of morphological characteristics (truss-morphometric) and their genetic (RAPD).

Based on truss-morphometrics analysis, the coefficient of diversity (CV) and the characteristics of *wader abang*, *wader kepe*, and *wader pari* in the Progo River have high averages, i.e., ranging between 12.03 and 56.6%. This CV value is higher than the CV of the other fish such as rice field eel (3.01–14.65%) [14], *tengadak* fish (2.24–12.76%) [15], *kelabau padi* fish (6.89–10.77%) [16], *nilem* (3.38–10.35%) [17], and gourami 10.5% [18]. The diversity coefficient value of a character indicates the diversity level of a population [15]. Reference [19] stated that morphological diversity is influenced by some factors, namely, genetic factors inherited by the parent, adaptation of body form, color, and fin at the environmental condition in which they live, and adaptation

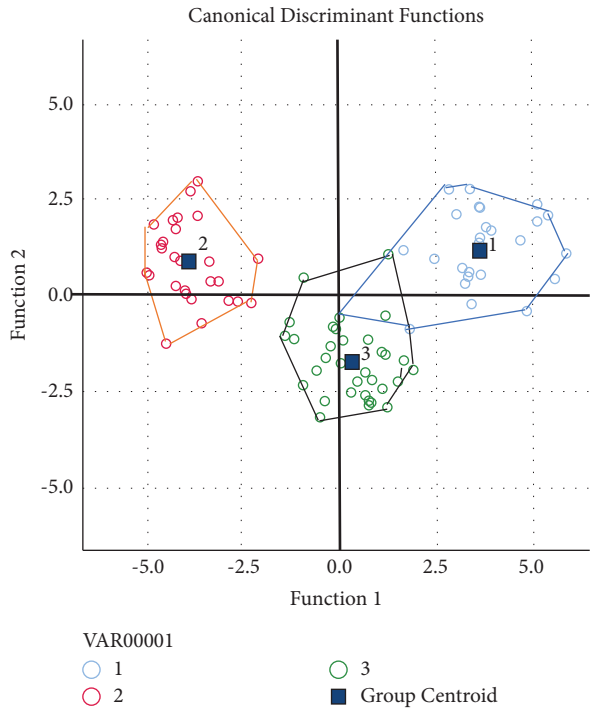

Figure 3: The discriminant canonical function of wader fish from Progo River. 1 = *wader abang* (blue); 2 = *wader pari* (red); 3 = *wader kepe* (green).

Table 3: Classification results of wader fish from the Progo River based on morphometric characteristics.

Groups	Species		Predicted group membership			Total
			Wader abang	Wader pari	Wader kepe	
Original	Count	Wader abang	25	0	2	27
		Wader pari	0	27	1	28
		Wader kepe	1	0	33	33
	%	Wader abang	92.6	0	7.4	100.0
		Wader pari	0	100	0	100.0
		Wader kepe	3.0	0	97	100.0

Note. 96.6% of original grouped cases were correctly classified.

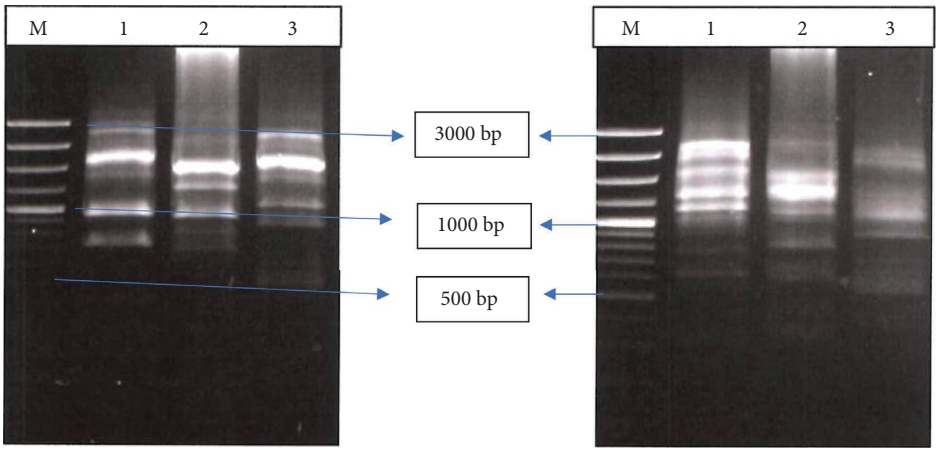

Figure 4: Amplification results of wader fish using OPA-1 primer (left) and OPA-2 primer (right). 1 = *wader abang*; 2 = *wader pari*; 3 = *wader kepe*; M = marker.

