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Research Article
Genetic Variation of *Limnonectes blythii* (Anura: Dicroglossidae) Using RAPD (Random Amplified Polymorphic DNA) Analysis in West Sumatra

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Abstract

Background and Objective: *Limnonectes blythii* population in west Sumatra sharply decline because of its trading activity and damaged habitat. Conservation effort is necessary to protect the species. One of important information that needs to be done is to understand the species genetic variation. The aim of this study was to analyze the genetic variety of *Limnonectes blythii* of west Sumatra using Random Amplified Polymorphic DNA (RAPD). **Materials and Methods:** Thirty frogs were collected from three location of wildlife sanctuary in west Sumatra. Genetic variation analysis using RAPD with 11 OPA primer. The electrophoresis results were marked as 1 if a band founded and 0 if there's no band. **Results:** Genetic variation of three population were low and the highest founded on Malampah population , next on Sijunjung and lastly Harau . However, genetic differentiation inter-population was considered moderate, which of half of overall genetic variation generated from differentiation inter-population and also a half from differentiation of intra-population which supported by lower heterozygosity inter-population than intra-population heterozygosity. Gen flow value of inter-population was observed moderate. **Conclusion:** Genetic variation of *L. blythii* intra-population is lower than inter-population and the gene flow between *L. blythii* population considered moderate.

Key words: *Limnonectes blythii*, species genetic variation, wildlife sanctuary in west Sumatra, malampah population, heterozygosity, intra-population, inter-population

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bobo frog, *Limnonectes blythii* is one of widely spread species in southeast Asian, from Myanmar, Thailand, Peninsular Malaysia and northern Sumatra[1-4]. In west Sumatra, this frog population decline because of over harvest by local community for export commodity and their damaged habitat[5].

This species status by IUCN[6] is almost threatened. Some effort needed to conserve this species. However, there was some difficulties because no information provided for genetic variation of this species.

Research related to biology of *L. blyhhii* west Sumatra is still a few in number. One of the information of is biology, that is crucial for conservation is genetic variation. Genetic variation information of species are important to understand because the higher variation leads to the higher chances of a species ability to adapt in their environment[7]. The RAPD (Random Amplified Polymorphic DNA) can be used to analyze the genetic variation of frog[8,9].

This research aimed to evaluate the genetic variation of *L. blyrhii* in west Sumatra using RAPD. The information obtained could be used to construct a future conservation plan for this frog species.

MATERIALS AND METHODS

Frogs collection locality: Thirty samples were collected from three location of wildlife sanctuary in west Sumatra i.e., Lembah Harau-Payakumbuh (Harau), Malampah-Pasaman Barat (Malampah) and Pangean II-Sijunjung (Pangean) and samples stored at Zoology Museum of Andalas University (Table 1, Fig. 1).

Table 1: Research sampling area of *Limnonectes blythii*

Locations	Regency	Altitudes (m)	Coordinates	Number of samples
Wildlife sanctuary of Lembah Harau-Payakumbuh	50 Kota	500-600	0°04'S and 100°39' E	10
Wildlife sanctuary of Malampah-Pasaman Barat	Pasaman Barat	456-763	0°11' S and 100°04' E	10
Wildlife sanctuary of Pangean II-Sijunjung	Sijunjung	300-600	0°90' S and 101°50' E	10
Total				30

