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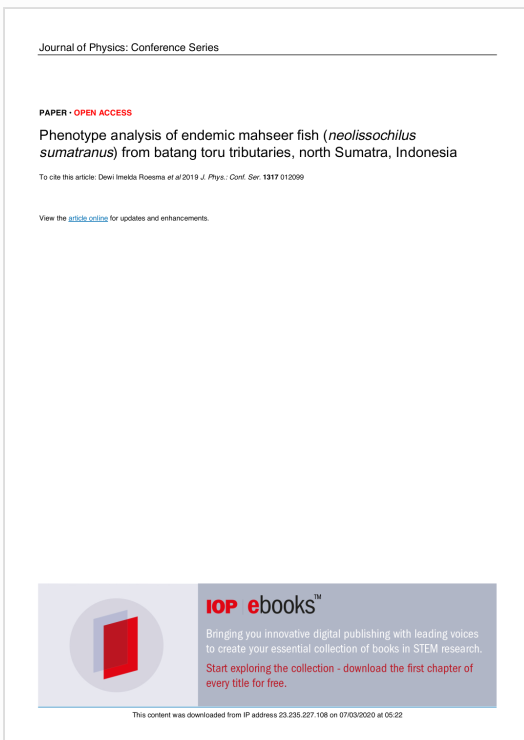

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Phenotype analysis of endemic mahseer fish (*neolissochilus sumatranus*) from batang toru tributaries, north Sumatra, Indonesia

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Abstract. A Phenotype analysis of *Neolissochilus sumatranus* (Cyprinidae) which is an endemic species to Sumatra Island has been carried from five tributaries of Batang Toru based on 23 morphometric and 14 meristic characters. The result showed the significance of characters differentiation in morphometric characters but no differentiation in meristic characters detected. Using Kruskal Wallis Test and Mann-Whitney U Test, the characters which showed significantly different among population related to body width and body depth, as well as to fin and eye diameter. Plot Principal Component Analysis (PCA) indicated the distinction clearly for each population. Phenotype variation between *N. sumatranus* in North Sumatra proving the local adaptation ability of fishes in different environments, and it probably related to the ability of morphological plasticity.

1. Introduction

More than 4,411 fishes species are recorded in Asia continent, includes freshwater fishes and brackish saltwater fishes (Le´ve^que et al. 2008). Among them, Indonesia contributed 1,172 native fish species which make up the high diversity in ichthyofauna. In average 400 species are endemic in Sundaland hotspot and currently the most threatened as its diversity faced the increases of threats (Hubert et al. 2015). The threats come over, but the knowledge of freshwater fishes are still incompletely known (Dudgeon et al. 2006). The study in Indonesian freshwater fishes has been challenged due to limited access to the island and, complicated political history. Furthermore, Indonesia fish fauna also hosts several large radiations of morphologically similar species (Myers, et al. 2000; Lamoureux et al. 2006; Hoffman et al. 2010; Hubert et al. 2015).

The largest family in Indonesia freshwater fishes is Cyprinidae with 241 species followed by the families of Gobiidae (122 species), Osphronomidae(81 species) and Bagridae (60 species) (Hubert et al. 2015). Mahseer is one of Cyprinidae family and are commonly known as economically important fishes. Kottelat et al. (1993) were grouping Mahseer fishes as *Neolissochilus* and *Tor*. While other researcher (Rainboth 1985; Talwar and Jhingran 199; Bhatt and Pandit 2015) wrote that Mahseer consist of the species of *Tor*, *Neolissochilus* and *Naziritor* genera.

Mahseer fishes have been used as local delicacies served at the cultural ceremony in North Sumatra, Indonesia. According to Kottelat et al. (1993) there are two species *Neolissochilus* identified

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in Sumatra, *N. thiennemanni*and *N. Sumatranus.* They are differ each otherby the presence of pores in operculum. In the Batang Toru river system survey, Roesma et al. (2016) recorded that the Cyprinidae is the largest family which consists of eleven species, and one of them is *N. Sumatranus,* local people called it as Jurung fish. As a Mahseer in general, the habitat of *N. sumatranus* is the fast-flowing river. This is a potamodromousfish, migrate towards the upper river with rocky and sand to lay their eggs. The maturation period is at downstream of the river. The Mahseer like the rich oxygen water with low temperatures. Under that condition, they are potentially isolated in the hilly regions and inter population breeding became restricted.

