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Genetic Variation at Microsatellite Loci in *Odorrana hosii* (Boulenger, 1891)

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ABSTRACT

The true frog species, *Odorrana hosii*, found in West Sumatera Indonesia, has high morphological differentiation and also estimated has high genetic variation. A total of 35 *O. hosii* at a seven location have analyzed using DNA microsatellite markers. Genetic variation of five microsatellite loci provided the highest value of expected heterozygosity (H_e) for the population in Padang (H_e = 0.618), while the lowest was the population in Merapi (H_e = 0.427). There are genetic differences in moderate levels among populations of *O. hosii* in West Sumatra (F_{ST} = 0.108) with inbreeding intrapopulation value (F_{IS} = -0.559), and high value of gene flow among the populations (*Nm* = 2.061). This study becomes the first molecular data for establishing effective population management conservation.

Keywords: gene flow; heterozygosity; *Odorrana hosii*; poisonous rock-frog; Ranidae

INTRODUCTION

The distribution of *Odorrana hosii* is wide, from Thailand, Malay Peninsula, Sumatra, Java, to Borneo. This species can live in an altitude interval from 300 masl to 1430 masl. The widespread and the altitude interval for survival provide a possibility of morphological and genetic variation to *O. hosii* (van Kampen, 1923; Inger *et al*., 2017).

According to a previous study, *O. hosii* in West Sumatra from different altitudes interval shows a high morphological differentiation and variation to the relative length of chromosomes (Agesi, 2011). Several environmental factors affected differentiation in phenotypes, such as temperature and altitude, genetic factor, or interaction of both factors (Conover & Schultz, 1995; Vences *et al*., 2002). Therefore, further research is necessary to examine the DNA level among *O. hosii* in West Sumatra to describe the species morphological variation that also happens genetically. This research aimed to analyze the genetic variation level of the *O. hosii* population in West Sumatra based on microsatellite markers. DNA Microsatellite markers are used because of these markers have a high polymorphism level. Hence, it is very appropriate to determine the genetic variation level within and between populations (Allendorf *et al*., 2012). Besides, the analysis of DNA variation using DNA microsatellite can also determine the genetic differentiation of a frog population-based on geographical location with a range of 10 kilometers (Morgan *et al*., 2008).

MATERIALS AND METHODS

The samples were collected at seven sites in West Sumatra (Alahan Panjang, Padang, Pasaman, Palupuah, Payakumbuh, Gunung Merapi, and Sijunjung). Those locations have been chosen based on altitude and distance shown in Figure 1 and Table 1.

DNA isolation. Analysis of genetic variation used DNA microsatellite markers (Funk *et al*., 2005; Arruda *et al*., 2017). DNA isolated from the liver tissue of five individuals from each population using QIAGEN kit protocol DNAeasy® Blood and Tissue Kit. PCR reaction was performed using PROMEGA PCR Core kit by mixing 2 µl isolate DNA, 12 µl GoTaq Green Master Mix, 1 µl forward primer, 1 µl reverse primer, and sterile water until the total volume is 25 µl. All loci have an optimal temperature. Information of nine microsatellite loci showed in Table 2. Amplification of DNA was programmed with an initial denaturation 95ºC for 2 min, 33 cycles of denaturation: 95ºC for 30 s, annealing: at optimal temperature 30 s, extension: 72ºC for 30 s, and final extension 72ºC for 5 min. The product was detected by electrophoresis in

agarose gel (1.2% of DNA isolation and 2% of the PCR product).

