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Genetic variation of *Rana parvaccola* (Inger, Stuart and Iskandar, 2009) based on DNA microsatellites in West Sumatra

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Abstract
Rana parvaccola is a new species of differentiated *Rana chalconota* complex in Sumatra. We have analyzed the genetic variation of 30 individuals *R. parvaccola* with Microsatellite DNA in West Sumatra. Five primers were used in the amplification process with PCR technique. The highest expected heterozygosity (He = 0.6533) was Padang population and the lowest (He = 0.2501) was Siberut population. There is a very high genetic differences between populations of *R. parvaccola* in West Sumatra (F_{ST} = 0,3452) with inbreeding coeffesient value F_{IS} = -0,4152 and F_{IT} = 0.0849. The value of gene flow between populations was very low (Nm = 2.0613).

Keywords: genetic variation, Microsatellite DNA, *Rana parvaccola*, gene flow

1. Introduction
Rana parvaccola is an amphibian species that identified as new species from *Rana chalconota* complex in Sumatra. R. chalconota is a species that is widespread in the Sundaland [1]. Geographical isolation can lead to changes morphotype between populations at the complex species such as size and color pattern [1-3]. *R. rufipes* and *R. parvaccola* are species of R. chalconota complex only found in Sumatra. *R. rufipes* is a large R. chalconota, have red swimming membrane, size of the tympanum is equal to the diameter of the eye in females and larger in males which on the inside a little depressed. *R. parvaccola* is a small R. chalconota, have black swimming membrane, tympanum approximately two-thirds the size of the diameter of the eye in females and larger in males [1]. Based on surveys and observations that have been done in West Sumatra, we know that *R. parvaccola* found frequently than the *R. rufipes*. Out of nine locations that have been surveyed we found *R. parvaccola* at six locations, while *R. rufipes* found in three locations. *R. parvaccola* found in habit an area with various heights and regions of West Sumatra.
Morphological form and genetic constitution of amphibian were affected by human-caused habitat destructions, altitude landscape and geographical factors [4-6]. Amphibia spend their entire life cycle in water and land so they are generally believed to be sensitive to enviroment perturbations, partly because of their central place in the food chain. Land use changes could be the most critical factor for the population decline and it's has been reported in various parts of the world [6, 7]. Microsatellite markers are used in interpopulation diversity and gene flow studies of natural populations and also provide relevant information for identifying conservation units and for investigating the genetic processes that take place in populations such as the incidence of genetic drift and generation of genetic neighborhood [8, 9]. According o the topography of West Sumatra we assumed genetic variation in population of *R. parvaccola*. This study aimed to analyze the genetic variation intra- and inter-population *R. parvaccola* in West Sumatra.

2. Materials and Method
Individual of *Rana parvaccola* and DNA samples collected during March-June 2013 at six locations in West Sumatra (Figure 1). Analysis of genetic variation used DNA Microsatellite markers [2, 10, 11]. We used the QIAGEN kit protocol DNAeasy® Blood and Tissue Kit (QIAGEN, Inc., Valencia, California, USA) for the DNA isolation. The quality of DNA was tested by 1,2% gel electrophoresis added 3 µl Ethium Bromida (EtBr). Amplification of DNA was programmed for 33 cycles with modified temperature and time (denaturation: 950C;120

minutes, annealing: modification time; 30 minutes, extention: 72^{0}C;10 minutes) using PROMEGA PCR Core Kit. Nine primers were used (RNH1, RNH2, RNH3, RNH4, RNH6, RNH9, RNH10, RNH12 and RNH13) refers to the Gong [12] with modification. Identification DNA amplification used 2% gel electrophoresis added 5 µl Ethium Bromida (EtBr). Quantification of the results analyzed using GENEPOP ver 4.0 program 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 (Pp), the average number of alleles per lokus (Na) and expected heterozygosity (HE). Genetic variation between populations can be seen from the F-statistic, genetic distance and similarity values dendogram based on Nei's genetic distance matrix (1978) and the value of gene flow (Nm) [13].

