



Comparative analysis of morphometric characters and genetic diversity of superior Nile tilapia, *Tilapia niloticus*, populations in Indonesia

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Abstract. Improved seed varieties from selective breeding play a crucial role in enhancing cultivation productivity. This study aimed to evaluate and establish baseline genetic information for several superior Indonesian tilapia varieties. Four superior Nile tilapia populations resulting from breeding have been observed for their morphometric characteristics and genetic diversity as essential baseline data for the continuation of the formation of national superior varieties. The four Nile tilapia varieties used were Nilasa (Yogyakarta), Sultanaa (Sukabumi), Srikandi (Sukamandi), and Larasati (Klaten). The morphometric characteristics measured were body weight (BW), head length (HL), body depth (BD), body thickness (BT), and standard length (SL). Data were analyzed in the form of the ratio of morphometric characteristics to standard length, body area (BA), and body volume (BV). The DNA analysis used was Random Amplified Polymorphism DNA with primers OPA-01, OPA-05, and OPA-16. The parameters observed included the genetic diversity values of the population, namely allele polymorphism and heterozygosity values. The phylogenetic relationships among varieties were shown using Nei's genetic distance. There were statistically significant differences in the effect of variety on the BD/SL, BT/SL, and HL/SL ratio values ($P < 0.05$), but not on the BW/SL, BA, and BV values. Significant differences were also observed in the BW/SL, BD/SL, BA, and BV parameters between male and female individuals. Nilasa tilapia had the largest average BA and BV values. Heterozygosity values ranged from low (0.090) in Nilasa tilapia to medium (0.1227) in Larasati tilapia. Loci polymorphism ranged from 21.05 to 34.21%. Nei's genetic distance values ranged from 0.2658 to 0.4011. The closest genetic distance was indicated between Nilasa and Larasati varieties. Fluctuations in heterozygosity values were similar to fluctuations in the coefficient of variation values in morphometric characteristics. Nilasa and Sultana tilapia are the best candidates for establishing a superior tilapia population.

Key Words: decline, diversity, application, breeding, tilapia.

Introduction. Tilapia is the second most commonly farmed fish species in the world, following carp (Miao et al 2020). Aquaculture of Nile tilapia (*Oreochromis niloticus*) has been steadily growing in many countries (El-Sayed & Fitzsimmons 2023) and is presently practiced in over 140 countries, including Indonesia (Zhang et al 2020). During the first half of 2023, Indonesia emerged as the second-largest global tilapia producer. However, the majority of the production is now directed towards meeting domestic demand. Indonesia exported 4,700 tons of tilapia in the form of frozen fillets during this period (FAO 2023). Anticipated factors, such as increasing inflation, escalating feed costs, and dwindling tilapia supplies, are likely to contribute to the downfall of this situation. Furthermore, the growing and undeniable influence of global warming is a matter of concern, as evidenced by Khallaf et al (2020) who discovered its impacts on the acceleration of sexual maturation and reduced reproductive capacity.

As a result, fish farmers have been working diligently to enhance the production and efficiency of tilapia farming. Multiple initiatives are underway to enhance the production of tilapia. Nugroho (2021) identified three key factors that contribute to the success of fish farming: the presence of high-quality water, effective feed, and superior seeds. The diminishing condition of public waters has been a challenge in ensuring the provision of high-quality water for maintenance purposes (Nugroho et al 2022). In their study, Nugroho et al (2020) discovered that tilapia farming in ponds utilizing the biofloc technology exhibits superior productivity in comparison to tilapia farming in floating cages.

