DOI: http://dx.doi.org/10.15578/iaj.19.2.2024.133-145 *Indonesian Aquaculture Journal, 19 (2), 2024, 133-145*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

MORPHOMETRIC AND MOLECULAR VARIATION OF NEON TETRA FISH (*Paracheirodon innesi*) FROM BOJONGSARI DISTRICT, INDONESIA

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(Received: May 2, 2024; Final revision: July 26, 2024; Accepted: July 26, 2024)

ABSTRACT

Neon tetra, Paracheirodon innesi, is endemic to rivers and streams in southeastern Colombia, eastern Peru, and western Brazil and is commercially traded as aquarium fish in the world. In Indonesia, neon tetras were mass produced in Bojongsari District, Depok, West Java as the centre of neon tetra fish production. Understanding their genetic variation is useful for implementing their selective breeding programs, environmental restoration, and estimating genetic contributions in stocks. The current study aimed to investigate the patterns of morphometric and molecular diversity using randomly amplified polymorphic DNA-polymerase chain reaction techniques among farmed broodstocks of P. innesi in Indonesia. Three populations, namely: the Bojongsari, Curug, and Pondok Petir derived from the Bojongsari District, Depok, west Java, were used in the study. Thirty live fish from each location were analyzed based on 25 truss morphometric characters. Sixty fresh fish samples were obtained for DNA analysis using the RAPD-PCR technique, which uses three random primers. Principal component analysis (PCA) was performed to distinguish morphometric variations among populations. Morphological and molecular analysis displayed a similar result that Bojongsari and Pondok Petir neon tetra fish had high similarities, while Curug neon tetra was distinguished from others. The closest genetic distance was between the Pondok Petir and Curug populations (0.4088), while the farthest genetic distance was between the Curug and Bojongsari populations (0.4138). The results will be useful in developing breeding programs to improve broodstock quality.

KEYWORDS: Molecular variation; neon tetra; *Paracheirodon innesi*; truss morphometric; RAPD

INTRODUCTION

Neon tetra, *Paracheirodon innesi*, is one of the most popular freshwater ornamental fish among hobbyists and is commercially traded as aquarium fish in the world (Balon, 2004; Dey, 2016). Neon tetra is highly valued for its tiny and small-bodied, vibrant color, calm behavior, and interactive displays when kept in groups (Avianty *et al*. 2017; Kucharczyk *et al*., 2010). Neon tetra belongs to the Characin family (family Characidae) of order Characiformes are endemic to the Amazon Basin of South America, covering rivers and streams in southeastern Colombia, eastern Peru, and western Brazil (Myers, 1936; Weitzman & Fink, 1983). The neon tetra is a brightly colored freshwater fish with a silver-white abdomen and a dark olive-green back. Characteristics of this fish are the specific color as an iridescent blue-green stripe along each side of the fish from its nose to the base of the adipose fin and the wide brilliant red stripe extends posteriorly, starting from the middle of the body to the base of the caudal fin (Chapman *et al*., 1998).

Neon tetras, which are obtained from wild catches in the Amazon River along the Peruvian-Colombian border, were first traded as ornamental fish in the 1950s (Balon, 2004). Soon after, it was extensively farmed and exported as an ornamental fish in several countries, including Indonesia (Kusumah *et al*., 2022). In Indonesia, neon tetras are mass-produced by farmers and the production center is in Bojongsari District, Depok, West Java. Nonetheless, neon tetra fish farming is still confronted with challenges such as inferior quality broodstock, inadequate supply of high-quality seed, poor water quality, and disease outbreaks (Nurlaili *et al., 2021;* Sugiani *et al*., 2020). Inbreeding and the limited number of

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broodstock used for spawning are the causes of the decline in genetic variation, which affects important aquaculture production traits such as fecundity, survival, growth, and abnormalities (Ariyanto, 2019). Aquaculture practices such as the breeding of related individuals also may unintentionally contribute to a decrease in genetic variation in farmed populations (Butt *et al*., 2024).

According to Rajora & Mosseler (2001), to reduce losses, maintain natural levels of genetic diversity, and genetic integrity, as well as increase the genetic diversity of species, genetic resource conservation is needed. In the context of sustainable aquaculture, understanding genetic variation is useful for implementing selective breeding programs, restoring the environment, and estimating genetic contributions in stocks (Sonesson *et al*., 2023). By selecting breeding stocks based on genetic diversity and performance traits, aquaculturists can improve the productivity, growth, disease resistance and survival, and overall health of cultured fish populations (Farias *et al*., 2017).