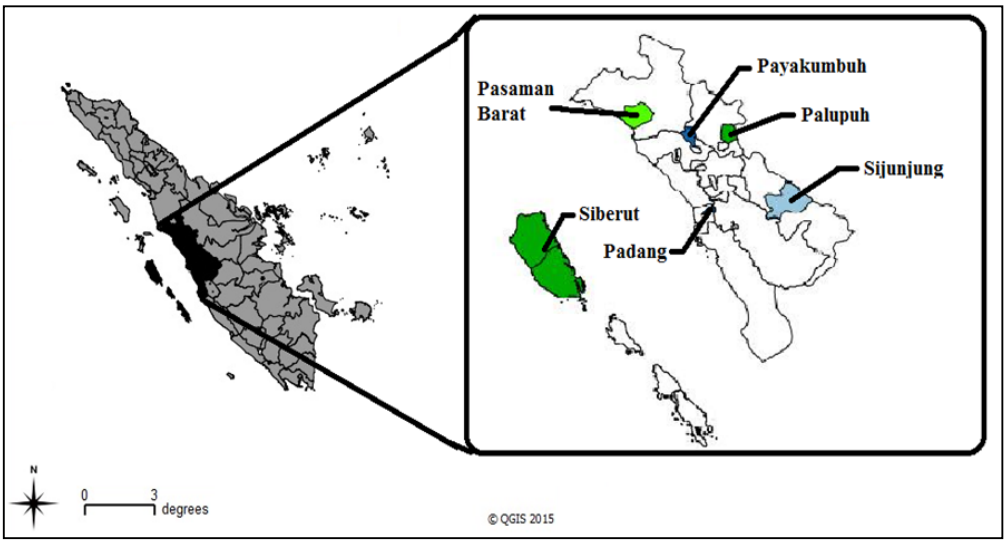

Fig 1: Research Location in West Sumatra

Table 1: Geographic position and altitude of reasearch location of *R. parvaccola*

No	Location	District	Altitude (mdpl)	Geographic Position
1.	Cagar Alam Lembah Harau-Payakumbuh	50 Kota	500-600	0º 04'LS dan 100º39' BT
2.	Pasaman Barat	Pasaman Barat	456-763	0^0 11' LS dan 100^0 04' BT
3.	Hutan Pendidikan Penelitian Biologi (HPPB) Unand Padang	Padang	260–300	0^0 21' LS dan 100^0 46' BT
5.	Cagar Alam Pangean I-Sijunjung	Sawahlunto- Sijunjung	300-600	0^0 90'LS dan 101^0 50' BT
6.	Cagar Alam Batang Palupuah-Agam	Agam	700-1000	0° 14' LS dan 100°21' BT

3. Results and Discussion

Based on the results of selection of the nine primers, we found that only five primer produces the amplicon that is the primer RNH3, RNH9, RNH10, RNH12 and RNH13. The number of alleles at each locus ranged from 4-13 alleles with total of as much as 41 alleles and an average of 8.2 alleles per primer. All alleles obtained a polymorphic alleles. A locus is usually considered to be polymorphic if the ferquency of the most common allele is less than either 95% or 99% [14, 15]. The value of *R. parvaccola* expected heterozygosity intra-population in West Sumatra ranged from 0.4044 to 0.6533 with the percentage of polymorphic loci (Pp) ranged from 80-100 (Table 2). Based on that value we know that population of Padang, Sijunjung, and Pasaman Barat have a high genetic variation while population of Payakumbuh, Palupuh and Siberut have a lower. The population that has the highest heterozygosity values is population of Padang with a value of heterozygosity (He) of 0.6533 and Shannon Index value (I) ± 0.0911 and a population that has the lowest heterozygosity values is Siberut population (H = 0.4044; I = 0.2501). High heterozygosity in the population due to large population sizes so that mating occurs randomly. In line [15, 16] a large population size will suppress the occurrence of inbreeding and increase mating randomly so that genetic variation and heterozygosity in the population will increase.

The lowest heterozygosity found in Siberut population. Siberut is an island separated from the mainland of Sumatra by the Indian Ocean. According to [17] reduced genetic diversity on oceanic islands is expected because island populations are typically founded by a few migratory individuals, resulting in extreme genetic bottlenecks. These conditions causes increase of inbreeding on the Siberut population. Low levels of genetic variation can be expected based on small population sizes in the islands. Island acts as a barrier to dispersal and migration of amphibians. Therefore, the effects of drift will be intensified when founding populations are small, when islands are small in area and when islands are far from the mainland source population [17, 18].

Table 2: Intra-population genetic variation of *R. parvaccola* based on DNA microsatellite

Population	Na	Ne	I	He	Pp (%)	N
Padang	3,0000 ±1,0000	2,5028 ±0,4761	0,9174 ±0,2657	0,6533 ±0,0911	100	5
Payakumbuh	2,0000 ±0,7071	1,8204 ±0,6160	0,5833 ±0,3776	0,4311 ±0,2688	80	4
Palupuh	3,4000 ±1,9494	2,7765 ±1,4796	0,9606 ±0,6435	0,5822 ±0,3462	80	4
Sijunjung	3,2000 ±1,6432	2,5393 ±0,7551	0,9714 ±0,3816	0,6444 ±0,1299	100	5
Siberut	2,2000 ±1,0954	1,7151 ±0,5234	0,5787 ±0,3923	0,4044 ±0,2501	80	4
Pasaman Barat	2,6000 ±0,8944	2,2819 ±0,3894	0,8555 ±0,2276	0,6133 ±0,0795	100	5