Enhancing production through the utilization of high-quality water can be further improved by employing superior breeding seeds (Nugroho et al 2023). Superior seeds have been utilized in various aquaculture methods, such as tilapia in floating net cages (Nugroho et al 2013), catfish in tarpaulin ponds (Nugroho & Haryadi 2017), salmon in concrete ponds (Janssen et al 2017), tilapia in biofloc systems (Luo et al 2017), and tilapia in earthen ponds (Murphy et al 2020). The distribution of utilizing high-quality seeds may also be accomplished by the utilization of male tilapia seeds obtained through the manipulation of male sex determination using natural substances (Wahidah et al 2023). Enhancing the quality of fish seeds can be achieved by utilizing high-grade broodstock, which encompasses broodstock with optimal body size and superior traits. Efforts to enhance broodstock quality has experienced swift progress. In Indonesia, the systematic implementation of superior seeds commenced in 2010 with the introduction of new fish varieties (Nugroho 2018). The released fish species were predominantly tilapia. Approximately 14 different varieties of tilapia, each possessing distinct advantageous characteristics, have been distributed to the general public. Four of these varieties are Nilasa (Yogyakarta), Srikandi (Sukamandi), Sultanaa (Sukabumi), and Larasati (Klaten). The Nilasa tilapia exhibits a genetic improvement of 14.74% in weight and 5.56% in survival rate per generation. The Srikandi tilapia has a heterosis value of 13.44% for weight, 4.55% for total length, and 20.33% for survival rate. The Sultanaa tilapia exhibits an average genetic improvement of 10.33% in weight and 7.64% in survival rate per generation. During a 150-day period, Larasati tilapia reaches a final weight of 560-620 g in earthen ponds and 930-955 g in fast-flowing ponds (MMAF 2009; MMAF 2012a; MMAF 2012b; MMAF 2012c).

The fall in the genetic diversity of released fish, which can result in a loss of their superior traits, has been attributed to the widespread use of low-quality seeds by many farmers. The utilization of high-quality strains has a direct impact on the quality of the tilapia seeds generated, thereby improving the performance of these superior seeds. Hence, it is imperative to conduct an inventory of the morphological traits of broodstock in breeding activities (Amrullah et al 2023). Nugroho et al (2014) observed a reduction in genetic diversity in selectively bred tilapia from the F1 to F4 generations, followed by a drop in growth. Previously, Widiyati et al (2007) indicated low diversity values and relatively high asymmetry values in tilapia used in several aquaculture centers in Indonesia. Shechonge et al (2018) tracked the decrease in genetic diversity of indigenous cichlid due to the spread of introduced fish varieties in natural waters. Johnson et al (2020) noticed alterations in the genetic structure of tilapia. Neto et al (2014) observed a decrease in morphometric characteristics from GIFT-G3 to G4 tilapia. Integrating both morphological and genetic information on tilapia is essential, enabling farmers to select fish based on desired traits for cultivation, leading to increased production.

Accurate knowledge on the genetic variety of tilapia strains is essential for the development of efficient and sustainable future breeding programs. To mitigate the negative effects of inbreeding depression, it is advisable to select breeders from genetically diverse populations. Inbreeding depression can result in decreased fitness and productivity (Pavlova et al 2024; Pavlova et al 2017). An understanding of genetic variation facilitates the identification and selection of individuals possessing desirable traits such as rapid growth, resistance to diseases, and environmental adaptability (Aswini et al 2023). Information on genetic diversity can be used to build and manage gene banks that conserve genetic material, ensuring the long-term availability of diverse genetic resources for breeding programs (Karaca & Ince 2019). By monitoring and

managing genetic diversity, breeding programs can avoid the loss of rare alleles and maintain a healthy, diverse gene pool (Restoux et al 2022). Precise genetic information enables the optimization of mating schemes, minimizing the risk of inbreeding and ensuring the long-term sustainability of the breeding program (Zhao et al 2023). By monitoring the genetic variety in publicly distributed and utilized tilapia varieties, we can enhance subsequent management programs and procedures. It is crucial to prevent the potential extinction of a variety or species (Thresher et al 2020) and to support aquaculture in producing high-quality seeds that can maintain a competitive advantage despite the challenges of increasing inflation and feed prices. The objective of this study is to observe and analyze the morphometric and genetic diversity of superior tilapia varieties that have been distributed and utilized by farmers.

Material and Method

Tested fish varieties. A total of 160 fish specimens, comprising four superior varieties, were used in this study. These fish were collected from the Fish Culture Technology Application and Assessment Center, Fisheries and Marine Affairs Office of the Special Region of Yogyakarta. The four varieties are Nilasa fish (Yogyakarta), Sultanaa (Sukabumi, West Java), Srikandi (Sukamandi, West Java), and Larasati (Klaten, Central Java). Nilasa and Sultanaa fish are the result of selective breeding methods, while Srikandi and Larasati fish are products of hybridization programs. The original locations of the collected fish are shown in Figure 1.