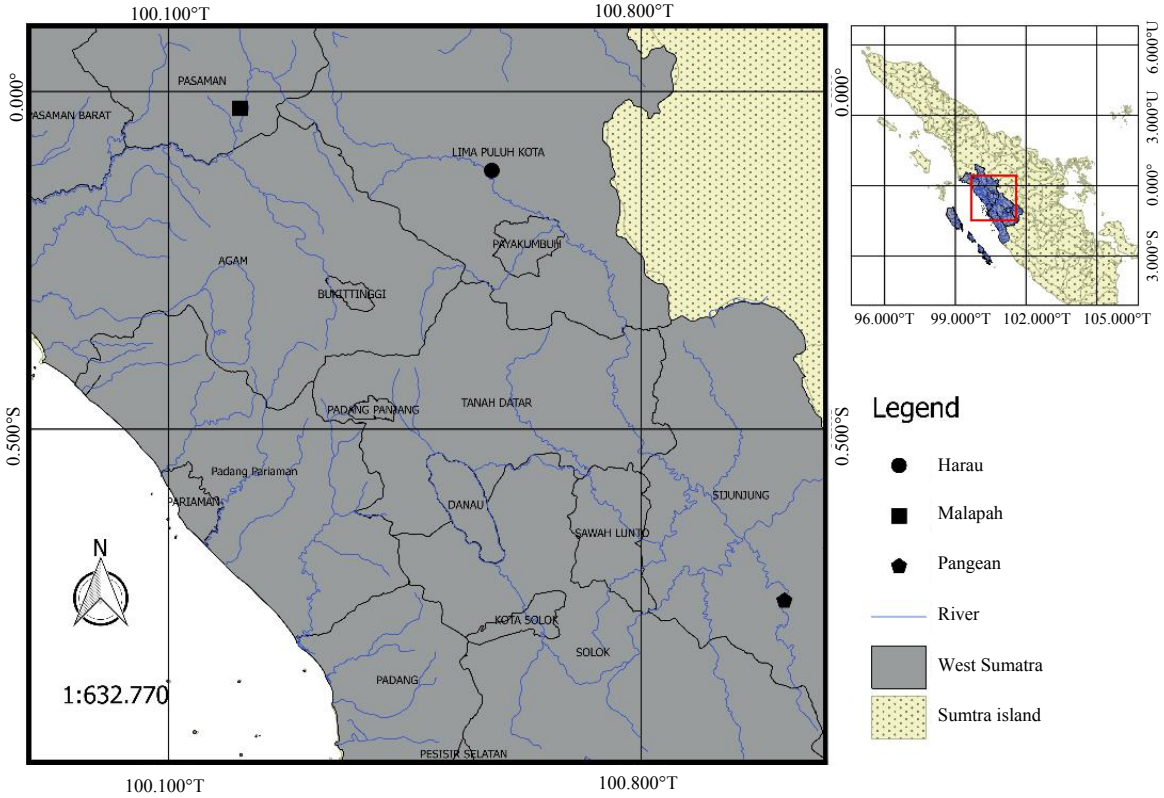

Fig. 1: Sampling location of *Limnonectes blythii*

DNA isolation dan DNA primer: The DNA isolations were done using kit protocol of Roche, Blood and tissue Kit Roche. Eleven RAPD primer used were from Operon Technologies Inc., Alameda, USA, i.e., OPA-01, OPA-02, OPA-03, OPA-04, OPA-05, OPA-06, OPA-07, OPA-09, OPA-11, OPA-12 and OPA-13.

PCR amplification: Kit *GoTaq* Green 12,5 µL+9,5 µL ddH_2O+2 µL DNA sample+1 µL primer used for DNA amplification. PCR cycle steps used were pre-denaturation at 94°C for 2 min, denaturation at 94°C for a minute, annealing at 35°C for a minute and elongation at 72°C for 2,5 min and repeated for 45 cycle[10]. The PCR results analysis then continued with gel electrophoresis using agarose 2% and colored with EtBr.

Data analysis: Bands of gel electrophoresis were analyzed by scoring based on the presence of DNA bands. Samples with DNA bands were mark as 1 and with no band founded mark as 0. After that, the data analyzed using POPGENE version[11] 1.31, including population genetic variation (Percentage of polymorphic locus (P), heterozygosity (H), Shannon diversity (I)) and genetic variation inter-population (population heterozygosity (H_S), heterozygosity intra-population (D_{ST}), heterozygosity total (H_T), genetic differentiation (G_{ST}) and gen flow (Nm).

RESULTS

Ninety five bands with size of 200-2.642 bp were founded, which consist of 82 polymorphic bands (85.77%) and 13 DNA monomorphic bands (14.23%) (Fig. 2).

The highest number of bands were founded from Malampah population (66 bands) and the lowest from Pangean population (42 bands). DNA bands resulted from each primers varies from 8-16 DNA bands and approximately 11.88 bands per primer. The highest numbers of polymorphic locus and percentage were found at Malampah population with 47 locus and 49.47% and Harau and Pangean population with 30 locus and 31.58% (Table 2).