According to Parenrengi *et al*. (2016), genetic variability can be determined by morphological characterization (morphometric analysis), allozyme electrophoresis (protein pattern), and DNA fingerprinting. Morphometry is widely recognized as the simplest and easiest technique for species identification, which can characterize morphological variation and assess any potential relationship between variation and genetic diversity (Ujjania & Chaudhari, 2021). Morphometric characterization visually demonstrates morphological differences based on landmarks and can explain the genetic differentiation and taxonomic status of a fish population based on similarities or differences in body shape (Asiah *et al*., 2019; Mohaddasi *et al*., 2013). Many previous studies have successfully used the morphometric methods to quantitatively evaluate the morphological differentiation among fish populations based on variations in external body morphology and structure such as African catfish *Clarias gariepinus* (Iswanto *et al*., 2015; Iswanto *et al*., 2016) and also to distinguish populations and species of fish, such as rasbora (Cyprinidae) (Muchlisin, 2013), shemaya *Alburnus chalcoides* (Mohaddasi *et al*., 2013), Indian major carp *Labeo rohita* (Amatya, 2021), and amphidromous goby (*Gobiiformes*) (Pasisingi *et al*., 2024).

At the molecular level, the valuation of genetic variations can be determined using information obtained from a variety of sensitive molecular techniques such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and microsatellites (Dudu *et al*., 2015). Although the microsatellites method has high sensitivity and accuracy, it is technically quite difficult to perform because it requires a lot of time and cost, especially in designing the primers (Mulyasari, 2007). As an alternative, PCR-based DNA fingerprinting techniques such as RAPD are still efficient in generating molecular markers that can be utilized to assess genetic variation both within and between stocks, populations, and species of ornamental fish (Khoo *et al*., 2011). Unlike other molecular markers, the RAPD approach does not require probes or knowledge of the genome (Babu *et al*., 2014; Mulyasari, 2007). The RAPD approach is low cost and easy to perform, give rapid results, required little DNA, yield high of DNA polymorphism, and easy to acquisition random primer (Ali *et al*., 2004; Nasution *et al*., 2021). Many studies of fish diversity using RAPD methods in many ornamental fish species such as rainbowfish *Melanotaenia ajamaruensis* (Hayuningtyas *et al*., 2018), giant snakehead *Channa micropeltes* (Muhajirah *et al*., 2021) and guppy (Mulyani *et al*., 2023) reported that this technique has successfully proven to differentiate populations and identify species.

The genetic diversity of *P. innesi* in Indonesian farms has not yet been assessed through morphological and molecular analysis although it is an important commercial species. Thus, for the first time, the current study aimed to investigate the phenotypic and molecular diversity among farmed broodstocks of *P. innesi* in Indonesia. Information on morphometric and genetic variations of neon tetra fish is important for their management to develop better conservation strategies, support breeding programs, and help to improve stock management of this species.

MATERIAL AND METHODS

Experimental species

The research was conducted over 6 months from January 2022 to June 2022. Neon tetra fish used in this study were domesticated broodstock from three different locations: Pondok Petir Village, Curug Village, and Bojongsari Village in Depok District, West Java Province (Figure 1). Thirty live fish from each location were collected for morphometric studies and twenty fresh fish samples from each location were preserved in 70% ethanol for isolation of genomic DNA. The fish sample used in this investigation had a total length of 3-4 cm.

Truss Morphometric Parameters

Each fish sample was placed on a petri dish and captured on camera with an Olympus SZX10 Stereo Microscope that was connected to a monitor, with a magnification of 0.8 × 0.5. Furthermore, truss points

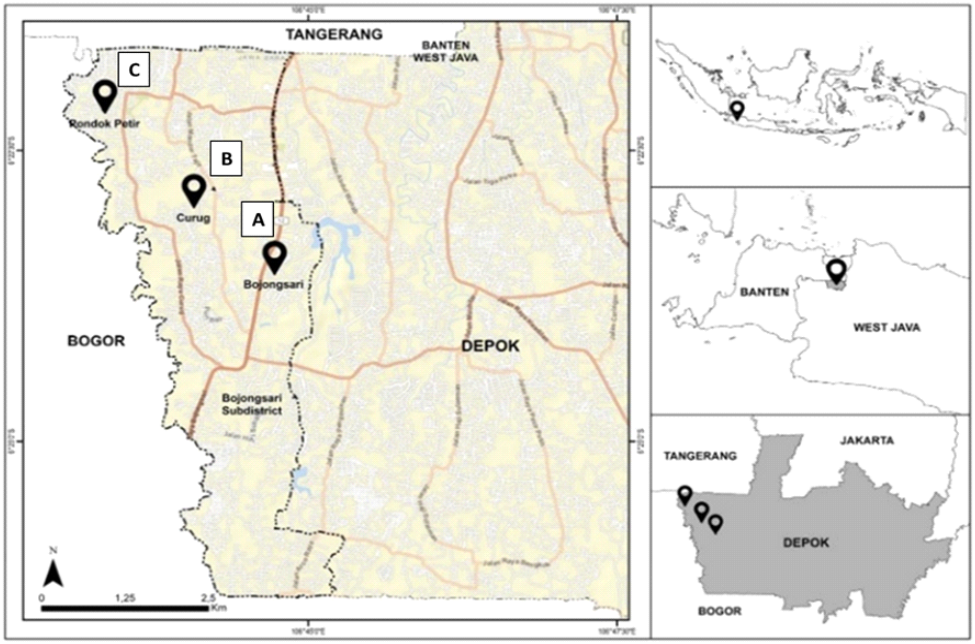

Figure 1. Three locations of neon tetra fish (*Paracheirodon innesi*) collection in Bojongsari District, West Java, Indonesia for the experiment (A: Bojongsari, B: Curug, C: Pondok Petir).

