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Domestication of Snakeskin Gourami (*Trichopodus pectoralis* Regan, 1910) in Indonesia: Characterization, Bioreproduction and Early Development

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Key words
***Trichopodus pectoralis*, Domestication, Bioreproduction, Indonesia, RAPD**

ABSTRACT

Domestication is the important step to increase the production and productivity of the snakeskin gourami in Indonesia. At the initial step of domestication, information on phenotype, genotype and early development stage are needed. This paper will describe the result of studies on phenotype, genotype and early development of snakeskin gourami from nine populations in Indonesia; Jambi, South Sumatra, and Lampung(Sumatra), West Java, Central Java and East Java (Java) and West Kalimantan, Central Kalimantan and South Kalimantan (Kalimantan) were carried out. The results showed that the highest intra-population similarity index value from sharing component analysis was Central Java population (73.3%), while the lowest was South Sumatra population (16.7%). The genetic relationship showed that the first cluster represented by populations from South Sumatra, Lampung, East and West Jawa. Even the hatching phase was the most critical phase in the early development of snakeskin gourami but the early development performance of fertilized eggs, embryos, and larvae showed no differences between observed populations. The survival rate of larvae for East Java, West Kalimantan and Lampung were 92%, 86% and 82%, respectively.

INTRODUCTION

The total fisheries production of the Southeast Asian in 2016 was 45.4 million metric tons (MT). The production was increased by 13% from 2012 to 2016 and gave 23% contribution to the total of world production (FAO, 2016). In the some period, Indonesia was the highest fish producer the Southeast Asian countries with more than 51% from total production (23,172,872 MT in 2016), and 12% of the total world production. Indonesia is a country with the largest area of tropical peat in the world, ranging from 13.5-26.5 million ha (20 million ha on average) or 50% of the tropical peat area in the world (Huwoyon and Gustiano, 2013). The culture based fisheries is one of the promisisng way to optimize the potential of peatlands through fisheries. Biological approach using high tolerance local fish that able to adapt with the low pH have to be applied for local fish culture in peatland area (Gustiano *et al*., 2011; Huwoyon *et al*., 2013). Peatland local fish is dominated by air breathing species such as climbing perch (*Anabas testudineus*), kissing gourami (*Helostoma temminckii*), snakeskin gourami (*Trichopodus pectolaris*), giant gourami (*Osphronemus gouramy*), stripped snakehead (*Channa striata*), and Indonesian snakehead (*Channa micropeltes*).

Snakeskin gourami is a member of the family Osphronemidae. Previously, snakeskin gourami were included in the genus *Trichogaster*, and after a taxonomic revision this fish group was included in the genus *Trichopodus*. In the world, there are 6 members of the

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genus *Trichopodus*: *Trichopodus cantoris* (Gunther, 1861), *Trichopodus leerii* (Bleeker, 1852), *Trichopodus microlepis* (Gunther, 1861), *Trichopodus pectoralis* (Regan, 1910), *Trichopodus poptae* (Low *et al*., 2014); *Trichopodus trichopterus* (Pallas, 1770). In Indonesia, there are three species of snakeskin gourami; 1) Pearl gourami (*T. leerii*), known as high commercial value freshwater ornamental fish. Currently, pearl gourami is categorized as endangered species in West Kalimantan; 2) Three spot gourami (*T. trichopterus*), a relatively cheap commercial ornamental fish; 3) Snakeskin gourami (*T. pectoralis*), the most common fish with the commercial value from peatland area. According to Schuster (1950), snakeskin gourami introduced in Indonesia around 1934 via the Malacca Peninsula. Based on the original habitat, the fish were distributed in swamp area of Sumatra, Kalimantan, Sulawesi and Java, then spread widely in Indonesia. With the rapid reproduction performance, the snakeskin gourami now become an important fish dominating swamp water area (Sukadi *et al*., 2009). In several area, the snakeskin gourami are dominating the catch of fish farmer with 60% compared with other types of swamp fish.