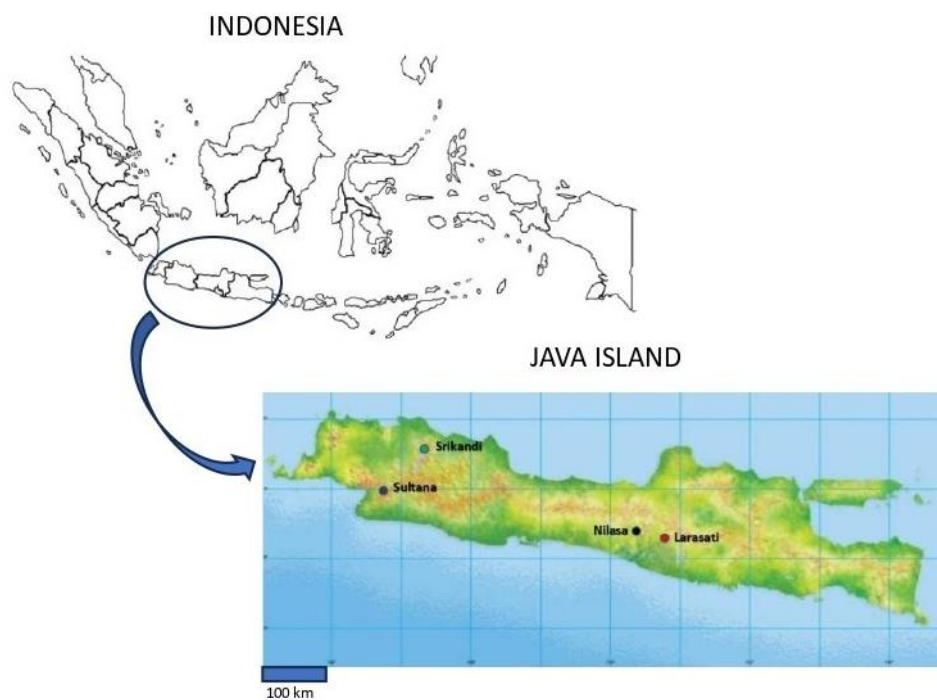


Figure 1. The location of sample collection.

Morphometric variation. Morphometric measurements were performed on parameters related to business aspects, namely body weight (BW), standard length (SL), body depth (BD), body thickness (BT), and head length (HL) individually. The number of fish measured for each variety was 20 males and 20 females at the age of 6 months. The ratio values of morphometric parameters to standard length were calculated to determine the characteristic traits of each Nile tilapia variety after being distributed and used by breeders. Additionally, body area (BA) and body volume (BV) values were also calculated as supporting data. Phenotypic variability was observed using the coefficient of variation (CV) of the morphological parameters. Scoring based on morphometric data (with equal percentage contributions) was conducted to identify the preferred varieties for breeders,

which included heavy body weight, long, tall, and thick bodies, and relatively small heads. The score values among varieties for each morphometric parameter were obtained as the absolute comparison of the observation value of one variety to the highest value in the related parameter.

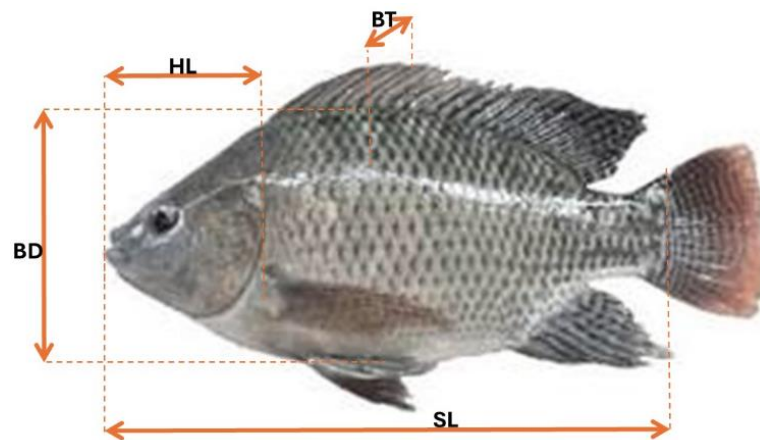


Figure 2. The morphometric characters measured in several varieties of tilapia (BD: body depth; BT: body thickness; HL: head length; SL: standard length).

