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PRELIMINARY STUDY ON THE MORPHOMETRIC AND GENETIC OF SHEAT CATFISHES POPULATION (SILURIDAE) FROM THE DOWN STREAM OF MUSI RIVER, SOUTH SUMATRA PROVINCE, INDONESIA

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ABSTRACT

Sheat catfishes is an economically important species group from open water in Indonesia. As a high demand in the local market, this species group was threatened from overfishing and habitat destruction. For its sustainability, sheat catfishes should have an attention to be conserved and domesticated. Population genetic study has been done to understand the diversity of sheat catfishes as the basic information for its aquaculture program. Objectives of the current study are to identify and characterize the phenotype and genotype of sheat catfishes population at Plaju, Mariana Ulu, Mariana Ilir and Meritai in the down stream of Musi River, South Sumatra Province. Phenotypic analysis based on truss morphometric used eight land-mark on the body of the observed specimens. Ten specimens from each site varying in size were measured with dial caliper with an accuracy of 0.01 mm. Meanwhile, the genetic analysis used PCR-RAPD method using OPA 06, OPA 08, and OPA 12 primers. DNA was extracted from the fin of the observed specimens. Discriminant analysis reflecting in the canonical function of truss morphometric data showed that six morphometric characters (A1, A2, A5, B2, B4, and C4) enabled to separate sheat catfishes population. The population of Mariana Ulu and Mariana Ilir spread on negative of Y axis, separately from population of Plaju and Meritai. The population of Meritai is on negative of the X axis. Genetic analyses found that the population of Mariana Ilir and Meritai have a percentage polymorphism of 22.00% which was higher than the population from Mariana Ulu and Plaju with a percentage of 18%. The population of Mariana Ilir has the highest heterozygosis value of 0.093. Kinship analysis showed that the population from Plaju and Mariana Ilir has the closest distance (0.3463) while the population of Meritai and Plaju has the farthest distance (0.5429).

Keywords: Siluridae; sheat catfish; morphometric; genetic; RAPD; Musi River

INTRODUCTION

The sheat catfishes or lais/silais in local name is a group of catfishes belonging to the family Siluridae. This group of catfish has similar morphology with a specific character as follow: no adipose in some species, no spine in dorsal fin, spine in pectoral fin, very short ventral fin, very long anal fin with 41-110 rays, and no barbell (Berra, 2007; Nelson, 2016). According to Eschmeyer *et al*. (1998), Siluridae consists of 12 genera with 107 species spread across Asia and Europe (only two species). The genera are *Silurus, Silurichthys, Ompok, Wallago, Belodontichthys, Hemisilurus, Kryptopterus, Phalacronotus, Micronema, Pterocryptis, Ceratoglanis,* and *Pinniwallago*. Generally, sheat catfishes live at the bottom of the river and lake and eat small fishes. In Indonesia, the extra-large size of sheat catfish is *Wallago leeri*. Many of the popular species of sheat catfishes belong to genera *Ompok* and *Kryptopterus,* which have different morphotypes. Those genera of sheat catfishes are known as an expensive freshwater fish consumed by people as fresh fish or as processed, such as smoked or salted fish.

By year, it is reported that the production of sheat catfishes in South Sumatra has decreased due to over exploitation in spawning season, catching fingerling, destruction of spawning ground, and land conversion for other purposes. This species should have an attention to be conserved and domesticated for its sustainability. The first report of the genus *Kryptopterus* was a study on the morphometric of the population of Kampar River in Riau Province, Sumatra (Pulungan *et al*., 1985). Thereafter, the biological aspect was reported on the population of "Lubuk

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Lampan" open water, South Sumatra (Utomo *et al*., 1990). Prasetyo (2005) observed the food habit of the population of a fishing sanctuary in Sambujur River, South Kalimantan. Study on the genus *Ompok* was begun after 2005 (Elvyra, 2009; Minggawati, 2010; Rahman, 2010). The focusing studies were bio ecology (Minggawati *et al*., 2010; Jusmaldi *et al*., 2018) and reproduction (Sjafei *et al*., 2008; Handayani *et al*., 2009; Elvyra *et al*., 2010; Minggawati *et al*., 2015; Jusmaldi *et al*., 2017). Various studies of population genetics from different rivers in Indonesia using molecular approach were carried out (Elvyra, 2009; Rahman, 2010).

To understand the diversity and relationship of sheat catfishes as the basic information for its aquaculture program, our current study aimed to identify and characterize the phenotype and genotype of sheat catfish population at Mariana Ulu, Mariana Ilir, Plaju, and Meritai in the down stream of Musi River, South Sumatra Province.