Snakeskin gourami is also one of the important freshwater fish species in other South East Asian Country such as Thailand (Yoonpund and Little, 1997) and Vietnam (Phong, 2014). In Vietnam, snakeskin gourami has higher price in 2014 (USD 2.5-3.5/kg) than pangasius (USD 1.0-1.2/kg) and red tilapia (USD 1.5-1.6/Kg). Indonesia and Thailand are two main producing countries for snakeskin gouramy comparing with the increasing trend of snakeskin gourami production in Thailand. The production of snakeskin gourami in Indonesia has decreased and even experienced scarcity in the last 10 years. Snakeskin gourami production in Indonesia from inland open waters was 45,873 tonnes in 2016 (Wibowo *et al*., 2018) and showed 25% decrease in 2017, with the estimated national snakeskin gourami production 34,384 tonnes (MMAF, 2018).

Domestication is one of the promising solution to prevent the extinction and to increase the production of snakeskin gourami in Indonesia. At the initial step of domestication, information on phenotype, genotype and early development stage are needed. This paper will describe the result of studies on phenotype, genotype, and early development of snakeskin gourami from nine populations in Indonesia; Jambi, South Sumatra, Lampung (Sumatra), West Java, Central Java East Java (Java), West Kalimantan, Central Kalimantan and South Kalimantan (Kalimantan).

MATERIALS AND METHODS

A series of studies on phenotypic, genotypic characterization, and early development of several snakeskin gourami populations in Indonesia were conducted to develop snakeskin gourami ex-situ culture for the domestication program. The research was conducted at the Population Genetics Laboratory of Institute for Aquaculture Research and Fisheries Extension and the Cijeruk Freshwater Fish Germplasm Research Station in Bogor, Indonesia. The samples used for truss morphometric and genetic analysis were the snakeskin gourami populations originating from three provinces in Sumatera: Jambi, South Sumatra, and Lampung; in Jawa: West Jawa, Central Jawa and East Jawa; In Kalimantan: West Kalimantan), Central Kalimantan and South Kalimantan. Each population consisted of 30 samples for truss morphometric and 10 samples for RAPD analysis. The specimens used for the RAPD analysis were fin samples stored in 70% alcohol solution.

Truss morphometric

The land marks on the body of measured fish were designated following Kusmini *et al*. (2019). The truss points in one truss area are connected to each other resultings 16 lines obtained as distinguishing characters (Fig. 1). Prior to the analysis, morphometric truss measurement data were divided by the standard length to eliminate the effect of different sizes. The analysis was carried out to obtain the value of coefficient of variance, the distribution of observed populations, and the level of similarity (sharing component). The level of similarity was illustrated in the form of a dendrogram.

Genetic analysis

DNA extraction was carried out by the phenol-chloroform method as used by Gustiano *et al*. (2013), Fin samples (5-10 mg) were taken in a microtube, to which 500 ml of TNES urea and 10 µl of protein kinase were added. Samples were homogenized with vortex for 1 min and then incubated at 37 ºC for 24 h. Then 1000 µl of phenol chloroform solution was added, homogenized for 1 min and then centrifuged at 10,000 rpm for 10 min. The supernatant was taken, to which was then added 1000 µl of 90% ethanol and 10 µl $CH_3$3COONa. The mixture was centrifuged at 10,000 rpm for 10 min. The seprnatant was discarded and dried to air at room temperature. The DNA pellets were then dissolved in 100 µl Tris-EDTA (TE) buffer.