were marked on the image of the body fish using the *ImageJ* program according to the truss morphometric pattern. The morphometric data were measured from the left side of the fish body. Twenty-five linear measures of morphometric characters were measured based on a previous study (Campos *et al*., 2003) (Figure 2), that is landmark 1-2 snout tip to upper jaw tip; 1-3 snout tip to occiput; 2-3 upper jaw tip to occiput, 2-4 Upper jaw tip to pectoral fin origin; 2-5 Upper jaw tip to dorsal fin origin; 3-4 Occiput to pectoral fin origin; 3-5 Occiput to dorsal fin origin; 4-5 Occiput to dorsal fin origin; 4-6 Pectoral fin origin to pelvic fin origin; 4-7 Pectoral fin origin to posterior insertion of dorsal fin; 5-6 Dorsal fin origin to pelvic fin origin; 5-7 Basal length of dorsal fin; 6-7 Pelvic fin origin to posterior insertion of dorsal fin; 6-8 Pelvic fin origin to anal fin origin; 6-9 Pelvic fin origin to adipose fin origin; 7-8 Posterior insertion of dorsal fin to anal fin origin; 7-9 Posterior insertion of dorsal fin to adipose fin origin ; 8-9 Anal fin origin to adipose fin origin; 8-10 Basal length of anal fin; 8-11 Anal fin origin to posterior insertion of adipose fin; 9-10 Adipose fin origin to posterior insertion of anal fin; 9-11 Basal length of adipose fin; 10-11 Posterior insertion of anal fin to posterior insertion of adipose fin; 10-12 Posterior insertion of anal fin to mid caudal base; 11-12 Posterior insertion of adipose fin to mid caudal base.

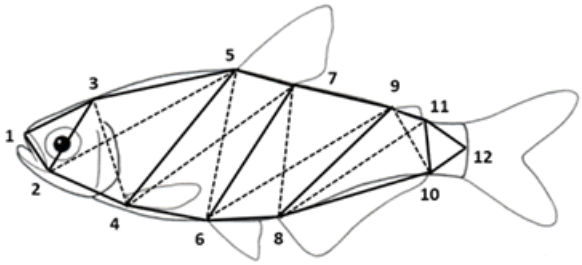

Figure 2. Truss morphometrics measured in neon tetra fish (*Paracheirodon innesi*) samples from Bojongsari District, West Java, Indonesia.

Molecular Analysis

The total genomic DNA was extracted from 10-25 mg muscle tissue according to the procedure of the gSYNC™ DNA Extraction Kit Quick Protocol (Geneaid, Taiwan). The extracted DNA was quantified by UV spectrophotometer analysis (*GeneQuant* 1300 *Spectrophotometer*) at 260 nm.

The PCR reaction was conducted in a thermal cycler (Applied Biosystems™ Veriti™ thermal cycler). For the RAPD analysis, three primers purchased from In-

tegrated DNA Technologies, Inc., USA were used (Table 1). A gradient thermocycler PCR was applied to obtain the best annealing temperature for each primer. PCR amplification was carried out at 94 °C for 2 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing for 1 min according to melting temperature (Tm) of each primer, extension at 72 °C for 2 min and final extension at 72°C for 7 min. Each reaction was performed in 25 µL reaction mixtures containing 12.5 µL Dream TaqMaster Mix 2x (Thermo Scientific, USA), 1 ìL primer (Operon Technologies Primer set A, 1st BASE Pte Ltd), 3 µL DNA, and 8.5 µL nuclease-free water.

A total of 6 µL of PCR product (containing loading dye in the master mix kit) from each sample was loaded into the wells of a 1.5% agarose gel containing 0.01% gel dye (PeqGreen, PeqLab Biotech, United Kingdom). Electrophoresis was performed with a 100 bp Plus marker (Vivantis) at a voltage of 100 volts for 60 minutes on 1´ tris borate EDTA (TBE)) media using PowerPac Basic (Bio-Rad). Furthermore, DNA visualization was performed using a UV transilluminator of the gel documentation system and the image was photographed using the gel documentation system (Gel Documentation-UV Transilluminator AlphaImager, Protein Simple).

The RAPD profile generated by each set of primers was scored based on the appearance of DNA fragments of an amplification product. The molecular weight of the amplification product was predicted using AlphaView SA software. Furthermore, each DNA fragment appearance was converted to binary data, the presence of a fragment was scored as 1 and the absence of it as 2.