MATERIALS AND METHODS

Ten specimens of each sampling at Mariana Ulu, Mariana Ilir, Plaju, and Meritai, were collected from March to December 2019 for truss morphometric and genetic analyses. Sampling sites were selected based on the fish landing location in Musi River. Fin of each observed population were collected and preserved in ethanol solution for PCR-RAPD analysis.

Phenotypic Characterization

Sheat catfish in each population were chosen randomly based on the completeness of their limbs and without separating between male and female. Truss morphometric following method developed by Strauss & Bookstein (1982) was applied in the current study. Sheat catfish specimens were laid on a water-proof paper, and eight land marks on the body were designated (Figure 1). Measurements were then taken by connecting spots of the land marks using a dial caliper with 0.01 mm accuracy.

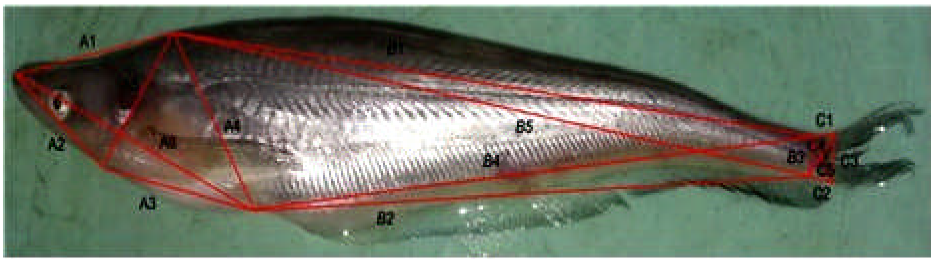

Figure 1. Truss morphometric on sheat catfish, locations of the eight landmarks are illustrated as the point to draw truss lengths. Each truss length is referred to by its corresponding cells (A, B, C). A1) The tip of the upper mouth to the end of cranial, A2) The tip of the upper mouth to the bottom part of operculum, A3) The bottom part of the operculum to the origin of anal fin base, A4) The origin of the anal fin base to the end of cranial, A5) The bottom part of the operculum to the end of cranial, A6) The tip of the upper part mouth to the origin of anal fin base, B1) The end of the cranial to the beginning of upper part peduncle, B2) The origin of the anal fin base to the end of anal fin base, B3) the beginning of the upper part of peduncle to the end of anal fin base, B4) The origin of the anal fin base to the beginning of the upper part peduncle, B5) The end of the cranial to the end of anal fin base, C1) The beginning of the upper part peduncle to the origin of top part caudal fin, C2) The end of the anal fin base to the origin of bottom part caudal fin, C3) The origin of the top part caudal fin to the origin of bottom part caudal fin, C4) The beginning of the upper part peduncle to the origin of the bottom part caudal fin, C5) The end of the anal fin base to the origin of top part caudal fin. Note: A5 is made on the outline of the fish at the point of the end of the cranial crossed to the end of operculum.

Genotypic Characterization

DNA was extracted using Genomic DNA Purification Kit (Fermentas) following Gustiano *et al*. (2013). Thereafter, DNA was amplified using the PCR method, starting with selecting 20 primers. PCR program was used including 94^{o}C pre-denaturation for 5 minutes, denaturasi of temperature 94^{o}C for 1 minutes, annealing of temperature 36^{o}C for 1 minutes, elongation of temperature 72^{o}C for 2.5 minutes, final elongation of 72^{o}C for 7 minutes, and stabilization process of 4^{o}C for 3 minutes. The PCR process was done in 45 cycles. The selected primers (OPA 6, OPA 8, and OPA 12) gave more amplification products than other primers. PCR results were then electrophoresed using 2% agarose gel in Tris Borate EDTA (TBE) buffer. The gels were visualized on a UV illuminator and documented with a polaroid camera.

Similarity and Genetic Analysis

All data of truss morphometric were converted into the ratio value by dividing the measurement with standard length. Intra and inter-population morphometric characters distribution were analyzed with ANOVA using SPSS version 19. The results of the analysis were presented in a canonical discriminant diagram sharing component. Genetic diversity was analyzed using TFPGA (*Tools for Population Genetic Analysis*) according to Nei & Tajima (1981). Inter-population kinship relationships were analyzed based on the UPGMA (Unweighted Pair Genetic Methods Arithmetic) and presented in the form of a dendrogram.

RESULTS AND DISSCUSSION

Results

Phenotypic Characterization

Coefficients of variation resulting from the data of 16 truss morphometric measurement characters are presented in Table 1 and the morphometric characters distribution of sheat catfishes are presented in Figure 2.