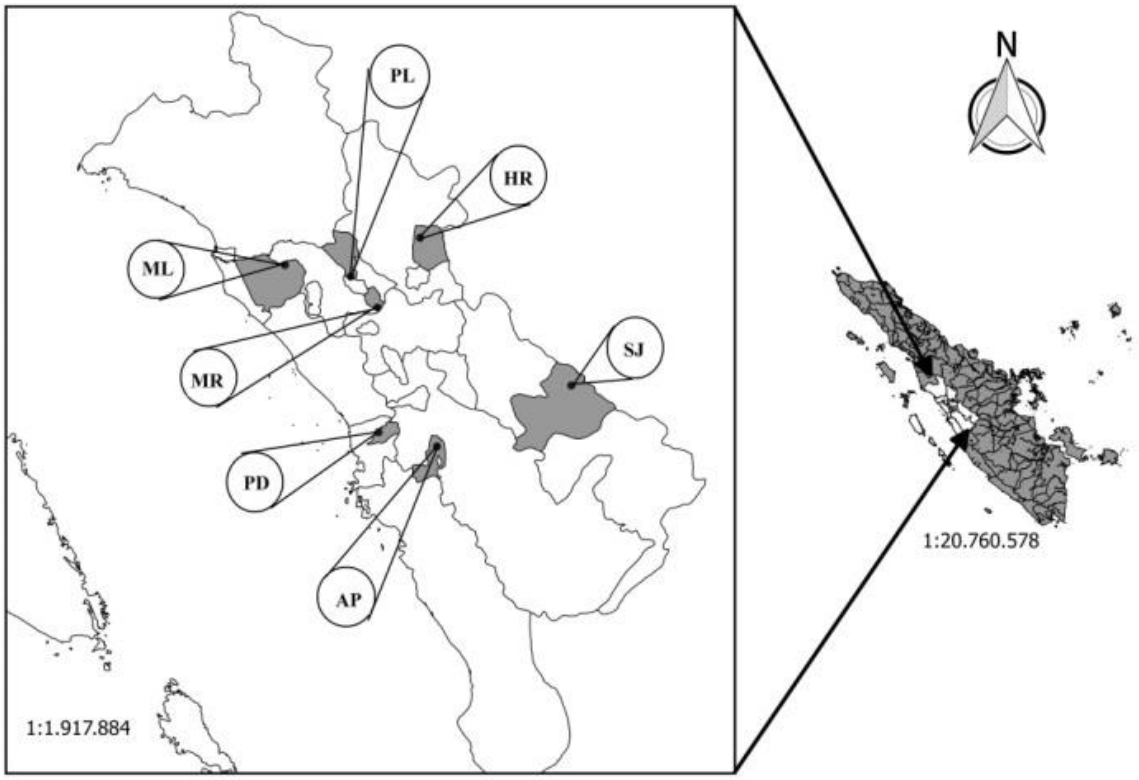

Figure 1. Research sites of *Odorrana hosii* in West Sumatra

Data analysis. Quantification of the results analyzed using the GENEPOP version 4.0 to determine the genetic variation within and between populations. The parameters used to determine the genetic variation within a population were allele frequencies, the percentage of polymorphic loci (P_p), the average number of alleles per locus (N_a), and expected heterozygosity (H_e). Genetic variation between populations can be seen from the F-statistic, genetic distance and similarity values dendrogram based on Nei's genetic distance matrix (1978) and the value of gene flow (*Nm*)(Raymond & Rousset, 1995).

Table 1. Geographic position and attitude of research sites of *O. hosii*

No.	Location	District	Altitude (m a.s.l)	Geographic Position
1	Alahan Panjang	Solok	1200-1400	1º 08' SL and 100º 07' EL
2	Cagar Alam Lembah Harau-Payakumbuh	50 Kota	500-600	0º 04' SL and 100º39' EL
3	Cagar Alam Malampah-Pasaman Barat	Pasaman Barat	456-763	0º 11' SL and 100º 04' EL
4	Hutan Pendidikan Penelitian Biologi (HPPB) Unand Padang	Padang	260–300	0º 21' SL and 100º 46' EL
5	Gunung Merapi	Agam	900-1500	0º 22' SL and 100º 28' EL
6	Cagar Alam Pangean I-Sijunjung	Sawahlunto Sijunjung	300-600	0º 90' SL and 101º 50' EL
7	Cagar Alam Batang Palupuah-Agam	Agam	700-1000	0° 14' SL and 100°21' EL

RESULT AND DISCUSSION

Amplification results of nine loci showed that only five loci produce the amplicon (*Rnh-3, Rnh-9, Rnh-10, Rnh-12,* and *Rnh-13*). The optimal temperature has been searched previously for the nine loci with different annealing temperatures. The annealing temperature for the five loci were modified from Gong *et al.* (2013) shown in Table 2.