Table 1. Primer used for RAPD-PCR amplification of neon tetra (*Paracheirodon innesi*) from Bojongsari District, West Java, Indonesia

Primer Code	Primer sequence (5' – 3')	Nucleotide length	G+C Content (%)	Temperature Melting (°C)
OPB-10	CTGCTGGGAC	10-mer	70	36.6
OPC-05	GATGACCGCC	10-mer	70	37.6
OPW-02	ACCCCGCCAA	10-mer	60	42.9

Data Analysis

In the present study, multivariate analysis PCA was conducted to discriminate the three populations and to observe the relationships among the fish samples and the 25 truss parameters. All the morphometric measurement data were divided by standard length (SL) and given as a ratio to standardize the variations in total body size among the individuals (Olopade *et al*., 2018). Statistical analyses for morphometric data were performed using the Statistical Program for Social Science (SPSS) version 16.1.0 software package and Microsoft Excel 2013. The level of genetic linkage between neon tetra fish populations was analyzed using the tools for population genetic analysis (TFPGA) program according to Wright (1978) modified from Rogers (1972) in Miller (1997). Dendrograms were constructed based on unweighted pair group arithmetic average (UPGMA) (Miller, 1997).

RESULTS AND DISCUSSION

Morphometric variation among the population

Based on truss morphometrics, we evaluated morphological variations among three populations of *P. innesi* collected in Subdistrict Bojongsari, Depok, Indonesia. The features in this analysis that had eigenvalues greater than one were kept, while the others were eliminated. In this study, seven components with eigenvalues >1 were found by PCA analysis of 25 morphometric measures, explaining 74.38% of the variance. The first principal component (PC1) accounted for 25.39% of the variation, second (PC2), third (PC3), fourth (PC4), fifth (PC5), and the sixth (PC6) for 13.22%, 9.29%, 7.48%, 7.09%, 6.48%, and 5.42%, respectively (Table 2).

According to Nimalathasan (2009), factor loading greater than 0.30 is considered significant, 0.40 is more important, and 0.50 or greater is very significant. Only the factors with loadings greater than 0.40 were considered significant in this investigation. This analysis confirmed that the variation in morphological measurements was evident in 11 landmarks, 2-5, 3-4, 4-5, 4-6, 4-7, 5-6, 6-7, 6-8, 6-9,7-8, 10-12 among different populations of neon tetra fish, that associated with head, anterior body length, mid-body length, and tail region. PC2 contributes nearly 13.22% of the total explained variance, which is much less compared to PC1. PC2 presented only eight strong loading characters (landmark 1-3, 2-3, 8-9,8-10, 8-11, 9-11,10-11, and 11-12) associated with head, posterior body length, and tail region (Table 2). Those characters are key morphological characters to discriminate between the three populations of neon tetra fish. These dis-

Table 2. Eigenvalues, percentage of variance, percentage of cumulative variance, and matrix structure for 7 PC in *Paracheirodon innesi* truss morphometric characters from three populations

Function	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalues	6.349	3.305	2.324	1.870	1.773	1.620	1.354
% of Variance	25.396	13.222	9.297	7.479	7.092	6.480	5.415
Cumulative %	25.396	38.618	47.915	55.394	62.486	68.966	74.380
1-2	-.144	.048	.426	-.004	.135	.606	-.165
1-3	-.237	.494	.291	.285	.139	.181	-.269
2-3	.056	.438	.232	.320	.305	.096	.209
2-4	-.198	.210	-.258	.497	.146	-.632	-.192
2-5	.490	.264	-.411	.169	-.222	-.282	.157
3-4	.552	.031	-.102	.381	.256	.013	-.003
3-5	.378	.134	-.199	-.480	-.326	.007	-.150
4-5	.873	-.013	.011	-.259	-.055	.198	.093
4-6	.566	-.178	-.220	-.442	.369	-.038	.262
4-7	.885	-.045	-.065	-.218	.099	.154	.091
5-6	.913	-.087	.061	.052	.101	-.042	-.043
5-7	.303	-.037	-.031	.328	.630	.135	-.016
6-7	.918	-.055	.207	.153	-.042	.053	-.116
6-8	.587	-.209	.381	.317	-.426	.057	-.285
6-9	.729	.070	.255	.306	-.430	.131	-.184
7-8	.759	.091	.169	.046	.242	-.230	.206
7-9	.151	.330	-.061	.128	-.543	-.134	.288
8-9	.085	.814	-.043	.066	-.105	.081	.318
8-10	-.287	.499	-.395	.083	-.104	.494	.192
8-11	-.011	.904	-.053	-.122	.041	.173	-.020
9-10	.176	.249	.599	-.139	.167	-.266	.339
9-11	.107	.433	-.011	-.448	.232	-.106	-.545
10-11	.043	.616	.476	-.278	-.056	-.308	-.067
10-12	-.404	-.023	.665	-.220	-.028	-.278	.023
11-12	-.367	-.405	.434	.090	-.110	.135	.479

Description: Bold types indicated factors with loadings above 0.40 were considered significant (Mir *et al*., 2013).

tance characters obtained in this study are involved in body depth shape variation in the dorsoventral axis. A similar result has been reported in Indian major carp *Labeo rohita* (Amatya, 2021) and crucian carp *Carassius carassius* (Brönmark & Pettersson, 1994), where body depth was significant in the cultured fish under good food conditions, primarily in the quantity, type of food, and feeding method.