Table 1. Coefficient of variation (CV) of 16 morphometric characters of the sheat catfishes of Plaju, Mariana Ilir, Mariana Ulu, and Meritai

Morphometric characters	Coefficient of variation (%)				Significant
	Plaju	Mariana Ulu	Mariana Ilir	Meritai	
A1	0.07	0.06	0.07	0.20	0.001*
A2	0.13	0.14	0.12	0.09	0.000*
A3	0.12	0.15	0.20	0.08	0.301
A4	0.16	0.13	0.19	0.18	0.123
A5	0.16	0.18	0.13	0.20	0.028*
A6	0.13	0.13	0.07	0.04	0.021*
B1	0.14	0.09	0.11	0.05	0.196
B2	0.19	0.12	0.12	0.11	0.080*
B3	0.16	0.07	0.12	0.06	0.537
B4	0.18	0.17	0.10	0.10	0.008*
B5	0.05	0.04	0.05	0.07	0.274
C1	0.16	0.17	0.13	0.23	0.714
C2	0.20	0.13	0.19	0.18	0.706
C3	0.11	0.10	0.06	0.07	0.174
C4	0.08	0.10	0.15	0.07	0.000*
C5	0.09	0.09	0.09	0.09	0.402

*) significantly at P< 0.01

Coefficient of variation indicates the level of character variability in a population in which level of phenotype variation reflects the genotype variation (Tave, 1993; Gjedrem, 2005). In Table 1, different characters among populations occur in the head (A1, A2, A5, A6), midsection (B2 and B4), and the base of the tail (C4).

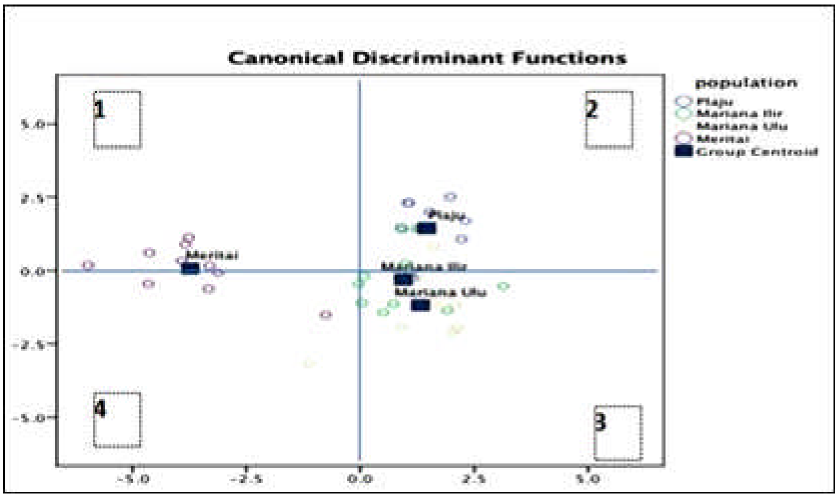

Figure 2. Morphometric characters distribution of silurid catfish.

The results of the canonical function analysis (Figure 2) show that the sheat catfish of the Mariana Ulu and Mariana Ilir are spread on the negative Y-axis and separate from the populations of sheat catfish from Plaju and Meritai, whereas the fish of the Meritai population are scattered on the negative X-axis. The sheat catfish of Mariana Ulu and Mariana Ilir are tangent which indicates a close relationship between the populations.

Genotypic Characterization

DNA amplification using OPA 06, OPA 08, and OPA 12 primers showed that the population of Meritai, Plaju, Mariana Ilir, and Mariana Ulu have varied amplified DNA at each locus (Figures 3, 4, and 5). The percentage of polymorphism and heterozygosity in the observed populations (Table 2) shows that the population of Mariana Ilir has a percentage of polymorphism and heterozygosity of 22.00% and 0.093, respectively, higher than the other fish population from other sampling locations, Mariana Ulu, Plaju and Meritai.

FST pairwise comparison (Table 3) showed significant differences in genetic diversity among the four populations of sheat catfish ($P<0.05$). The sheat catfish population of Plaju and Mariana Ilir have the closest genetic distance (0.3463), while Meritai and Plaju population value fish have the farthest genetic distance (0.5429) (Table 4 and Figure 6).