Table 2 showed that highest number of heterozygosity found at Malampah population (H = 0.1602±0.020) followed by Pangean (H = 0.1274±0.020) and the lowest was Harau (H = 0.1263±0.019). Average Index Shannon (I) Harau population (I = 0.184±0.028), Pangean (I = 0.185±0.028) and Harau (0.184±0.028).

Limnonectes blythii of west Sumatra Barat had lower population heterozygosity (H_S = 0.138) than heterozygosity inter-population (D_{ST} =0.143), therefore the total population heterozygosity (H_T = 0.281). This results showed that genetic variety of *L. blythii* intra-population was smaller than inter-population. Inter-population genetic differentiation of *L. blythii* (G_{ST} = 0.5091) was considered moderate and gen

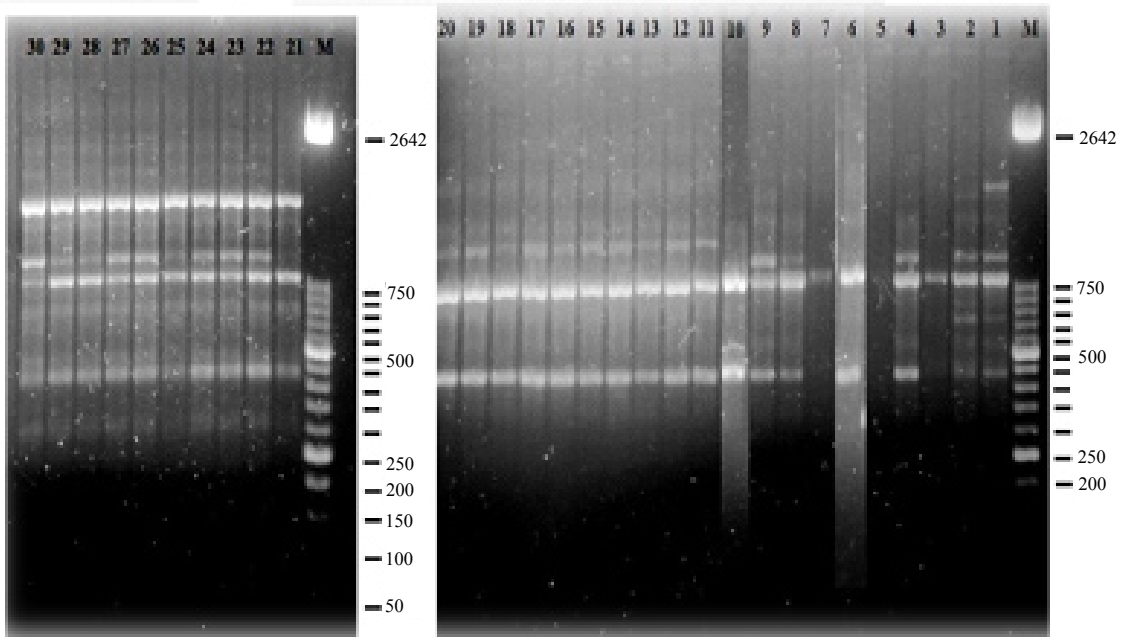

Fig. 2: DNA bands profile as the result of amplification using primer OPA-01 *L. blythii* on agarose gel 1.2%. M: Marker of 100 bp, 1-10: Pangean population, 11-20: Harau population and 21-30: Malampah population

Table 2: Genetic variation of *L. blythii* on west Sumatra

Parameters	Populations		
	Lembah Harau-Payakumbuh	Malampah-Pasaman Barat	Pangean II-Sijunjung
Band number	64.00	66.00	42.00
Average bands	8.00	8.250	5.250
Polymorphic locus (N)	30.00	47.00	30.00
Polymorphic locus percentage (Pp) (%)	31.580	49.470	31.580
Number of allele observed (Na)	1.326±0.467	1.496±0.503	1.316±0.473
Number of effective allele (Ne)	1.225±0.362	1.280±0.379	1.230±0.371
Average Heterozygosity (H)	0.126±0.019	0.160±0.020	0.127± 0.020
Average Shannon index(I)	0.184±0.028	0.240±0.028	0.185±0.028