Table 4: Genetic distance (D) of three types of *waders* in the Progo River.

Fish species	Genetic distance (D)		
Wader abang	*****		
Wader pari	0.7809	*****	
Wader kepe	0.6439	0.7963	*****

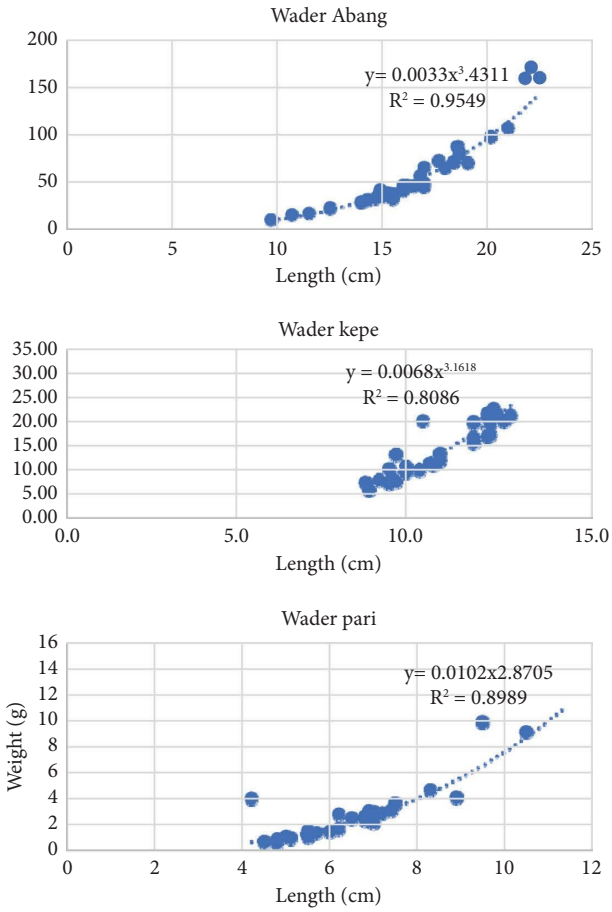

Figure 5: Length-weight relationship of *wader* fish in the Progo River.

Table 5: The growth pattern of *wader* fish in the Progo River is based on the length-weight relationship.

Fish species	N	A	b	r	R^2	Growth pattern
Wader abang	35	0.0033	3.4311	0.984	0.9549	Positive allometric
Wader kepe	32	0.0068	3.1618	0.918	0.8086	Positive allometric
Wader pari	32	0.0102	2.8705	0.852	0.8989	Negative allometric

Table 6: Condition factor of Fulton (K) for *wader* fish in the Progo River.

Fish species	Conditions factor			
	Fulton (K)		Relative weight (Wr)	
	Range	Mean	Range	Mean
Wader abang	0.819–1.574	1.07 ± 0.17	76.09–125.56	99.47 ± 10.74
Wader kepe	0.793–1.736	1.02 ± 0.20	81.87–174.54	101.56 ± 19.59
Wader pari	0.524–1.162	0.77 ± 0.14	62.998-151.370	95.437 ± 25.27

Table 7: Water quality of the Progo River.

Replications	Parameters					
	Temperature (°C)	pH	DO (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Ammonia (mg/L)
1	26.9	6.5	7.7	5	0.5	0.25
2	27	6	7.4	5	0.5	0.25
3	27	6	7.1	5	0.5	0.25

of head form for feed processing. The high morphological diversity becomes a chance to increase fish genetic variation. As mentioned by [20], the higher the CV value of a species, if there is crossbreeding among populations, the higher the chance of gene exchange among the populations.