Genetic diversity. Genetic diversity of the Nile tilapia varieties was observed using the Random Amplification Polymorphism DNA (RAPD) method. Whole DNA from forty samples was extracted from fin clips of four Nile tilapia varieties. After screening with 20 OPA (Operon Technology type A) primers, the best DNA fragment amplification was achieved using primers OPA-01 (5'-CAG GCC CTT C-3'), OPA-05 (5'-AGG GGT CTT G-3'), and OPA-16 (5'-AGC CAG CGA A-3'). The presence or absence of amplification bands was used to calculate polymorphism, heterozygosity, and Nei's genetic distance.

Data analysis. The morphometric parameters analyzed were the ratio values to standard length using the Compare Means analysis method in SPSS software version 16. The coefficients of variation for the measured parameters were analyzed descriptively. Genetic diversity parameters, indicated by polymorphism and heterozygosity values, were calculated using the Tools for Population Genetic Analysis (TFPGA) software. The genetic distance matrix between populations was calculated using Nei's unbiased genetic distances (Nei 1978) with the PopGene software program (Pfeifer et al 2014). The dendrogram resulting from the analysis was viewed using the TreeView software program (Page 1996). The equation used are (Neto et al 2014):

$$BA = SL \times BW$$

$$BV = BA \times BT$$

Where:

BA-body area (cm²);
 BV-body volume (cm³);
 SL-standard length (cm);
 BW-body weight (g);
 BT-body thickness (cm).

$$\text{Total Score} = (\text{Score (BW/SL)} + \text{Score (BD/SL)} + \text{Score (BT/SL)} + \text{Score (HL/SL)} + \text{Score (BA)} + \text{Score (BV)})$$

$$\text{Score}_{(\text{parameter- } i)} = (\text{Observation value}) / (\text{The highest value of the related parameter-} i)$$

Results. There is a statistically significant difference in the effect of variety on the ratios of body depth to standard length (BD/SL), body thickness to standard length (BT/SL),

and head length to standard length (HL/SL), but not on the ratio of body weight to standard length (BW/SL) and the values of BA and BV. The effect of sex differences is significantly observed in the values of BW/SL, BD/SL, BA, and BV, but not in BT/SL and HL/SL. Generally, the ratios of body depth and body weight to standard length, as well as BA and BV, are smaller in female Nile tilapia compared to male Nile tilapia of the same variety. The highest and lowest BD/SL values were found in male Sultana Nile tilapia (0.441) and female Larasati Nile tilapia (0.396), respectively. The highest BT/SL value was found in female Sultana Nile tilapia. The lowest HL/SL ratio was in male Nilasa Nile tilapia, and the highest in male Larasati Nile tilapia (Table 1).

Table 1
Morphometric character of four superior tilapia populations

Variety	Sex	BW/SLs	BD/SL	BT/SL	HL/SL	BA	BV
Nilasa	M	16.904 ±2.343	0.434± 0.040	0.180± 0.014	0.289± 0.028	223.74 ±28.55	915.08± 141.32
	F	15.210 ±3.040	0.415± 0.025	0.184± 0.011	0.308± 0.016	187.3± 29.87	734.78± 164.18
Srikandi	M	19.445 ±2.762	0.438± 0.027	0.188± 0.008	0.302± 0.017	219.95 ±24.44	926.72± 140.24
	F	13.001 ± 2.050	0.400± 0.027	0.187± 0.011	0.299± 0.015	159.03 ±27.74	597.56± 156.97
Sultana	M	17.985 ± 3.601	0.441± 0.036	0.196± 0.013	0.295± 0.024	225.28 ±26.34	997.58± 159.41
	F	15.192 ± 4.052	0.420± 0.034	0.197± 0.015	0.303± 0.020	165.88 ±25.06	649.85± 132.63
Larasati	M	14.775 ±4.255	0.411± 0.025	0.180± 0.019	0.327± 0.022	197.60 ±46.30	789.04± 257.61
	F	16.010 ±3.298	0.396± 0.034	0.183± 0.014	0.302± 0.027	185.89 ±30.01	741.08± 169.77

BW-Body Weight; SL-Standard Length; BD-Body Depth; BT-Body Thickness; HL-Head Length; Body Area; BV-Body Volume.