Table 3: Genetic differentiation and gene flow of *L. blythii* on west Sumatra

Samples number	H_T	H_S	D_{ST}	G_{ST}	N_M
30	0.281	0.138	0.143	0.5091	0.4821

H_T : Total population heterozygosity (H_S+D_{ST})
H_S : Heterozygosity intra-population
D_{ST} : Heterozygosity inter-population
G_{ST} : Genetic differentiation inter-populasi
N_M : Gen flow

flow was N_M (0.4821). Genetic differentiation showed that 50.91% of total genetic variation was inter-population and 49.09% from intra-population as shown in Table 3.

DISCUSSION

Wildlife Sanctuary of Lembah Harau-Payakumbuh (Harau), Malampah-Pasaman Barat (Malampah) and Pangean II-Sijunjung (Pangean) are conservation area protected by Indonesian government. Outside sanctuary, *Limnonectes blythii* were rarely found. Higher genetic variety in Malampah population was most likely influenced by undisturbed river condition of this species habitat from human activities compared with Harau and Pangean. This species habitat in Harau was already disturbed by human activities for tourist attraction which lead to changes in vegetation and habitat destruction because of land fragmentation and pollution. Likewise with Pangean area, the river of *L. blythii* habitat is close with community settlement which caused pollution and habitat fragmentation. *Limnonectes blythii* used to live and reproduce at shallow and clear river flow in tropical forest[12]. Genetic variation of *Hylarana parvicola* on Malampah was higher than Pangean and Harau because both of the species habitat on already fragmented and polluted[13]. Anthropogenic factor greatly influence organism's habitat quality, including amphibians[14].

Genetic variation on the three population were considered low with average heterozygosity between 0,160-0,126. One of the reason behind this low genetic variety is intensive capture since 1970's until 2000's for trade and export goods. This condition leads to low possibility to find this species population outside conservation area. Small population would increase inbreeding and non-random mating thus decreasing genetic variation[7]. Small population also lower genetic mixing among individual in population that bring down genetic variation and fitness of that population[15]. The low genetic variation of *L. blythii* in north Thailand caused by captures for export[16]. *Limnonectes blythii* as one of the biggest frog species in the world and able to reach 90-260 mm. The great size made this species often captured intensively and exported by local people for its legs for consumption[5].

Genetic differentiation (G_{ST}) and gen flow (N_M) on all population in this research were considered moderate G_{ST} (0.5091) and N_M (0.4821). This result showed that genetic variation intra-population was lower than inter-population. Moderate inter-population genetic differentiation correlated with how this species living. The three population of this species live on different river habitat, which eliminate possibility to cross mating between populations. Higher probability of inbreeding inside population, lowering genetic variation and lead to moderate gen flow of three *L. blythii* populations. The number of gen flow shows the level of migration and dispersal between populations[6]. The that lower genetic differentiation and gen flow of *H. parvacola* population of west Sumatra caused by changes of vegetation types and microhabitat of their each population[13].

Low genetic variation of three population observed from RAPD analysis implied that future sustainability of this species would be endangered. However, moderate genetic differentiation of *L. blythii* inter-population bring through the possibility to construct a conservation strategy. Another

challenge to conserve frog species is how control human activities such as, land clearing for forestry, mining, expansion for residential areas and capture for trading, which lead to habitat deforestation, fragmentation and pollution. Damaged habitat cause *L. blyhtii* distribution obstructed and no migration occur. Inbreeding would increase and lead to decreasing genetic variation and species viability. Anthropogenic activities such as, urbanization, intensive agriculture are some of activities that damaged habitat structures and lead to forest fragmentation[17]. Anthropogenic activities have great influence to organism habitat quality, including amphibians[14]. Genetic variation determine organism ability to adapt for environmental changes[18].

Based on low genetic variation of *L. blythii* in the west Sumatra both on inter and intra-population, *in situ* and *ex situ* conservation effort need to be planned involving collaboration between government, academics and community.

CONCLUSION

Present study concluded the genetic variation of *L. blyhtii* was mostly found at Malampah population and the lowest at Harau population. However, overall genetic variety from sampling sites were considered low. The intra-population genetic variety of *L. blythii* was lower than inter-population and genetic differentiation and gen flow of *L. blythii* inter-population were considered moderate.