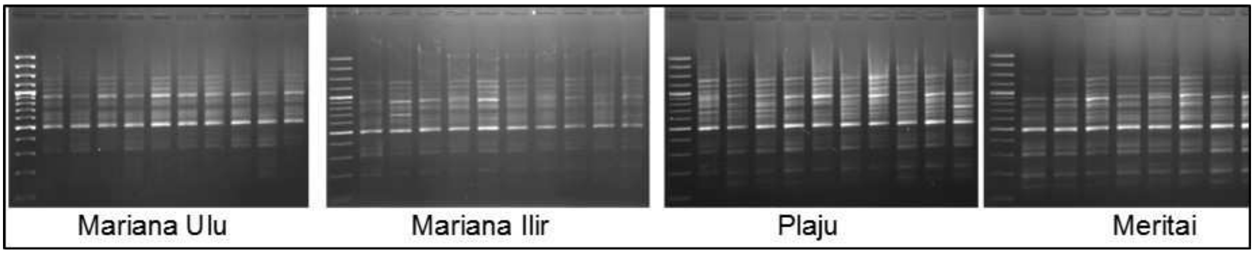

Figure 3. DNA amplification of OPA-06 primer on four population of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia.

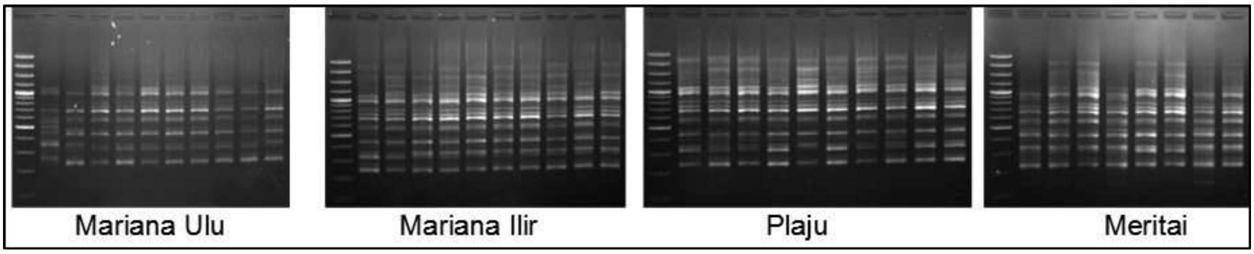

Figure 4. DNA amplification of OPA-08 primer on four population of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia.

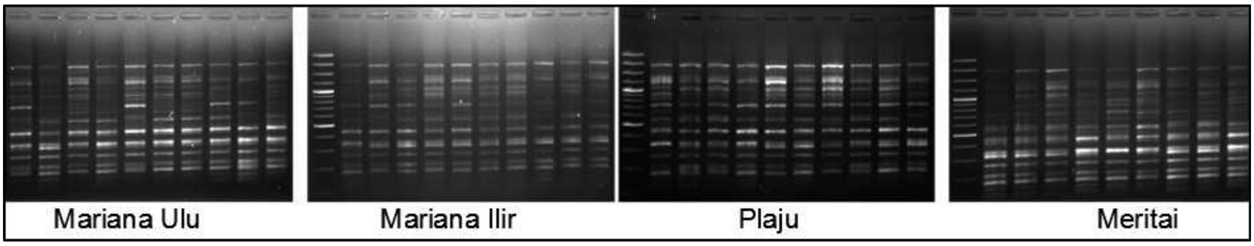

Figure 5. DNA amplification of OPA-12 primer on four population of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia.

Table 2. Percentage of polymorphism and heterozygosity on four population of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia

Population	Polymorphism (%)	Heterozygosity
Mariana Ulu	18.00	0.073
Mariana Ilir	22.00	0.093
Plaju	18.00	0.086
Meritai	22.00	0.091

Table 3. Fst pairwise comparison of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia

Population	Mariana Ulu	Mariana Ilir	Plaju	Meritai
Mariana Ulu	*****			
Mariana Ilir	0.0000*	*****		
Plaju	0.0000*	0.0002*	*****	
Meritai	0.0000*	0.0000*	0.0000*	*****

*) significantly at P< 0.05

Table 4. The genetic distance of sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia

Population	Mariana Ulu	Mariana Ilir	Plaju	Meritai
Mariana Ulu	*****			
Mariana Ilir	0.4328	*****		
Plaju	0.4829	0.3463	*****	
Meritai	0.5196	0.5302	0.5429	*****