Table 2. Nine primer pairs of microsatellite markers produces amplicons and annealing temperature

Locus ID	T_a	Repeat Motif	Primer Sequences (5'-3')	Amplicon
Rnh-1	-	(TGC)7	F:TGAAGTATTCAGGTACAACAGGT R:GGGCCAAAAGAGAGGGT	X
Rnh-2	-	(TGC)4TGTG(GTT)3T T(TGC)6	F:GCTTCGGGCTATAAATCAAACA R:GCCTGGCCGACTACACG	X
Rnh-3	52	(AAC)7	F:CCGGAAGGCAGTGGAGGACA R:ATGGACATGCGGTGGGGTAGG	V
Rnh-4	-	(GA)5	F:CGCTTACTATGGGGGGATA R:GCCTGAGAAGGGTGGTGCT	X
Rnh-6	-	(AAC)5	F:TCTCGGGAGGAAAGCAATGG R:AAGGAGCCTGGGACTATGGTAAAC	X
Rnh-9	59.5	(GCA)7	F:GCACAGTTAGCGAGATGGA R:CTCACTAGAGCTGGGTGGTAT	V
Rnh-10	61	(GCT)6	F:AGTGCAACATCAACTTGGGTG R:GCAGAGTCGCTGTCGGGA	V
Rnh-12	58.5	(AC)20	F:ATGTTATTGAGCCCAGAG R:GGTCAGCAGCAGGTAA	V
Rnh-13	55.3	(GCA)5	F:GATACGGGAGGCAAACG R:TCCACAGCCCAGCACTC	V

Notes: T_a= annealing temperature; V= amplicon produces; X= not amplicon produces

The number of alleles at each locus ranged from five to seven, with as much as 27 alleles and an average of 5.4 alleles per locus. All alleles microsatellite showed a high polymorphism with the percentage of polymorphic loci (P_p) ranged from 75 (*Rnh-13*) to 100% (*Rnh-9).* Analysis of genetic variation showed that population of Padang have a high genetic variation with 17 allele per locus, 0.618 expected heterozygosity (H_e), Shannon Index of 1.00, and 100% of polymorphic loci (P_p), and Merapi have a lower (N_a = 11; H_e = 0.427; I = 0.616; P_p = 80%)(Table 3).

Table 3. Analysis of the genetic variation of *O. hosii* based on microsatellite DNA in seven populations

No.	Population	N_a	H_e	I
1	Padang	17	0.618	1.000
2	Alahan Panjang	13	0.529	0.767
3	Malampah	13	0.533	0.783
4	Harau	11	0.444	0.619
5	Palupuh	13	0.596	0.846
6	Sijunjung	12	0.520	0.733
7	Merapi	11	0.427	0.616
	Mean	12.86	0.524	0.766

Notes: N_a = the number of alleles per locus; H_e = expected heterozygosity; I = the average of Shannon index

The high value of H_e in Padang because of large population sizes. A large number of *O. hosii* populations were based on criteria of high-level occurrence of *O. hosii* whilst collecting samples. Environmental conditions supported the local adaptation of populations (Arens *et al*., 2006; Alberto *et al.*, 2013). It has known that Padang was likely to become the habitat of *O. hosii*. According to Iskandar (1998), *O. hosii* was likely to live on a clear and strong water stream and a river streamline in the primary forest. A large population will reduce

the chances of inbreeding and increase random mating so that heterozygosity will also be increased (Cervantes *et al*., 2011; Frankham, 2015; Hedrick & Garcia-Dorado, 2016).

The lowest H_e value was found in the Merapi population. It can expect these values a small number of population, which will increase the occurrence of inbreeding (Frankham *et al*., 2002). Inbreeding associated with the loss of genetic variation, drift and less heterozygous levels related to the loss of some alleles and low levels of polymorphism. As a result, it would increase inbreeding depression (Arens *et al*., 2006; Taylor *et al*., 2010; Jamieson, 2011).

Inbreeding depression is mating between individuals from the same parental offspring, which produce offspring with lower live quality level (Arens *et al*., 2006). Besides, Allendorf *et al*. (2012) also stated that a low level of heterozygosity could reduce viability because most of the population recessive alleles were lethal. Lethal recessive alleles, predominantly covered by dominant alleles in a heterozygote locus, have a big opportunity to express in a few populations. The extinction will probably led faster in a small number of frog populations (Rog *et al*., 2013; Soto-Azat *et al*., 2013). The categorization of a population can be looked at from its frequency of occurrence. The frequency of occurrence of *O. hosii* from Merapi is relatively lower than another population.