The environmental variables are known contribute to the morphology changes of both aquatic and terrestrial animals. Body morphology for aquatic animals is largely responded to the environmental pressures derived from the medium in which of the organism live (Knouft 2003; Moyle and Cech 2004). This is due to the morphological traits (meristic and morphometric) are under the influence of the interaction between genotype and environment (Swain and Foote 1999). Batang Toru as a nature reserve hasa complex water flows river systems with different physical environment flowing through the typical evergreen rainforest (Roesma et al. 2016). There for the study of morphometric variation becomes necessary to investigate whether morphological variation occurred among populations N. *sumatranus* from several tributaries in Batang Toru river system.

2. Materials and Methods

Fish sampling was conducted from 2014-2015 during the wet season from five localities in Batang Toru river system (Figure 1) consist of six individuals from AekSitandiang, five individuals from Batang Paya, six individuals from AekSirabun, 19 individuals from AekMalakut and 10 individuals from Aek Na Pot Pot. All of this tributaries are flows into Batang Toru main river systems. Many different methods were used to collect the fishes depending upon the circumstances, e.g., fishnet, backpack electrofishing, and hand net. Fish specimens in the field were stored in 10% formalin and transferred into 70% alcohol after one week for a long-term preservation and further investigation. Each individual was labeled, and identification process follows Weber and Beaufort (1916) and Kottelat et al. (1993). Measurements for 23 morphometric characters in total was followed Haryono and Tjakrawidjaja (2006); Roesma and Santoso (2011), as follows : TL: Total Length, SL: Standard Length, HL: Head Length, PDL: Pre Dorsal Length, PPL: Pre Pelvic Length, PAL: Pre Anal Length, , HD : Head Depth, BD: Body Depth, DCP: Depth of Caudal Peduncle, LCP: Length of Caudal Peduncle, SNL: Snout Length, BW: Body Width, ED: Eye Diameter, IW: Interorbital Width, LDB: Length of Dorsal Base, LAB : Length of Anal Base; LPVF : Length of Pelvic Fin; LPCF : Length of Pectoral Fin: LUCL : Length of Upper Caudal, LMCR: Length of Middle Caudal, LLCL : Length of Lower Caudal, MXB : Length of Maxillary Barbell and SMB : Length of Small Mandibular Barbell. The meristic character was counted based on de Silva et al. (2006). Statistical analysis was carried out separately for morphometric and meristic character due to the two types of variables being different concerning statistical. Morphometric data are continues while meristic data are discrete. Standard length is given as original values of average, while other morphometric characters are given as a percent of standard length. The Kruskall-Wallis test was performed to identify the characters that showed significant differentiation of the overall population (multivariate population ratio). The statistical test with Mann-Whitney U test is intended to identify morphological differentiation between two different populations (one population with other populations) of *N. sumatranus* species found in five rivers. Because of the number of samples is few and not uniform then we used Kruskal-Wallis test and Mann-Whitney U Test for all character used IBM SPSS ver. 22. Principle Component Analysis (PCA) to identify patterns of *N. sumatranus*phenetic differentiation among populations in five localities in Batang Toru river system. The data analyzed by PCA are morphometric data which have been standardized by length and transformed with Log^{10}. Further data is processed using MVSP 3.1 software program to obtain PCA plot ordination. Unweighted Pair Group Method Aritmatic Average (UPGMA) was constructed to looking for the divergence between species used NTSystVer.2.02i program.

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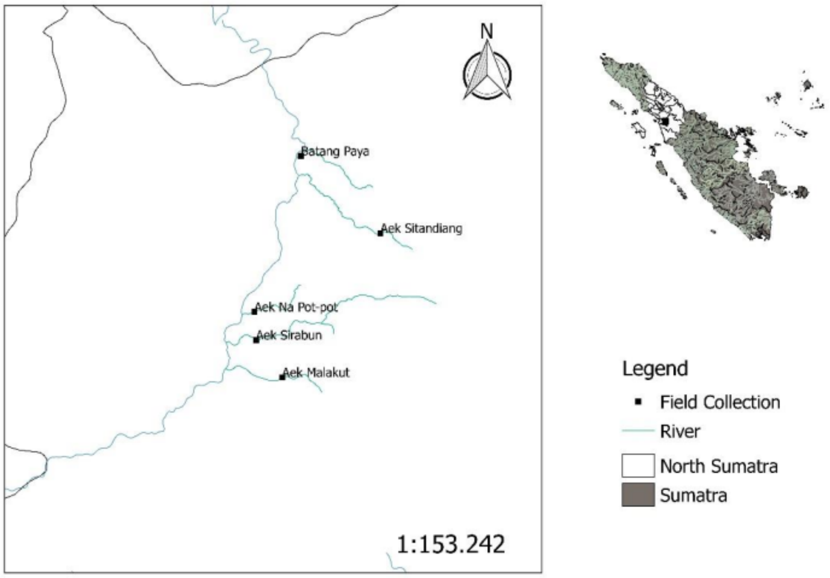