The primers used in the study were OPC 02, OPC 05 and OPA 9. The DNA amplification process was carried out using the Polymerase Chain Reaction (PCR) method, using the composition of the reaction: 2 µl DNA, 2 µl primers with a concentration of 10 pmol, 12.5 µl KAPA2G Robust HotStart Ready Mix, and 21 µl H2O; with a

total volume of 25 µl.The PCR process used a TAKARA thermocycler with pre-denaturation at 94ºC for 2 min, 35 multiplication cycles consisting of denaturing at 94ºC for 1 min, annealing at 36ºC for 1 min and elongating at 72ºC for 2.5 min; and final extension at 72ºC for 7 min. Furthermore, electrophoresis results from PCR using 1% agarose gel in 1% Tris-Boric-EDTA (TBE) buffer and the results were observed with a UV illuminator. The values of polymorphism and heterozygosity were statistically tested. The genetic relationship was contructed using Tools for Population Genetic Analysis (TFPGA) ver. 1.3 (Miller, 1997) and the results are presented in dendrogram form.

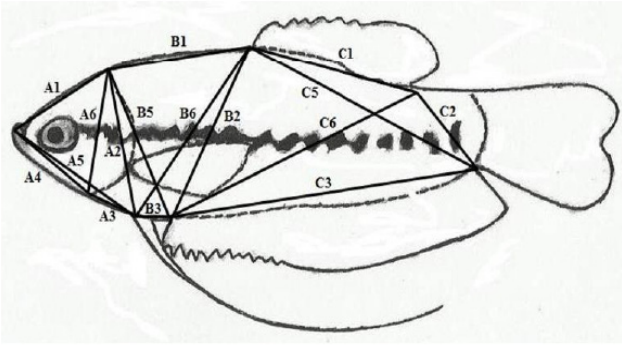

Fig. 1. Truss morphometric on snakeskin gourami, location of the eight landmarks are illustrated as the point draw truss lengths. Each truss length is referred to by its corresponding cells (A, B, C). (A1) The tip of the upper mouth to the end of cranial, (A4) The tip of the upper mouth to the bottom part of operculum, (A3) The bottom part of the operculum to the origin of anal fin base, (A2) The tip of the upper mouth to the origin of the anal fin base, (A5) The tip of the upper mouthto the origin of the anal fin base, (A6) The bottom part of the operculumt to the end of cranianl, (B1) The end of cranial to the origin of dorsal fin base, (B2) The orogin of the anal fin base to the origin of dorsal fin base, (B3) The origin of ventral fin base to the origin of anal fin base, (B5) The end of cranial to the origin of anal fin base, (B6) The origin of ventral fin baseto the origin of dorsal fin base, (C1) The origin of dorsal fin base to the end of the dorsal fin base, (C2) The end of dorsal fin base to the end of anal fin base, (C3) The origin of anal fin base to the end of the anal fin base, (C5) The origin of dorsal fin base to the end of anal fin base, (C6) The origin of anal fin base.

Reproduction and embryonic development

Three wild type populations of snakeskin gourami were collected from Lampung, East Jawa and West Kalimantan. For spawning activity, the number of breeders used were three pairs for each population. The female had a length of 17.1 ± 1.32 cm and a weight of 96.2 ± 1.81 g, while the male parent has a length of 15.7 ± 1.34 cm and a weight of 94.6 ± 1.12 gram. The maturation of the gonads of female and male snake skin gourami was carried out separately in an aquarium (100 cm x 30 cm x 30 cm). Before being used for spawning, the pH of water was lowered by adding 50g of 5 dried ketapang leaves (*Terminalia catappa*). The styrofoam was provided in the aquarium as a place for foam to be attached to fertilized eggs by male. Spawning was done by first inserting male fish into the aquarium. If foam has formed on the surface of the water and Styrofoam, approximately 20% of the surface of the water, then the female gourami parent was united with the male, ratio of 1: 1. Spawning lasted 2-3 days. The reproductive parameters evaluated on this reproductive performance are fecundity, degree of fertilization, degree of hatching and larval survival. The early development of fish (embryo phase) is observed after fertilization until the larvae are two days old using an Olympus BX-51 microscope equipped with Olympus DP-12 digital imaging. Data from one hundred embryos observed were recorded on the embryo survival rate with respect to the developmental phase.