Discriminant function analysis on the morphometric data aims to determine if a character can be used to differentiate a species from a population which is very hard to differentiate [12]. In this study, discriminant function analysis was able to differentiate *wader* fish in the Progo River into three groups/populations. *Wader abang*, *wader kepe,* and *wader pari* form their character groups though there is an intersection of *wader abang* and *wader kepe* (Figure 2 and Table 4). This intersection shows that an individual has the same morphometric character between *wader abang* and *wader kepe*. Based on the classification result, there is a 7.4% similarity between *wader abang* and *wader kepe* and there is a 3% similarity between *wader kepe* and *wader abang*. In line with morphometric analysis, the results of RAPD analysis show that the three wader fish from the Progo River have a long genetic distance, i.e., 0.6439–0.7890 (Table 7). This result supports the assumption that the three-wader types in the Progo River are different species. Genetic distance can describe the pattern of kinship among populations. The smaller the genetic distance (D), the closer the kinship between two populations and vice versa [21]. The genetic distance of *wader* fish in this study is longer than that of *ringau* fish (*Datnioides microlepis*), i.e., 0.1248 [22], giant snakehead (*Channa micropeltes*), i.e., 0.2471 [23], black and red *nila* (*Oreochromis* sp), i.e., 0.1414–0.6553 [24], *tengadak* fish (*Barbonymus schwanenfeldii*), i.e., 0.48–0.55 [25], *butini* fish (0.2114–0.6272) [26], and Malaysian river catfish (*Mystus nemurus*), i.e., 0.390–0.635 [27]. The genetic distance of the three wader fish in this study is close to those of *nilem hijau* and *nilem were* or *nilem beureum panon* in West Java, which suggests that the three types of the *nilem* fish are three different species [28].

Based on canonical function discriminant and classification results, *wader abang* fish has a higher morphological similarity with *wader kepe* than *wader pari*. This result is in line with the result of RAPD analysis in which, based on molecular analysis, the genetic distance between *wader abang* and *wader kepe* is more than that of *wader pari*. It shows that, genetically, *wader abang* fish has higher similarity with *wader pari*. This difference is suspected that *wader kepe* and *wader abang* have the same genera of *Barbus*, but *wader pari* belongs to the genera of *Rasbora*. On observation of color and body shape based on the existing references, *wader abang* has morphological similarity with *balar/brek* (*Barbonymus ballaroides* or *Barbus bramoides*) and *wader kepe* is similar to *Barbus marginatus* or *Mystacoleucus marginatus,* while *wader pari* has morphological similarity with *Rasbora* sp (Figure 6).

4.2. Length-Weight Relationship and Condition Factor of Wader Fish. Length-weight relationship character of fish becomes an important morphometry parameter in fisheries resource management [32–34]. This information can be used as basic knowledge for explaining the differences between the application of length size and standardization of length size in growth comparison studies [35, 36]. They are also used to compare different populations or fish species [37].

The length-weight relationship of wader fish in the Progo River has been conducted to determine their growth pattern. *Wader abang* and *wader kepe* have an allometric growth pattern positive with a "*b*" value of 3.4311 and 3.1618, respectively, while *wader pari* has an allometric negative with a "*b*" value of 2.8705. The differences in the growth pattern of *wader abang* and *wader kepe* with *wader pari* are suspected due to the difference in activity that *wader abang* and *wader kepe* are passive swimmers, while *wader pari* is an active swimmer which needs much energy to move, so it has negative allometric growth. According to [38], active swimmer fish have a *b* value relatively lower than passive swimmer fish. This is related to the energy used for movement and growth [39]. Other than movement, gonad development, feed availability, and body size variation have been reported to affect the *b* value [40, 41]. The positive allometric growth pattern of *wader abang* and *wader kepe* in this study is similar to that of female *Bader* fish (*Barbonymus ballaroides*) [42], lalawak fish (*B. ballaroides*) which have been domesticated [43], *wader bintik dua* [44], *bilih* fish (*Mystaecoleucus padangensis*) [45], and male *gengehek* fish (*M. marginatus*) [46, 47]. Furthermore, the growth pattern of *wader pari* with the allometric negative is in line with the growth pattern of *depik* fish (*Rasbora tawarensis*) [48] and *wader pari* fish (*R. argyrotaenia*) [39, 49–52] as well as *Rasbora* fish [53].

The length-weight relationship chart (Figure 5) shows the value of the correlation coefficient (*r*) of *wader abang* fish, *wader kepe,* and *wader pari* which are close to 1 (0.852–0.984). It shows that there is a strong correlation between the length and weight of *wader* fish in which the length increment affects the weight of the fish. This is in line with the opinion of [44] that the correlation coefficient value of the length-weight relationship is close to 1 showing that the increment of length will affect the increment of weight. The determination coefficient (R^2) of wader abang fish in this study is 0.9549, meaning that 95.49% of the fish weight is