Figure 1. Map showing the sampling sites in Batang Toru river system in Sumatra

3. Results and Discussion

The total of 46 individuals have been collected from five sampling site. The analyzed and measured based on 23 morphometric characters. The minimum and maximum total length of the average value of five populations for the whole samples were 114.5-149. In comparison to standard length, it has the minimum and maximum length as follows, 129 % and 133.40% (Table 1.). The result of analysis indicated that there was five characters which showed significantly different among the population, those are body depth, body width, eye diameter, length of anal base and length of middle caudal. Those significant in $p<0.05$ based on non-parametric Kruskall Wallis Test. We did not examined the rank data of Standard length (SL), then we provides them in exact measurement value. Other characters were showed in ratio to standard length. For each character, the significant value was in range 0.01-0.03 which is lower than $p<0.05$.

The five significant characters are correlated to Body forms, such as body width and body depth. That character might reflect general environmental condition. Morphometric characters are measurable linear measurements of a fish and are known to vary with the factors like the river, altitude range, and environmental conditions of the habitats. For a long-term, it also reflected the local condition and adaptation then it drives intra-specific morphological plasticity (de Silva et al. 2006).

Table 1. The value of Kruskall Wallis Test, minimum and maximum value in each characters for each locality which are statistically different.

Characters	A. Sitandiang N = 6	A. Batang Paya N = 5	A. Sirabun N = 6	A. Malakut N = 19	A. Na Pot Pot N = 10	*H*	
TL	132 ± 0.06	133.10 ± 0.03	133.10 ± 0.02	133.40 ± 0.05	129 ± 0.06	*H:* 5.96	
	119.90 - 138	128.90 - 135	129.50 - 134	126 - 149	114.50 - 139.4	χ: 0.2	ns
SL	112.60 ± 20.30	113.20 ± 34.80	97.48 ± 7.22	67.57 ± 12.90	92.76 ± 22.56	*H:* -	
	91.60 - 139	52.70 - 137	91.27 - 110	55.64 - 101	62.55 - 138	χ: -	
BD	28.17 ± 0.01	28.70 ± 0.02	26.88 ± 0.07	26.09 ± 0.02	27.79 ± 0.02	*H:* 11.9	

	26.42 - 30.14	25.12 - 30.93	12.46 - 31.24	22.34 - 29.18	24.89 - 29.60	χ:	0.02	*
BW	14.39 ± 0.00	13.42 ± 0.01	13.62 ± 0.03	12.74 ± 0.01	13.53 ± 0.01	H:	10.9	
	14.14 - 15.06	12.60 - 14.96	7.18 - 16.60	10.45 - 16.23	12.16 - 14.94	χ:	0.03	*
ED	6.60 ± 0.01	6.54 ± 0.01	7.55 ± 0.01	7.89 ± 0.02	7.58 ± 0.01	H:	14.1	
	5.49 - 7.38	5.84 - 7.41	6.19 - 10.27	5.90 - 14.10	6.69 - 8.89	χ:	0.01	*
LAB	8.58 ± 0.01	7.36 ± 0.01	6.74 ± 0.01	8.78 ± 0.02	8.42 ± 0.01	H:	11.5	
	7.61 - 9.47	6.14 - 8.96	5.23 - 8.67	6.49 - 15.42	6.89 - 9.93	χ:	0.02	*
LMCR	13.16 ± 0.01	14.42 ± 0.02	14.00 ± 0.01	15.66 ± 0.02	15.53 ± 0.02	H:	11.8	
	11.19 - 14.35	12.82 - 17.11	12.67 - 15.26	12.66 - 19.71	12.54 - 18.73	χ:	0.02	*

Further analysis of looking at two different population provided in Table 2 and the number of characters which showed significantly different provide in Table 3.

Table 2. Mann Whitney Test result to compare the significant character between two different populations