RESULTS

Truss morphometric

The data of morphometric truss data is presented in Table I. The coefficient of variance (CV) ranged from 2.92-12.99%, with the highest mean CV in character B3 and lowest in character C3. Interpopulation significance test showed that the A3 character is not significantly different among the 9 populations observed. Meanwhile, the other 15 characters were significantly different ($P<0.05$). Canonical discriminant analysis showed that the distribution of intrapopulation phenotypes in all quadrants and intersect between populations (Fig. 2). The existence of this intersection indicates the similarity of several morphometric characters between populations. The sharing component analysis (Table II) showed that the highest intra-population similarity index value was found in the snakeskin gourami population from Central Java (73.3%) and West Kalimantan (66.7%), while the lowest was the snakeskin gourami population from Palembang (16.7%). Meanwhile, the interpopulation similarity index ranged from 0-23.1%. Based on the population center (group centroid), all populations originating from Kalimantan are in the positive x-axis quadrant.

Genetic analysis

DNA amplification done by 3 primers, OPC-02, OPC-05 and OPA-09 produced 9-28 fragments with sizes ranging from 1500-1800 bp (Table III). Tools For Population Genetic Analysis (TFPGA) showed the highest

percentage of polymorphism and heterozygosity of 65.62% in the population from East Java (Table IV). Meanwhile, populations with low genetic diversity, less than 10%, are found in the populations of Central Kalimantan, South Kalimantan and Jambi.

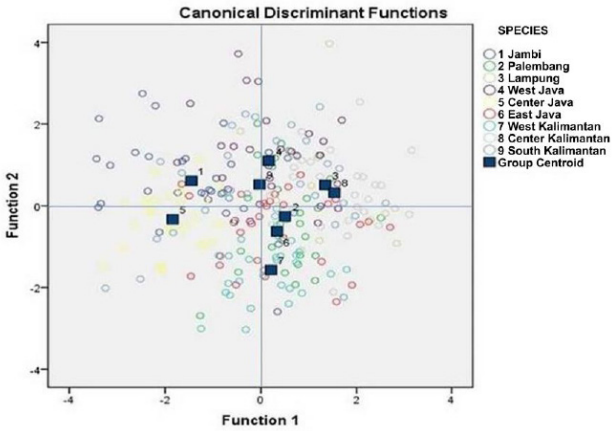

Fig. 2. The distribution of 9 snakeskin gourami population (*Trichogaster pectoralis*) from Sumatra, Java and Kalimantan Island based on truss morphometric analysis. Note: ■, centroid.

Statistically, 3 populations from the island of Sumatra (Jambi, South Kalimantan and Lampung) showed no significant differences in allelic distribution, as did 3 populations from the island of Kalimantan (West Kalimantan, Central Kalimantan and South Kalimantan). Meanwhile, 3 populations from the island of Java (West Java, Central Java and East Java) showed allelic differences between the populations of East Java and West Java, but the population of Central Java is not different from all other populations. Over all, the allelic distribution in the population of South Kalimantan was different from Jambi and Lampung, West Java and East Java (Table V). The genetic relationship of nine populations showed that there are two clusters exist (Fig. 3). The first cluster represented by populations from South Sumatra, Lampung, East and West Jawa. The second one belongs to the rest populations.

Reproduction and embryonic development

Data of snakeskin gourami broodstock and spawning are shown in **T**able VI and Figure 4. The freshly fertilized eggs had a diameter of 950.6 ± 52.54 µm. Observations of the early development of fertilized eggs, embryos, and larvae of the three populations observed did not show differences descriptively as reported by Ath-thar *et al*. (2014). The critical phase in the early development occuredbefore and after the hatching phase. The larvae of East Jawa population had a survival rate of 92%, the population West Kalimantan was 86% and the population from Lampung was 82% (Fig. 4).

Table I. Coefficient of variance resulted from 16 truss morphometric characters on 9 populations of the Snakeskin gourami (*Trichochopodus pectoralis*) from Sumatra, Jawa and Kalimantan.