A definition of the population may be shown visually using score plots of samples that relate the first and second principal components. According to the PCA, the populations of Bojongsari and Pondok Petir are more similar than the population of Curug (Fig. 3). Similar trends were also observed in the cluster analysis for the Curug population, which could be differentiated from the Bojongsari and Pondok Petir populations (Fig. 4). It was considered that genetic isolation had occurred in the Bojongsari Subdistrict and that genetic structure had been effectively maintained to create distinct populations. Bojongsari and Curug populations are geographically remarkably close to each other, but they demonstrate different body shapes. This relation isn't affected by the geographical distance. A similar result was reported by Saad *et al*. (2009) on some of the populations of *C. gariepinus* collected from the wild. It could be explained by the possibility that transportation and mixing processes may dilute any differences in general phenotypic and genotypic characters.

This study suggested that morphologically distinct populations may result from local adaptation in response to environmental variables. In the previous study, Hossain *et al.* (2010) investigated three populations of *L. calbasu* from the Jamuna and Halda rivers as well as a hatchery using DFA and PCA analysis and found that morphological differentiation among them

was due to environmental conditions and local migration of the fish. According to Kusumah *et al.* (2021) and Mohaddasi *et al.* (2013), such variation in morphology is commonly due to the isolation of portions of a population within local habitat conditions, such as temperature, pH, turbidity, food availability, water depth, and flow. In the other study, Amatya (2021) reported that the body shape of fish can also be influenced by culture conditions, such as food type, quantity, and feeding schedule. For further, Oladimeji *et al.* (2020) and Zhang *et al.* (2023) reported that factors such as geographical barriers, geographic distance, watershed connections, environmental variations, and life history characteristics could all be factors contributing to population differentiation.

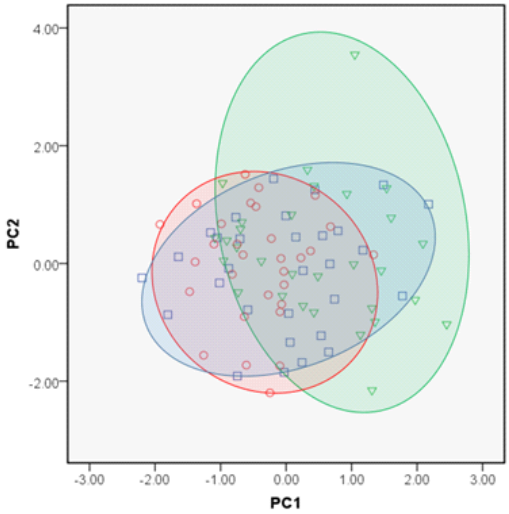

Figure 3. Principal component score plot of morphometric data of Neon Tetra (*Paracheirodon innesi*) in three populations (□Blue for Bojongsari, ▽Green for Curug, ○ Red for Pondok Petir).

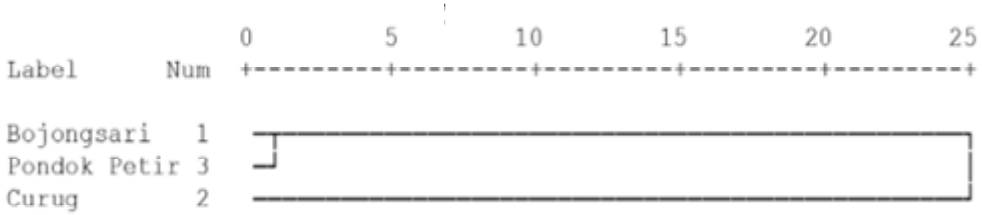

Figure 4. Cluster analysis of neon tetra (*Paracheirodon innesi*) from three different locations in Bojongsari District, Depok, Indonesia.