Different varieties of Nile tilapia can have different body sizes. Some varieties have larger overall body part ratios or specific character traits. Sultana Nile tilapia has average morphological character values more aligned with breeder or consumer preferences compared to other varieties tested. This is shown in the total score based on morphometric measurements. Sultana Nile tilapia has the highest average ratios of weight, body thickness, and body depth to standard length, which are 16.59, 0.196, and 0.431, respectively. Nilasa Nile tilapia has the smallest average head size ratio to standard length, namely 0.299. The largest average BA and BV were found in Nilasa Nile tilapia, namely 205.5 cm² and 824.9 cm³, respectively (Table 2).

Table 2
Scoring value of tilapia based on the morphometric measurements

Variety	BW/SLs	BD/SL	BT/SL	HL/SL	BA	BV	Total score
Nilasa	<i>16.06</i> (0.968)	<i>0.425</i> (0.99)	<i>0.182</i> (0.929)	<i>0.299</i> (1.00)	<i>205.52</i> (1.00)	<i>824.93</i> (1.00)	5.884
Srikandi	<i>16.22</i> (0.978)	<i>0.419</i> (0.973)	<i>0.187</i> (0.955)	<i>0.300</i> (0.995)	<i>189.49</i> (0.922)	<i>762.14</i> (0.924)	5.746
Sultana	<i>16.59</i> (1.00)	<i>0.431</i> (1.00)	<i>0.196</i> (1.00)	<i>0.299</i> (0.999)	<i>195.58</i> (0.952)	<i>823.71</i> (0.999)	5.949
Larasati	<i>15.39</i> (0.928)	<i>0.403</i> (0.937)	<i>0.182</i> (0.019)	<i>0.315</i> (0.050)	<i>191.74</i> (0.933)	<i>765.06</i> (0.927)	5.601

* for the HL/SL ratio the lowest value is used as the maximum value. Point in italic letter; Score value in bracket.

Morphometric variability using the coefficient of variation (CV) approach shows that SL and BT parameters have average CV values below 10%, while the BW parameter has the highest average CV value at 27.30%. Srikandi Nile tilapia populations have relatively lower values for body weight, body depth, body thickness, head length, body area, and body volume parameters. Larasati Nile tilapia have a relatively low variation in the head length parameter (Table 3).

Table 3
Coefficient of variation of weight, standard length, body depth, body area and body volume of superior tilapia populations

Variety	BW	SL	BD	BT	HL	BA	BV
Nilasa	0.3126	0.1032	0.1136	0.1075	0.1236	0.2032	0.2833
Srikandi	0.2251	0.1032	0.1049	0.0709	0.0886	0.1666	0.2141
Sultana	0.2332	0.0947	0.1233	0.0943	0.1017	0.2012	0.2767
Larasati	0.3222	0.0882	0.1349	0.0940	0.0996	0.2122	0.2915
Average	0.273	0.097	0.119	0.092	0.103	0.196	0.266

BW-body weight; SL-standard length; BD-Body Depth; BT-Body Thickness; HL-Head Length.

The genotype variability analysis used RAPD markers. Nilasa Nile tilapia have the lowest polymorphism and heterozygosity values (21.0256 and 0.0900, respectively), followed by Sultana Nile tilapia (31.5789 and 0.1128, respectively). Srikandi and Larasati Nile tilapia have relatively higher genetic variability. The average Nei's genetic distance is 0.3312. The largest Nei's genetic distance was found between the values found in Sultana and Srikandi Nile tilapia specimens. The smallest Nei's genetic distance was between the values found in Nilasa and Larasati Nile tilapia specimens (Table 4). The dendrogram graph (Figure 3) summarizes the phylogenetic relationships among Nile tilapia varieties.