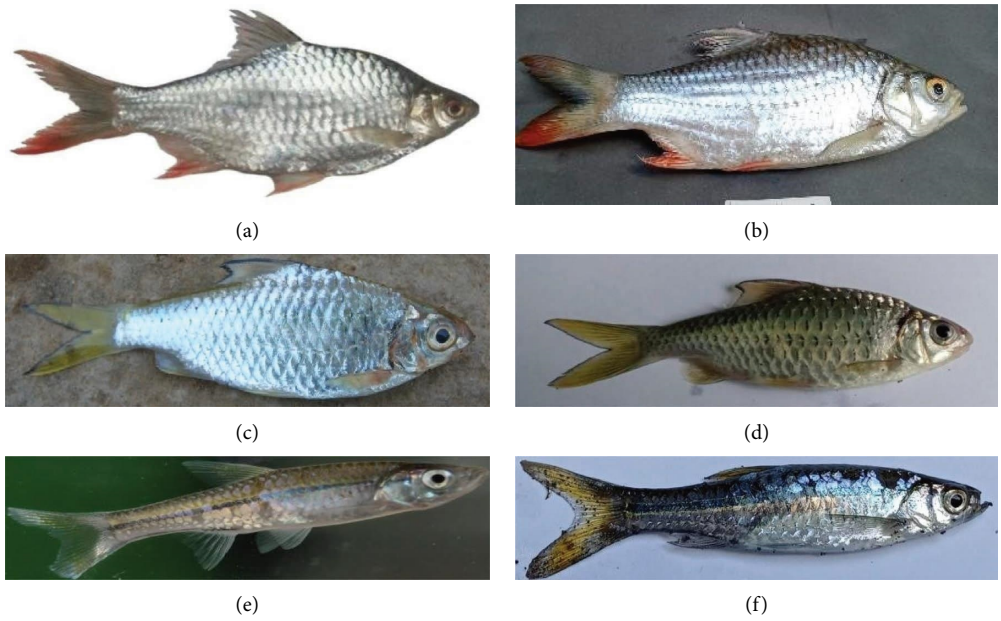

Figure 6: Observation of color and body shape of wader fish based on the existing references. (a) Source: [29]. (b) Source: primary data, 2022. (c) Source: [30]. (d) Source: primary data, 2022. (e) Source: [31]. (f) Source: primary data, 2023.

affected by the length of the fish while 4.51% is affected by some other unknown factors. *Wader kepe* has an R^2 value of 0.8086 meaning that the fish weight is affected by the fish length of 80.86% and 19.14% is affected by some other unknown factors. *Wader pari* fish has an R^2 value of 0.8989 meaning that 89.89% of the fish weight is affected by its length and 10.11% by some other unknown factors.

The condition factor is a very important factor used to evaluate fish population and can be used as an indicator to estimate fish body shape [54, 55]. The mean value of the Fulton condition factor (KTL) of *wader abang* fish is 1.07 and *wader kepe* fish is 1.02, higher than the mean value of the condition factor of *wader pari* (0.77). The shape of *wader pari* which is long and flat probably becomes a reason why its *K* value is low; this is to the finding of [9], who stated that fish with flat shapes tend to have a smaller condition factor.

Other than predicting the fish's shape, condition factors can be used to describe a fish's health status [56]. The mean value of relative condition factor (Kr) *wader* fish from the Progo River is 95.435%–101.56%. According to [57], the relative condition factor is close to 100, showing that the fish are in very good condition and there is a balance between prey and predator in the fish habitat environment. Kr value of the three types of *wader* from the Progo River which is close to 100 indicates that the waters in the Progo River are still in good condition and stable enabling support for the growth of the *wader* fish. This can be seen from the physicochemical parameters of waters in Progo River which are suitable for a fish environment with an average pH of 8.8–8.9, temperature of 26-27°C, dissolved oxygen (DO) of 7.4 mg/L, ammonia of 0.25 mg/L, nitrate of 5 mg/L, and phosphate of 0.5 mg/L (Table 7).

5. Conclusion

Based on the results of discriminant function analysis, all wader fish from the Progo River used in this study were classified into three species with the highest differentiation character D4 (distance between the starting point of the upper tail fin and the starting point of the lower tail fin). Results also show that *wader abang* fish have similar morphometric characteristics and DNA closer to *wader kepe* than to *wader pari*.

The length-weight relationship of wader fish from the Progo River shows that the growth pattern of *wader abang* and *wader kepe* is allometric positive, while that of *wader pari* is allometric negative. The value of the condition factor for wader fish from the Progo River indicates that the environmental habitat of wader fish is excellent and stable so that it can support the fish growth.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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