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REFERENCES

1. Emerson, S.B., R.F. Inger and D. Iskandar, 2000. Molecular systematics and biogeography of the fanged frogs of Southeast Asia. Mol. Phylogenet. Evol., 16: 131-142.
2. Tjong, D.H., D.T. Iskandar and D. Gusman, 2010. Phylogentic relationship among *Limnonectes* (Ranidae: Amphibia) found in West Sumatra with other species from South East Asia based on the based on the 16S rRNA Gen. Makara J. Sci., 14: 79-87.
3. Inger, R.F. and D.T. Iskandar, 2005. A collection of amphibians from West Sumatra, with description of a new species of *Megophrys* (Amphibia: Anura). Raffles Bull. Zool., 53: 133-142.
4. Kurniawan, N., D.R. Ahmadlia, D.S. Nahari and A.S. Firdaus, 2015. Speciation and zoogeography of amphibian in sundaland. J. Biol. Res., 21: 1-7.
5. Iskandar, D.T. and W.R. Erdelen, 2006. Conservation of amphibians and reptiles in Indonesia: Issues and problems. Amphibian Reptile Conserv., 4: 60-87.
6. IUCN., 2018. IUCN red list of threatened species. International Union for Conservation of Nature (IUCN), Gland, Switzerland.
7. Allendorf, F.W. and G.H. Luikart, 2007. Conservation and the Genetics of Population. Blackwell Publishing, Malden, MA., USA., ISBN-13: 9781405121453, Pages: 664.
8. Trakimas, G., M. Matsui, K. Nishikawa and K. Kasugai, 2003. Allozyme variation among populations of *Rana pirica* (Amphibia: Anura). J. Zool. Syst. Evol. Res., 41: 73-79.
9. Zeisset, I. and T.J. Beebee, 2003. Population genetics of a successful invader: The marsh frog *Rana ridibunda* in Britain. Mol. Ecol., 12: 639-646.
10. Mendez, M.A., E.R. Soto, C. Correa, A. Veloso, E. Vergara, M. Sallaberry and P. Iturra, 2004. Morphological and genetic differentiation among Chilean populations of *Bufo spinulosus* (Anura: Bufonidae). Rev. Chilena Historia Nat., 77: 559-567.
11. Yeh, F.C., T. Boyle, R. Yang, Z. Ye and J.M. Xian, 1999. POPGENE version 1.31: A Microsoft window-based freeware for population genetic analysis. University of Alberta, Edmonton, Canada, August 1999.
12. Iskandar, D.T., 1998. Amfibi Jawa dan Bali. Puslitbang Biologi-LIPI, Bogor, Indonesia, ISBN-13: 9789795790150, Pages: 117.
13. Yuliatmy, P., D.I. Roesma and D.H. Tjong, 2016. Genetic variation of *Rana parvaccola* (Inger, Stuart and Iskandar, 2009) based on DNA microsatellites in West Sumatra. J. Entomol. Zool., 4: 1068-1071.
14. Spear, S.F. and A. Storfer, 2008. Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected vs. managed forests. Mol. Ecol., 17: 4642-4656.
15. Phillipsen, I.C., 2011. Population genetics of Ranid frogs: Investigating effective population size and gene flow. Ph.D. Thesis, Oregon State University, Corvallis, OR., USA.
16. Suwannapoom, C., W. Wongkham, N. Sitasuwan, C. Phalaraksh and T. Kunpradid *et al*., 2012. Genetic Structure and Diversity of the Giant Frog (*Limnonectes blythii*) in Northern Thailand. Res. J. Applied Sci. Eng. Technol., 4: 2564-2568.
17. Johansson, M., C.R. Primmer, J. Sahlsten and J. Merila, 2005. The influence of landscape structure on occurrence, abundance and genetic diversity of the common frog, *Rana temporaria*. Global Change Biol., 11: 1664-1679.
18. Pertoldi, C., R. Bijlsma and V. Loeschcke, 2007. Conservation genetics in a globally changing environment: Present problems, paradoxes and future challenges. Biodivers. Conserv., 16: 4147-4163.