Chara	STD-BTP			STD-SRB			STD-MLK			STD-NPP			BTP-SRB			BTP-MLK			BTP-NPP			SRB-MLK			SRB-NPP			MLK-NPP		
cter	*U*	*χ*		*U*	*χ*		*U*	*χ*		*U*	*χ*		*U*	*χ*		*U*	*X*		*U*	*χ*		*U*	*χ*		*U*	*χ*		*U*	*χ*	
TL	15	1.00	ns	17	0.87	ns	57	1.00	ns	15	0.10	ns	12	0.58	ns	45	0.86	ns	11	0.09	ns	56	0.95	ns	11	0.04	*	52	0.05	ns
HL	9	0.27	ns	16	0.75	ns	34	0.14	ns	26	0.66	ns	11	0.47	ns	46	0.92	ns	21	0.62	ns	40	0.28	ns	24	0.52	ns	84	0.61	ns
PDL	10	0.36	ns	12	0.34	ns	52	0.75	ns	22	0.39	ns	15	1.00	ns	46	0.92	ns	25	1.00	ns	49	0.61	ns	27	0.74	ns	86	0.68	ns
PPL	12	0.58	ns	15	0.63	ns	21	0.02	*	18	0.19	ns	15	1.00	ns	21	0.06	ns	18	0.39	ns	24	0.04	*	21	0.33	ns	80	0.49	ns
PAL	9	0.27	ns	17	0.87	ns	30	0.09	ns	23	0.45	ns	10	0.36	ns	46	0.92	ns	19	0.46	ns	29	0.07	ns	26	0.66	ns	68	0.22	ns
HD	12	0.58	ns	18	1.00	ns	33	0.13	ns	20	0.28	ns	13	0.72	ns	42	0.70	ns	22	0.71	ns	40	0.28	ns	20	0.28	ns	93	0.93	ns
BD	11	0.47	ns	11	0.26	ns	18	0.01	*	24	0.52	ns	15	1.00	ns	19	0.04	*	17	0.33	ns	24	0.04	*	17	0.16	ns	49	0.03	*
DCP	15	1.00	ns	17	0.87	ns	47	0.52	ns	15	0.10	ns	14	0.86	ns	40	0.59	ns	12	0.11	ns	49	0.61	ns	14	0.08	ns	54	0.06	ns
LCP	15	1.00	ns	17	0.87	ns	44	0.41	ns	13	0.07	ns	14	0.86	ns	36	0.41	ns	15	0.22	ns	50	0.66	ns	18	0.19	ns	63	0.14	ns
SNL	5	0.07	ns	10	0.20	ns	55	0.90	ns	28	0.83	ns	13	0.72	ns	24	0.09	ns	14	0.18	ns	46	0.48	ns	27	0.74	ns	84	0.61	ns
BW	5	0.07	ns	15	0.63	ns	20	0.02	*	11	0.04	*	9	0.27	ns	28	0.17	ns	23	0.81	ns	29	0.07	ns	17	0.16	ns	51	0.04	*
ED	13	0.72	ns	9	0.15	ns	12	0.00	*	12	0.05	ns	6	0.10	ns	10	0.01	*	5	0.01	*	39	0.25	ns	21	0.33	ns	86	0.68	ns
IW	8	0.20	ns	7	0.08	ns	25	0.04	*	7	0.01	*	13	0.72	ns	32	0.27	ns	10	0.07	ns	54	0.85	ns	22	0.39	ns	84	0.61	ns
LDB	14	0.86	ns	6	0.05	ns	23	0.03	*	12	0.05	ns	9	0.27	ns	38	0.50	ns	17	0.33	ns	54	0.85	ns	24	0.52	ns	88	0.75	ns
LAB	5	0.07	ns	4	0.02	*	56	0.95	ns	28	0.83	ns	10	0.36	ns	22	0.07	ns	10	0.07	ns	15	0.01	*	7	0.01	*	91	0.85	ns
LPVF	6	[illegible]	ns	13	0.42	ns	35	0.16	ns	15	0.10	ns	10	[illegible]	ns	38	0.50	ns	22	0.71	ns	53	0.80	ns	25	0.59	ns	87	0.71	ns
LPCF	12	0.58	ns	15	0.63	ns	30	0.09	ns	20	0.28	ns	12	0.58	ns	22	0.07	ns	16	0.27	ns	29	0.07	ns	21	0.33	ns	67	0.20	ns
LUCL	8	0.20	ns	15	0.63	ns	56	0.95	ns	26	0.66	ns	6	0.10	ns	23	0.08	ns	14	0.18	ns	51	0.70	ns	20	0.28	ns	72	0.29	ns
LMCR	10	0.36	ns	11	0.26	ns	12	0.00	*	8	0.02	*	14	0.86	ns	28	0.17	ns	16	0.27	ns	25	0.04	*	16	0.13	ns	87	0.71	ns
LLCL	10	0.36	ns	18	1.00	ns	56	0.95	ns	26	0.66	ns	11	0.47	ns	30	0.21	ns	20	0.54	ns	56	0.95	ns	25	0.59	ns	81	0.52	ns
MXB	13	0.72	ns	18	1.00	ns	53	0.80	ns	24	0.52	ns	11	0.47	ns	40	0.59	ns	12	0.11	ns	57	1.00	ns	17	0.16	ns	70	0.25	ns
SMB	14	0.86	ns	11	0.26	ns	46	0.48	ns	23	0.45	ns	8	0.20	ns	30	0.21	ns	18	0.39	ns	56	0.95	ns	21	0.33	ns	73	0.31	ns