Description : Na : the average number of alleles perlokus; Ne : the average of efective alleles; I : the average of Indeks Shanon (Lewontin, 1972); He : heterozigosity (genetic diversity Nei); PP: the percentage of polymorphic loci; N: Number of polymorphic loci Inter-population genetic structure of *R. parvaccola* based on microsatellite DNA can be described by the total inbreeding value (F_{IT}: 0.0849), inbreeding intra-population (F_{IS}: -0.4152), coefficient of genetic differentiation (F_{ST}: 0.3534) and value gene flow (Nm: 0.4575). F_{IS} positive indicates the decrease of heterozygosity intra-population and negative F_{IS} indicate increased heterozygosity intra-population. So, we know that the heterozygosity intra-population is higher than heterozygosity inter-populations. F_{IT} value indicated that the mating of *R. parvaccola* among population is low. The value of genetic differentiation between populations (F_{ST}) can also be used to determine the genetic variation between populations. Value of the F_{ST}> 0.25 indicates a very high genetic differentiation [15]. Genetic differentiation inter-population of *R. parvaccola* in West Sumatra determined by F_{ST} value, known that the genetic variation interpopulation lower than intrapopulation.

Levels of migration or gene flow is high if value of Nm>1 [15, 19]. Our results also show that the gene flow (Nm) between populations is low (0.4575 ; Nm ≤ 0.5). Gene flow is caused by the movement or dispersal of whole organisms or genomes from one population to another. After entering a new population, immigrant genomes may become incorporated due to sexual reproduction or hybridization, and will be gradually broken up by recombination, so can reduce the genetic differentiation between populations and increases genetic variation in the population [15, 20]. From the value of gene flow in populations of *R. parvaccola* in West Sumatra we predicted that the migration of individuals between populations is low. This condition make the population of *R. parvaccola* in West Sumatra devided into subpopulation. The dendogram of *R. parvaccola* population in West Sumatra showed in Figure 2 and the genetic distance Nei 1978 showed in Table 3.

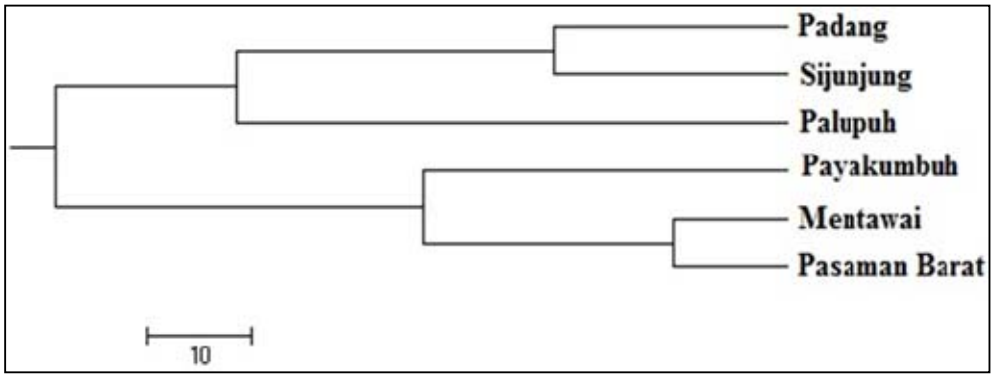

Fig 2: Dendogram of *R. parvaccola* population in West Sumatra.

Table 3: Nei genetic distance matrix 1978 in six populations of *R. parvaccola* based on microsatellite DNA markers.

No.	Population	1	2	3	4	5	6
1	Padang	-					
2	Payakumbuh	1,4314	-				
3	Palupuh	0,9457	0,9251	-			
4	Sijunjung	0,5253	2,2031	1,2988	-		
5	Siberut	1.7838	0,7389	1,2864	1,3061	-	
6	Pasaman Barat	1,4986	0,7393	1,5299	1,1599	0,2638	-

The grouping of *R. parvaccola* by UPGMA cluster analysis and the value of genetic distance between populations (Table 3) more than 0.5 (D> 0.5) indicate that the genetic structure of populations *R. parvaccola* in West Sumatra have been separated [2, 15, 21]. We preddicted that microhabitat factors such as habitat structure damage, antropogenic and type of vegetation change causeing genetic differentiation.in *R. parvaccola* We suggest to study about microhabitat and ecological factors of *R. parvaccola*.

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