Table 4. The value of F-statistik (F_{ST}, F_{IS}, F_{IT}) and gene flow (*Nm*)

Samples number	F_{ST}	F_{IS}	F_{IT}	*Nm*
35	0.108	-0.559	-0.3904	2.061

Notes: F_{ST} = coefficient of genetic differentiation; F_{IT} = the total inbreeding value; F_{IS} = inbreeding intra population; *Nm* = gene flow

F_{ST} value (Table 4) can determine the level of genetic differentiation between the *O. hosii* population in West Sumatra. According to Wright (1938), there are four categories of F_{ST} values that ranged from 0 to 0.05; 0.05-0.15; 0.15-0.25; and >0.25. Each showed a level of genetic differentiation, which indicated low, moderate, high, and very high genetic differentiation. Based on the statements above, we can conclude that *O. hosii* between populations in West Sumatra has genetic differentiation value that shows moderate genetic differentiation (F_{ST} = 0.108). From the F_{ST} value, we can determine that 10.82% of total genetic variation is among populations, and 89.18% is within the population.

The F_{IT} value that is less than 0 indicated that inbreeding in a population is low and random mating still occurs, so the probability for closely related species cross is low (Charlesworth & Willis, 2009). It is also supported by the value *Nm* among populations (2.061; $Nm \leq 0.5$). Based on that value, we know the migration of individuals among the population of *O. hosii* in West Sumatra is high (Slatkin, 1981). According to the obtained value, it can be concluded that *O. hosii* among populations in West Sumatra does not have genetic differentiation significantly based on microsatellite DNA.

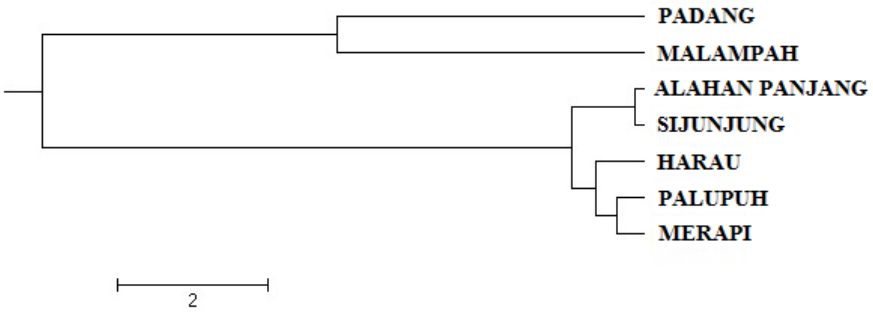

Figure 2. Dendogram of *O. hosii* population in West Sumatra by microsatellite markers

The pattern of *O. hosii* grouping among populations is presented in the dendrogram (Figure 2). Based on the UPGMA cluster analysis, *O. hosii* grouping in West Sumatra is divided into two major groups and provides a unique grouping pattern. *O. hosii* populations form groups following Bukit Barisan highlands (Padang and Malampah populations create a group on the western side of Bukit Barisan highlands; Alahan Panjang, Sijunjung, Harau, Merapi, and Palupuah populations create a group on the eastern side of Bukit Barisan), as well as the existence of rivers that facilitate the migration of individuals among populations. *O. hosii* grouping based on UPGMA cluster analysis is supported by genetic distance value among populations (Table 5).

Table 5. Nei (1978) genetic distance matrix in seven populations of *O. hosii* based on microsatellite markers

No.	Population	1	2	3	4	5	6	7
1	Padang	0.000						
2	Alahan Panjang	0.070	0.000					
3	Malampah	0.080	0.126	0.000				
4	Harau	0.094	0.014	0.134	0.000			
5	Palupuh	0.144	0.027	0.227	0.017	0.000		
6	Sijunjung	0.127	0.002	0.240	0.038	0.009	0.000	
7	Merapi	0.118	0.025	0.291	0.009	0.007	0.003	0.000

Table 5 presents that several populations are showing the great value of genetic distance differences among the population. It is caused by factors such as geographical isolation in the form of physical barriers, environmental factor differences, and reproduction. One of those factors will affect genetic structure formation (Arioli *et al*., 2010; Fouquet *et al*., 2012; Twomey *et al.*, 2016)). It can be concluded that geographical isolation factor, which is Bukit Barisan highlands locating from northern to the southern side of Sumatra that serves as a physical barrier separating among populations, has affected genetic structure formation of *O. hosii*.

CONCLUSION

The highest genetic variation of *O. hosii* is from the population in Padang (N_a = 17, H_e = 0.618, I = 1.000), while the lowest genetic variation is from the population in Merapi (N_a = 11, H_e = 0.427, I = 0.616). The existence of Bukit Barisan Mountain potentially becomes a barrier as well as the classification of *O. hosii* in West Sumatra. The analysis of the population's genetic structure has shown a moderate genetic variation (F_{ST} = 0.108).

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