Table 4
Genetic diversity and Nei's genetic distance

Variety	Genetic diversity		Nei's genetic distance			
	Polymorphism	Heterozygosity	Nilasa	Srikandi	Sultana	Larasati
Nilasa	21.0256	0.0900	xxxx	0.3421	0.3135	0.2658
Srikandi	34.2105	0.1161		xxxx	0.4011	0.3609
Sultana	31.5789	0.1128			xxxx	0.3020
Larasati	34.2105	0.1227				xxxx

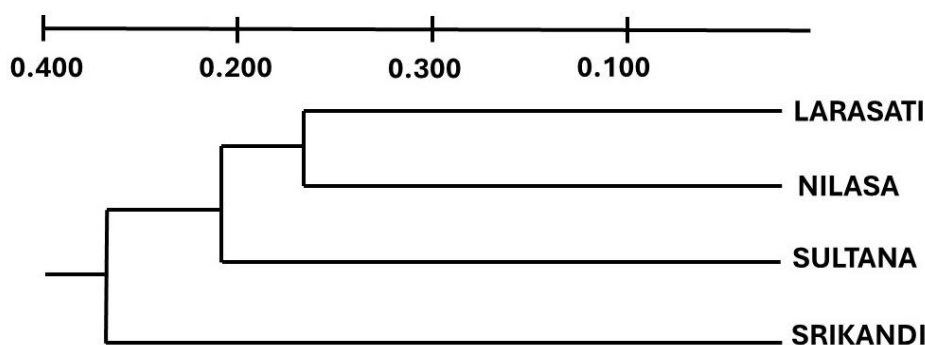


Figure 3. Dendrogram of relationships between the studied superior tilapia varieties.

Discussion. There are varying results regarding the influence of variety and sex of tilapia on morphological parameters. Generally, the ratios of body depth and body weight to standard length, as well as BA and BV, are smaller in female Nile tilapia compared to male Nile tilapia of the same variety. Wahidah et al (2023) obtained similar results in the evaluation of the morphological characteristics of Sultana Nile tilapia in several breeding centers, in South Sulawesi. Kwikiriza et al (2023) found average ratio values of 12.98

(BW/SL), 0.228 (BD/SL), and 0.185 (HL/SL) in Nile tilapia populations from aquaculture centers in Uganda. El-Zaeem et al (2012) found average BD/SL and BT/SL values ranging from 0.400 to 0.451 and 0.174 to 0.181 in wild Nile tilapia populations, and 0.385 and 0.163 in aquaculture populations. Neto et al (2014) found average BA and BV values in GIFT-G5 tilapia from a selective breeding program to be 171.0 cm² and 639.8 cm³, respectively. It is known that the different varieties of Nile Tilapia can have different body sizes. Some varieties have larger overall body part ratios or specific character traits. Generally, breeders or consumers prefer Nile tilapia with thicker and larger body shapes (Wibowo et al 2021). Sultana Nile Tilapia has average morphological character values more aligned with breeder or consumer preferences compared to other varieties tested. This is shown in the total score based on morphometric measurements. Sultana Nile Tilapia has the highest scoring value. Kwikiriza et al (2023) found average ratio values of 12.98 (BW/SL), 0.228 (BD/SL), and 0.185 (HL/SL) in Nile tilapia populations from aquaculture centers in Uganda. El-Zaeem (2012) found average BD/SL and BT/SL values ranging from 0.400 to 0.451 and 0.174 to 0.181, respectively, in wild Nile tilapia populations, and reaching 0.385 and 0.163, respectively, in aquaculture populations. Neto et al (2014) found average BA and BV values in GIFT-G5 tilapia from a selective breeding program to be 171.0 cm² and 639.8 cm³, respectively. Wahidah et al (2021) found non-significant ratios of body depth (BD) to total length (TL) of 27.80±0.48 to 28.48±0.35% in Sultana Nile tilapia fry from sex-directed breeding.