Genetic Variation among Population

In this study, the RAPD marker amplification patterns of the different *P. innesi* populations were investigated. The number of DNA fragments formed is determined by the sequence of nucleotides. In the present study, three different primers used in RAPD analysis- OPB-10 (Fig. 5), OPC-05 (Fig. 6), and OPW-02 (Fig. 7) -produced distinct polymorphic banding patterns in all the fish (N=60). A total of 51 scorable bands were observed using three random primers in three populations (Table 3). Neon tetra fish using primer OPW-02 had the greatest number of bands (23 bands), whereas primers OPB-10 and OPC-05 had the fewest bands (14 bands). The amplification results showed that the number of fragments and DNA size varied between 0-13 with a range of 0-2,800 base pairs. The number of fragments of neon tetra fish in the Bojongsari population is 0-12 fragments, the Curug population is 3-13 fragments, and the Pondok Petir population is 2-11 fragments. The highest fragment length range was obtained in the Bojongsari population (0-2,700 bp), followed by the Pondok Petir population (160-2,800 bp) and the Curug population (350-2,800 bp) (Table 3). The size, quality, and suitability of the location as well as the primer utilized are the main factors that affect the amplified band (Mulyani *et al.*, 2023). Due to variations in the primer attachment site nucleotide sequence, these fragments are generated in a variety of ways. The primary attachment sites are dispersed randomly over the genome, and variations in this region due to polymorphism will produce different amplifications. Thus, the primer and DNA template source contribute to the specificity of randomized DNA fragments (Hassan & Naeem, 2023).

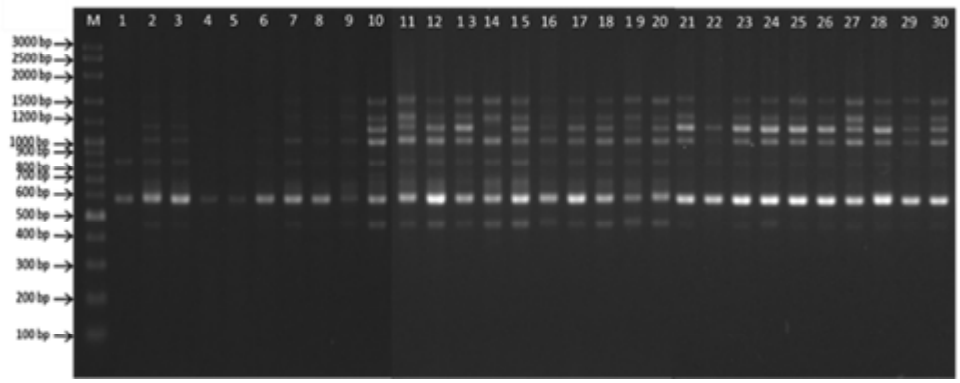

Figure 5. RAPD profile of DNA amplification resulted in three populations of neon tetra fish using primer OPB-10 (Lane 1-10 Bojongsari population, Lane 11-20 Curug population, Lane 21-30 Pondok Petir population.

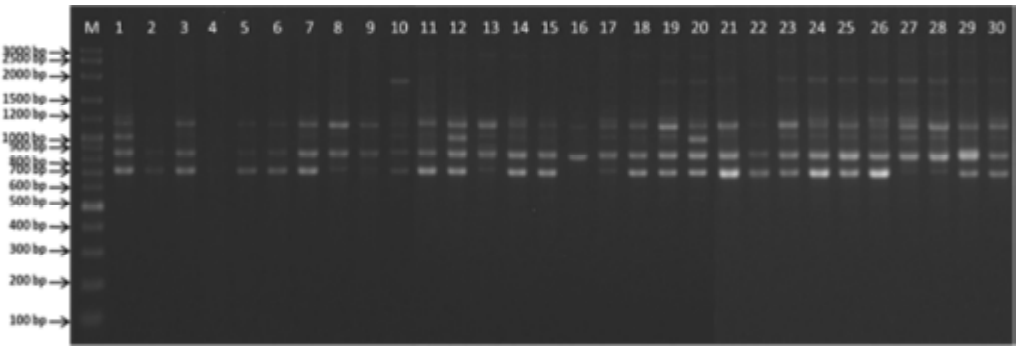

Figure 6. RAPD profile of DNA amplification resulted in three populations of neon tetra fish using primer OPC-05 (Lane 1-10 Bojongsari population, Lane 11-20 Curug population, Lane 21-30 Pondok Petir population).

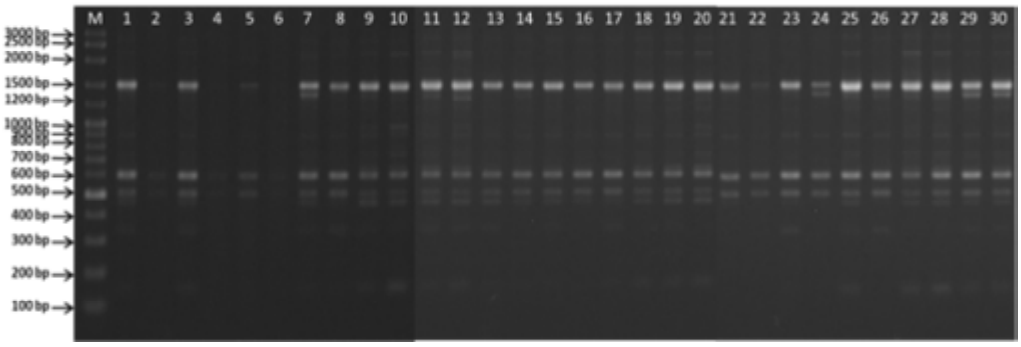

Figure 7. RAPD profile of DNA amplification resulted in three populations of neon tetra fish using primer OPW-02 (Lane 1-10 Bojongsari population, Lane 11-20 Curug population, Lane 21-30 Pondok Petir population).