Morphometric characters	Sumatra			Java			Kalimantan			Ave-rage	Significant Anova
	Jam	SS	Lam	WJ	CJ	EJ	WK	CK	SK		
A1	9.04	7.64	6.66	5.85	5.86	5.88	7.37	6.72	6.71	6.86	0.00
A2	4.34	3.32	3.98	4.23	2.72	3.72	3.42	3.62	3.43	3.64	0.00
A3	9.99	8.18	7.81	7.91	10.27	7.38	7.93	7.19	7.47	8.24	0.14*
A4	8.23	6.06	7.46	8.26	6.78	6.00	5.02	6.34	6.37	6.72	0.00
A5	4.56	3.87	4.33	4.43	5.06	4.15	3.62	4.45	3.78	4.25	0.00
A6	7.57	5.92	7.50	5.38	4.59	5.13	4.18	4.41	4.91	5.51	0.00
B1	5.52	5.56	5.78	4.56	4.32	6.48	4.98	4.93	6.51	5.40	0.03
B2	4.86	4.21	3.27	3.39	3.95	5.16	4.06	4.61	7.84	4.59	0.00
B3	15.56	8.54	15.26	12.68	13.75	13.56	17.88	8.83	10.84	12.99	0.00
B5	3.48	3.05	3.35	3.33	3.68	3.26	3.30	3.69	2.61	3.30	0.02
B6	4.06	3.85	2.93	3.16	3.31	4.94	3.15	3.93	6.37	3.97	0.00
C1	4.86	5.23	5.00	4.29	4.72	5.10	4.65	5.15	3.32	4.70	0.00
C2	4.65	4.06	5.08	4.31	4.62	4.92	3.83	3.33	3.56	4.26	0.00
C3	3.84	2.56	3.06	2.29	2.03	2.66	3.99	2.35	3.55	2.92	0.00
C5	3.18	3.01	4.10	1.92	2.21	3.83	3.07	2.66	2.51	2.94	0.00
C6	3.70	2.86	2.50	1.96	2.88	3.17	3.04	2.97	4.71	3.09	0.00

Note: *not significantly different. Jam, Jambi; SS, South Sumatra; Lam, Lampung; WJ, West Java; CJ, Central Java; EJ, East Java; WK, West Kalimantan; CJ, Central Kalimantan; SK, South Kalimantan.

Table II. Percentage of sharing component 9 snakeskin gourami populations (*Trichopodus pectoralis*) from Sumatra, Java and Kalimantan based on morphometric truss characters. Keterangan: 1, Jambi; 2, South Sumatra; 3, Lampung; 4, West Java; 5, Central Java; 6, East Java Timur; 7, West Kalimantan; 8, Central Kalimantan; 9, South Kalimantan.

Population	1	2	3	4	5	6	7	8	9	Total (%)
1	40	13.3	0	10	20	0	3.3	0	13.3	100
2	3.3	16.7	3.3	13.3	3.3	10	20	16.7	13.3	100
3	0	7.7	46.2	23.1	0	15.4	0	7.7	0	100
4	3.3	13.3	3.3	43.3	3.3	3.3	6.7	10	13.3	100
5	10	0	0	10	73.3	0	3.3	0	3.3	100
6	10	10	3.3	3.3	3.3	33.3	13.3	13.3	10	100
7	0	13.3	0	0	0	6.7	66.7	6.7	6.7	100
8	0	20	13.3	16.7	0	3.3	3.3	36.7	6.7	100
9	13.3	3.3	3.3	13.3	6.7	3.3	3.3	20	33.3	100

Table III. Number of fragments and size range of DNA amplification of 9 snakeskin gourami (*Trichogaster pectoralis*) population from Sumatra, Java and Kalimantan using primers OPC-02, OPC-05 and OPA-09.