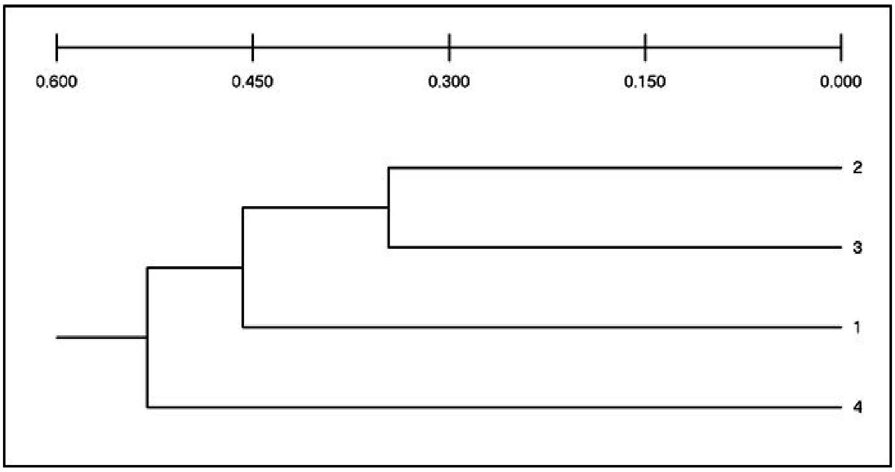

Figure 6. Kinship relationship among sheat catfishes from the down-stream of Musi River, South Sumatra, Indonesia based on OPA 06, OPA 08, and OPA 12. 1=Mariana Ulu, 2= Mariana Ilir, 3= Plaju, 4=Meritai.

Disscussion

Phenotypic Characterization

Few characters differ among populations showing the four observed populations live in the relatively same environment and the possibility of genetic material introgression has occurred among populations (Hallerman, 2003; Kapuscinski & Miller, 2007). According to Gustiano (2003), in *Pangasius*, the absence of differences in environmental conditions is the factor causing no emergence of phenotype differences. In the current study, coefficient of variation ranged from 0.04 to 0.23% classified as low and commonly found in freshwater fish such as, tinfoil barb (*Barbonymus schwanenfeldii*) ranged from 0.03 to 0.103 (Radona *et al.*, 2016), in snakehead ranged from 0.024 to 0.329 (Gustiano *et al.*, 2013), and in dwarf gourami ranged from 0.0275 to 0.1252 (Iskandariah *et al.*, 2015). The low coefficient of variation means that the chance of gene exchange with other populations is relatively small (Ryman & Utter, 1987).

This finding supported the assumption that sampling sites in the close of the same river flow have a high degree of similarity. Distribution of the centroid groups showed that populations of Mariana Ulu and Mariana Ilir are located in quadrant III and different from Plaju populations located in quadrants II and Meritai located between quadrants I and IV. Besides the biological and physiological aspects, other factors such as maternal effects, diet, growth compensation. and communal stocking can also influence the diversity level of population phenotypes as reported on several generations of redtail catfish (*Hemibagrus nemurus*) that have been domesticated (Kusmini *et al.*, 2019).

Genotypic Characterization

The percentage of polymorphism is determined by the number of amplified DNA fragments. The difference in polymorphism of DNA bands produced depends on the primary attachment site and can be used to provide a picture of the level of genetic diversity of a population (Vos *et al*., 1995). Polymorphism value is a picture of genetic diversity of a population that correlates with the level of heterozygosity and plays a role in the ability of the population to adapt to their environment (Allendorf & Utter, 1979). The higher heterozygosity, the more genes involved in contributing to the fitness level of a population.

Genetic diversity is a key parameter of population fitness that will guarantee the ability to respond passively to natural or artificial selection (Lorenzen *et al*., 2012). According to Tave (1993), differences in genetic diversity enable to increase genetic distances among populations. Thus, the population of Mariana Ilir has better adaptation ability to the environment and can be developed as a candidate for aquaculture. Mariana Ilir site is one of the water catchment areas for flooding retention in Palembang city. This location is also called lebak based on its specific character: deep basin river and overgrown with weeds (PSWG, 2010).

The populations of Plaju and Mariana Ilir with lower levels of genetic diversity compared to the population of lais from Mariana Ulu and Meritai have the closest genetic distance. As for the Meritai and Plaju fish populations with large differences in the level of genetic diversity in this study has the furthest genetic relationship. The results of the current study show that morphometric and genetic truss analysis are congruent with each other in separating different populations. Thus, data and information obtained can be used as a consideration to choose the population for domesticating prior to aquaculture as done in *Osteochilus kelabau* (Kusmini *et al*., 2011), snakehead *(Channa striata)* (Gustiano *et al*., 2013), dwarf gourami (*Trichogaster pectoralis*) (Iskandariah *et al*., 2015), and tinfoil barb (*Barbonymus schwanenfeldii*) (Radona *et al*., 2016).

CONCLUSION

Truss morphometric and genetic analyse s support each other, reflecting in separating the observed populations into different groups. Data and information obtained can be used as a consideration to choose the population for domesticating prior to aquaculture. Based on the level of diversity belonging to the population of Mariana Ilir, this population can be proposed as a candidate for new aquaculture.

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