Heterozygosity and the percentage of polymorphic locus between the three populations of neon tetra fish were displayed in Table 4. Neon tetra fish from the Bojongsari population had higher heterozygosity (0.20) compared to the Curug (0.18) and Pondok Petir (0.14) populations. A similar trend was also shown in the percentage of polymorphic, neon tetra fish in the Bojongsari population, which was larger (58.82%) than in the Curug (49.02%) and Pondok Petir (35.29%) populations. It indicates that neon tetras from the Bojongsari and Curug populations have higher genetic diversity than the Pondok Petir population. It indicates that neon tetras from the Bojongsari and Curug populations have higher genetic diversity than the Pondok Petir population. A different result has been reported by Hayuningtyas *et al*. (2018) on the ajamaru rainbowfish (*Melanotaenia ajamaruensis*), ajamaru rainbowfish cultured in Depok showed the higher genetic diversity with polymormic value was 73.43%, while wild rainbow ajamaru and

Table 3. Number and fragment length RAPD-PCR amplification product of neon tetra fish population, *Paracheirodon innesi.*

Primer	Fragment number			Fragment length (bp)			Number of Locus
	Bojong sari	Curug	Pondok Petir	Bojongsari	Curug	Pondok Petir	
OPB 10	1-9	7-8	4-7	450-1,600	450-1,600	450-1,500	14
OPC 05	0-6	3-10	3-9	0-1,900	700-2,800	550-1,900	14
OPW 02	2-12	6-13	2-11	350-2,700	350-2,800	160-2,800	23
Total	0-12	3-13	2-11	0-2,700	350-2,800	160-2,800	51

Table 4. Number of samples, heterozygosity, and percentage of polymorphic locus of three populations of neon tetra fish

Parameters	Population			Total sample/ Between population
	Bojongsari	Curug	Pondok Petir	
Number of samples (*ind.*)	20	20	20	60
Heterozygosity	0.20	0.18	0.14	0.26
Polymorphism (%)	58.82	49.02	35.29	78.43

ayamaru rainbowfish cultured in Papua with polymormic value was 62.5% and 70.31%, respectively. Ajamaru rainbowfish population have higher variation than neon tetra fish population, indicates that no inbreeding occurred when it's domesticated from the wild to the cultured condition. According to (Kusumah, 2020), the low variation in neon tetra populations in the Bojongsari Region can be caused by gene drift from inbreeding due to a limited number of broodstock or uncontrolled spawning. The observations of this study showed that broodstock from a single population and a few number of families was consistently employed in the recruitment process for the three neon tetra populations in the Bojongsari district region. This is considered to be the trigger for a decline in genetic variety, which has impacted the performance of the most important traits involved in aquaculture production, such as fecundity, survival, growth, and body abnormalities (Ariyanto, 2019; Fessehaye *et al.*, 2009; Fessehaye *et al.*, 2007; Tave, 1999).

Based on the value of heterozygosity and polymorphism, the genetic diversity of neon tetra fish from the present study is in the low-medium category (Zamroni *et al.*, 2023). Information on genetic diversity and genetic status is the basis for implementing fish breeding programs, such as selection and crossbreeding. According to (Mulyasari *et al.*, 2010), selection programs can be applied if the genetic diversity of fish is high, and if the genetic diversity is low, crossbreeding can be conducted.

The results of the paired F_{ST} test are shown in Table 5. The statistical analysis of AMOVA reveals a significant genetic variation ($P<0.05$) between the three populations of neon tetra fish. This is similar to the report of Hayuningtyas *et al.* (2018) on the AMOVA for RAPD marker analyzed on wild and cultured populations of *M. ajamaruensis* which indicated a significant difference ($P<0.05$) between the three populations. Although the neon tetras are still the same species and the three populations of fish from the Bojongsari district are located close together, there are significant differences between the three populations. This is a result of the three populations being isolated from one another due to no mixing of broodstock for spawning and disconnection in seed trading.