No	Population	Number of fragments	Size range
1.	Jambi	25-27	150-1600 bp
2.	South Sumatra	9-27	150-1500 bp
3.	Lampung	13-25	150-1500 bp
4.	West Java	19-25	150-1600 bp
5.	Central Java	25-27	150-1500 bp
6.	East java	13-26	190-1800 bp
7.	West Kalimantan	21-27	200-1600 bp
8.	Central Kalimantan	24-26	200-1500 bp
9.	South Kalimantan	26-28	200-1600 bp

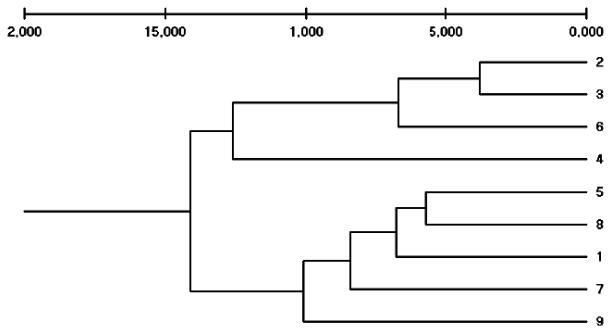

Fig. 3. Genetic distance of ninsenake skin gourami (*Trichogaster pectoralis*) from the Island of Sumatera, Jawa and Kalimantan. Note: 1, Jambi; 2, South Sumatra; 3, Lampung; 4, West Java; 5, Central Jave; 6, East Java; 7, West Kalimantan; 8, Central Kalimantan; 9, South Kalimantan.

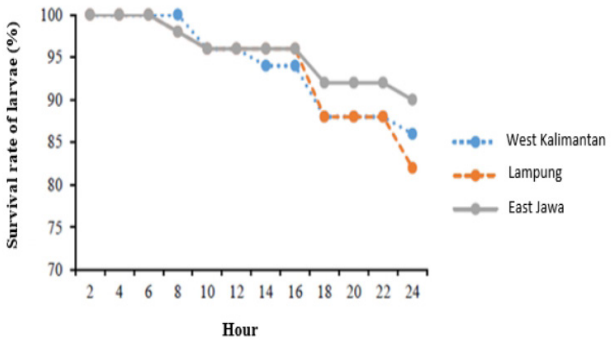

Fig. 4. Survival rate of snakeskin gourami larvae from west Kalimantan, Lampung and East Java on at 24 hours observation.

Table IV. Percentage of polymorphism and heterozygosity of 9 snakeskin (*Trichopodus pectoralis*) populations from the Islands of Sumatra, Java and Kalimantan.

No.	Population	Polimorfism (%)	Heterozigositas
1.	Jambi	6.25	0.02
2.	South Sumatra	56.25	0.27
3.	Lampung	59.37	0.25
4.	West Java	31.25	0.13
5.	Central Java	12.50	0.06
6.	East Java	65.62	0.29
7.	West Kalimantan	21.87	0.09
8.	Central Kalimantan	9.37	0.03
9.	South Kalimantan	6.25	0.02

Table V. Pairwise comparison test Fst of 9 snakeskin gourami (*Trichogaster pectoralis*) population from the Island of Sumatra, Java and Kalimantan.

Population	1	2	3	4	5	6	7	8	9
1	xxxx								
2	1.00*	xxxx							
3	0.62*	1.00*	xxxx						
4	0.04	0.20*	0.18*	xxxx					
5	0.99*	1.00*	0.78*	0.18*	xxxx				
6	0.14*	0.99*	0.97*	0.01	0.58*	xxxx			
7	0.35*	0.83*	0.04	0.00	0.89*	0.07	xxxx		
8	0.83*	0.99*	0.78*	0.22*	0.99*	0.42*	0.93*	xxxx	
9	0.03	0.21*	0.01	0.00	0.49*	0.02	0.96	0.94*	xxxx

Note: *not significantly different (P≥0.05); Note: 1, Jambi; 2, South Sumatra; 3, Lampung; 4, West Java; 5, Central Java; 6, Esat Java; 7, West Kalimantan; 8, Central Kalimantan; 9, South Kalimantan.