The morphometric variation indicated by the CV value of the BW character is greater than in the SL and BT characters. Breeding activities will have a greater influence on the target selection of BW characters compared to others. Kwikiriza et al (2023) found CV values for SL, BT, and BW parameters in Nile tilapia populations in breeding centers to be 13.04%, 10.32%, and 37.92%, respectively. Furthermore, separating male and female populations shows that male Nilasa tilapia have the smallest coefficient of variation in BW and BT parameters, which are 0.174 and 0.058, respectively. Male Srikandi tilapia have relatively more stable BD, SL, HL, BA, and BV parameters, with CV values of 0.075, 0.049, 0.066, 0.111, and 0.151, respectively. This suggests that these parameters should no longer be targeted for selection activities due to the relatively low potential for success in the respective populations, compared to targeting parameters with higher CV values for future selection.

When the phenotypic and genotypic variations are compared, there is an indication that the anomalies in phenotypic variability are due to environmental influences or genetic introgression during the breeding phase. Nilasa and Sultana Nile tilapia are products of selective breeding aimed at reducing the variability in targeted morphometric parameters. Conversely, Srikandi and Larasati Nile tilapia were developed through hybridization to increase the targeted phenotypic variability. This is shown in the results of genotype variability analysis using RAPD markers. Nilasa tilapia have the lowest polymorphism and heterozygosity values (21.0256 and 0.0900, respectively), followed by Sultan tilapia (31.5789 and 0.1128, respectively). Srikandi and Larasati tilapia have relatively higher genetic variability. According to Kusmini et al (2015), heterozygosity and polymorphism values indicate the ability to adapt to the environment. At higher heterozygosity values, more genes contribute to increasing the fitness of a population. In the Nilasa variety, when the heterozygosity value of a cross-bred variety is low, it is suspected that there is an incompatible combination of alleles so that the hybrid cannot adapt to its ecological environment. This incompatibility also affects the fitness of the variety as shown by its low genetic diversity value (Thompson et al 2022).

The genetic distance value resulting from crossing tilapia fish is quite high compared to cultivated fish in general. According to Hayuningtyas et al (2021), the genetic distance produced by betta rubra fish from three generations of cultivation ranges from 0.2415 to 0.3559. This indicates that by crossing fish varieties, new allele variations will be added which will increase genetic diversity so that the resulting genetic distance will be greater than that of uncrossed fish. The furthest genetic distance is the Srikandi tilapia fish, which is a fish that can live in saline habitats. Apart from that, it is a tilapia fish resulting from a cross between the Nirwana tilapia of *Oreocromis niloticus*, and the blue tilapia of *Oreocromis aureus* species, which are from different species but still in

the same genus. According to Yu et al (2022), Srikandi Nile Tilapia has the closest relationship to blue tilapia so that the proportion of blue tilapia offspring is more dominant than Nile tilapia.

Based on the dendrogram image, it can be seen that the Srikandi Nile Tilapia forms a much separate cluster compared to the other three varieties. Even though the Larasati Nile tilapia is the result of a cross, like the Srikandi Nile tilapia, the generation that formed is still from the same species, namely *O. niloticus*, descending from parents who are more closely related, while the Srikandi Nile tilapia which is formed comes from two different species with a higher chance of increasing allelic variability. More allelic variability collected will maintain the level of genetic variability of farmed fish (Gjedrem 2005; Ye et al 2022). Nilasa and Sultana tilapia were produced through selective breeding, while Larasati and Srikandi tilapia were developed through crossbreeding. The Nilasa and Sultana Nile tilapia resulting from selection form a cluster close to the Larasati Nile tilapia resulting from crosses because they are still from the same ancestor, namely Nile tilapia or belonging to the species *O. niloticus*.

Conclusions. Nilasa Nile tilapia has the largest average BA and BV values. Heterozygosity values ranged from low (0.090) in Nilasa, to medium (0.1227) in Larasati Nile tilapia. Genetic diversity values range between 21.05 and 34.21%. The closest genetic distance is the Larasati-Nilasa Nile tilapia and the furthest is Srikandi-Sultana Nile tilapia with a distance range of 0.2658–0.4011. The Larasati Nile tilapia, which is the result of a cross, forms a cluster together with the Nilasa and Sultana selection varieties; all three descend from the same ancestor and the same species, *O. niloticus*, while the Srikandi variety forms a separate cluster because it is the result of a cross with *Oreochromis aureus*.

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Conflict of interest. The authors declare no conflict of interest.

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