Table 5. F_{ST} comparison pairing test of three populations of neon tetra fish from Bojongsari District

Population	Bojongsari	Curug	Pondok Petir
Bojongsari	*****		
Curug	0.0000^{s}	*****	
Pondok Petir	0.0000^{s}	0.0000^{s}	*****

s = significantly different on the significance level of ($P<0.05$)

The value of genetic distance in this study is included in the medium category (Yuliani *et al.*, 2017) (Table 6). The genetic distance between the Curug and Bojongsari Populations was higher (0.4138) than the Pondok Petir and Curug Populations (0.4088). The high genetic distance of the Curug and Bojongsari Populations could be explained by the historical introduction of neon tetra broodstock. The trade of

Table 6. Genetic distance of three populations of neon tetra fish from Bojongsari District based on Wright's (1978) modification of Roger's (1972) distance

Population	Bojongsari	Curug	Pondok Petir
Bojongsari	*****		
Curug	0.4138	*****	
Pondok Petir	0.2883	0.4088	*****

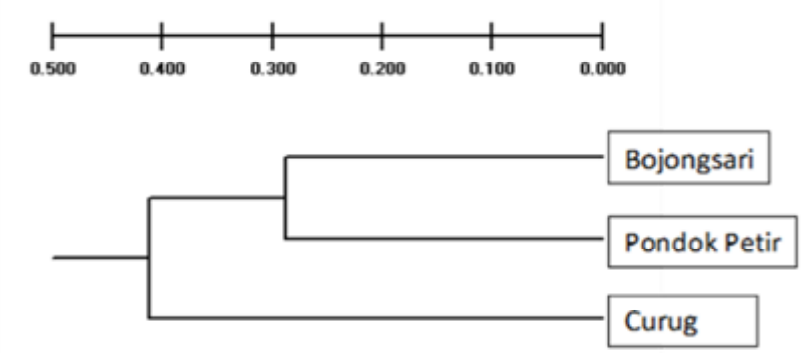

Figure 8. Dendrogram of three populations of neon tetra fish from Bojongsari District, Depok, Indonesia.

neon tetras from Bojongsari with Curug has been discontinued since 1994, while the farm from Pondok Petir has not been trading neon tetra fish to Curug farm since 2014 (Kusumah, 2020). Therefore, there is no mixing of neon tetra broodstock between the Curug and Bojongsari populations much longer than neon tetra broodstock from the Pondok Petir and Curug populations. According to Kokkinias *et al.*, (2023) mixing populations can reduce genetic distance by increasing genetic similarity between populations. Genetic distance can be impacted by individual migration across populations or mixing populations, which can introduce new genetic variants or homogenize existing ones. When migration is high, genetic distance tends to decrease as populations become more genetically similar due to the exchange of genetic material. On the other hand, when migration is low or restricted, genetic distance tends to increase as populations diverge genetically in isolation. High genetic distance observed between several pairs of populations provides a good opportunity to exploit it for genetic improvement purposes through the mean of hybridization or cross-breeding, such as in prawn species, giant freshwater prawn *Macrobrachium rosenbergii* (Imron *et al.*, 2009).

The dendrogram displaying the genetic relationship between three populations of neon tetra fish showed the formation of two clusters (Fig. 8). The results of the cluster analysis indicated that the Curug population could be distinguished from the Bojongsari and Pondok Petir. This result is similar to the cluster analysis on morphometric analysis (Fig. 4). It is indicated that morphometric and molecular analyses effectively distinguished three populations of neon tetra fish, *Paracheirodon innesi*.

Population genetic differentiation may be due to ecological, geographical, environmental conditions/pollution, various human-based activities, and evolutionary factors (Hasan & Goswami, 2015; Hassan & Naeem, 2023). Similar research using different RAPD markers in *Prochilodus marggravii* (Hatanaka & Galetti, 2003) and Silurisasotus Korean catfish (Yoon & Kim, 2001) revealed significant population differences, which were attributed to ecological isolation as a result of differing habitat conditions from various sampling sites.

Information on genetic and morphometric diversity from this study is preliminary data to support breeding activities to develop potential broodstock, which can be utilized by breeders in improving population quality through selection and crossbreeding programs. For information, a breeding program on neon tetra fish has never been carried out. According to this study, the Curug and Bojongsari neon tetra fish populations demonstrated higher genetic variety than the Pondok Petir populations, so they can be used in crossbreeding programs to improve the quality of neon tetra fish populations.

CONCLUSIONS

Morphometrics and molecular analyses effectively distinguished three populations of neon tetra fish, *Paracheirodon innesi*, where Bojongsari and Pondok Petir populations had high similarities, while the Curug population was distinguished from others.

ACKNOWLEDGMENTS

We would like to thank the National Research and Innovation Agency (BRIN), Agency for Marine and Fish-

eries Research and Human Resources (BRSDMKP) of the Ministry of Marine Affairs and Fisheries (KKP), Research Institute for Ornamental Fish Culture (BRBIH) Depok, our fellows at BRBIH Depok, and neon tetra fish farmer in Bojongsari District (Mr. Jaenal, Mr. Budi, Mr. Madun), Mr. Hasan, and Miss Yani for their assistance during the research. We would also like to extend our gratitude to the reviewers, whose insightful comments have improved and focused the presentation of the research results in this publication.

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