Table VI. The length and weight of snakeskin gourami gourami, and fertilization and hatching rate from 1:1 spawning mate snakeskin gourami.

Population	Size		Fecundity (n)	Fertilization rate (%)	Hatching rate (%)
	Length (cm)	Weight (g)			
Lampung	18.7	95.13	12 88 9	82.0	89.4
East Jawa	17.0	98.31	12 583	91.3	90.1
West Kalimantan	16.1	92.25	13 600	85.0	87.8

DISCUSSION

The coefficient of variance (CV) obtained (2.92-12.99%) provide an overview that improvement prog can be carried out on snakeskin gourami fish in Indonesia. The high CV value is a critical success factor in genetic improvement (Tave, 1993). Therefore, it is important to implement a strategy to increase the CV value and conduct the genetic improvement. Kusmini *et al*. (2019) reported that the sharing components between close generations of domesticated fish have a greater value than further generation. Thus, a large CV value will also support successful domestication and can reduce the possibility of inbreeding depression.

The highest intrapopulation in Central Java (73.3) indicated isolation from the population of other areas. The position of the population center from Central Java also supports the presumption of isolation, separated from West Java and East Java in the x-axis positive area. The intersections between the Sumatra and Kalimantan populations were also reported by Ath-Thar *et al*. (2016). A high index of similarity is presented by the main snakeskin gourami producing regions (Palembang and Kalimantan) with the main fish consuming regions (West Java and Central Java). Based on the population centers, all populations from Kalimantan are in the positive x-axis area, indicating that the Kalimantan population are not mixed with the population from another population. On the other hand, the population center from Java and Sumatra, are scattered in different quadrants. Jambi and central Java population have high intrapopulation index and appeared in same cluster with Kalimantan population rather than with Sumatra or Java population.

Polymorphism and heterozygosity data showed that the snakeskin gourami from consuming areas have a higher value (represented by East Java) than the producing areas (Kalimantan). The movement of fish from producing areas to consumption areas for aquaculture activities is one of the factors. However, South Sumatra showed high polymorphism and heterozygosity indicating fish origin was from another areas, especially Kalimantan. Tana *et al*. (2019) reported similar phenomenon, populations snakeskin gourami from Sarawak exhibited low genetic diversity, which is a typical sign of colonies introduced from a single source. To ensure that gene flow occurs between populations of different regions, a more capable genetic analysis with cytochrome oxidase I (COI) mitochondria DNA is required (Bachry *et al*., 2019). In general, genetic analysis showed that the Java population is closer to the Sumatra population than Kalimantan, except for the populations from Central Java and Jambi. The similar result is reported for kissing gourami (Sundari *et al*., 2012) and tinfoil barb (Radona *et al*., 2016; Kusmini *et al*., 2016). Result from truss morphometric measurements and genetic analysis appear to support each other.

The fertilization, hatching and survival rate shows the fitness level of a population. Observations showed that high values for the above parameters are found in populations from East Java. This information is closely related to the high polymorphism and heterozygosity values of this population based on genetic analysis (Table IV) due to interactions with external populations (Table II). The high value of polymorphism and heterozygosity in snakeskin gouramy populations from East Java can be considered as the candidate for the development of snakeskin gourami. Overall analyses enable to synthesis that similarity index has high correlation with the genetic relationship and showed the founder population. The finding of promising population should be supported by the appropriate strategies for further development. The precise genetics improvement strategies to increase the

productivity will provide a sustainability for snakeskin gourami development in the future.

CONCLUSION

Similarity index showed high correlation with the genetic relationship and revealed the founder population. High performances of early stage development are most influenced by the fitness of the population reflecting in the high polymormorphism and heterozygosity value. The present study enabled evidence to propose East Java population as candidate to develop snakeskin gourami farming in Indonesia.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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