Table 3. Number of character which showed significantly different between two populations based on Mann hitney U Test

	A.Sitandiang	**A.Batang.Paya**	**A.Sirabun**	**A.Malakut**	**A.Napotpot**
A.Sitandiang	0				
A.Batang.Paya	0	0			
A.Sirabun	1	0	0		
A.Malakut	7	2	4	0	
A.Napotpot	3	1	2	2	0

AekMalakut and AekSitandiang show the most significant characters, in total seven characters are significantly different. Those characters are PPL, BD, BW, ED, IW, LDB and LMCR. Following by AekSirabun and Aek Malakut which shows significant in four characters, those are PPL, BD, LMCR, and LAB. The characters which showed significantly different between AekSitandiang and Aek Na Pot Pot are BW, IW, and LMCR. AekBatangPaya and AekMalakut significant in two characters ED and BD.AekSirabun and Aek Na Pot Pot significant in two characters TL and LAB.AekMalakut and Aek Na Pot Pot showed significantly different in two characters. AekSitandiang and AekSirabun different in one character (LAB), and AekBatangPaya and Aek Na Pot Pot showed significantly

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different in only one character (ED). Mostly the characters which showed almost in all population are Body Depth and Eye Diameter (BD and ED). We also recorded some population does not show significantly different among populations, for example between population *N. sumatranus* in AekSitandiang and AekBatangPaya, and between AekBatangPaya and AekSirabun.

The result in this study suggested that the morphology character might be affected by environmental pressure. This thing also happens in perch, the extension of individual diet specializations are mainly correlated to morphological variation but not always. The contribution between genotype and environmental interacting each other also play a role in morphological variation. From the number of the study suggested that morphology differentiation correspondent to foraging activity, sometimes due to morphology plasticity (Svanback 2004). Morphological plasticity can be measured according to the morphometric character. Length-weight and character width are useful in fishery management for both applied and basic uses (Hussain et al. 2012). The morphometric measurement of fishes and the study of the statistical relationship among them are essential for the taxonomic study of a species (Tandon et al. 1993; Behnke 1980; Doherty and McCarthy et al. 2002). Suggested that some geographical regions may have suitable environments, factor such substrate availability and water depth also contributed to morphometric variation. In this study, we commonly found body depth and body width as the significantly different in each population. We expect that body form is also correlated to feeding behavior and physical forms of the river, such as water current, depth, and pH. The body depth and width may reflect the water flow and substrate; we assume that individual living in fast-water flow may have stream body compared to the who live in the deep and small river with the slow-water flow. However, we did not test the hypothesis, and this may need further analysis. Also, there is no significant difference in the quantitative analysis of meristic characters. *N. sumatranus* has 21-23 transverse scales, ten dorsal fin rays, nine ventral fin rays, 13 pectoral fin rays, 6-7 anal fin rays, 7-8 predorsal fin scales, 11-12 post dorsal fin rays, three scales around caudal peduncle, one dorsal fin spines, 19-20 caudal fin rays and five dorsal fin scales. There are no fin spines in ventral, pectoral and anal fin.

Plot Principal Component Analysis showed each population divergent in a different quadrant. The population of Aek Na Pot Pot and AekSirabun indicated in the same Quadrant (Figure 1). This result showed the same indication in Figure 2, in this cluster, Aeksirabun and Aek Na Pot Pot showed many similarities in characters than other population. Differ with those two populations, each of the population of N. sumatranus in AekBatangPaya, AekSitandiang and AekSirabun placed in the different quadrant. According to UPGMA cluster analysis, we can assume that AekMalakutare most diverge to other population. The population of *N. sumatranus* in AekBatangPaya and AekSitandiang showed many similarities and less in the distance than to the population of AekMalakut. We can divide the clade into three groups, those are AekSitandiang and AekBatangPaya in the first group (distance = 2), the second group are AekSirabun and Aek Na Pot Pot (distance = 5) and the third group is AekMalakut. The distance between the first and second group is 17.5, the third cluster between two populations to AekMalakut is 36. This result is in line with the PCA and non-parametric statistical test, which showed that the population of *N. sumatranus*in AekMalakut diverged both in the number of character significant and PCA plot. We need to measure the environmental variable which might be affected directly into each population. In addition, we also expected that morphological plasticity as the most reason for the grouping of *N. sumatranus*in the population. Further study is recommended for further decision making in that locality.

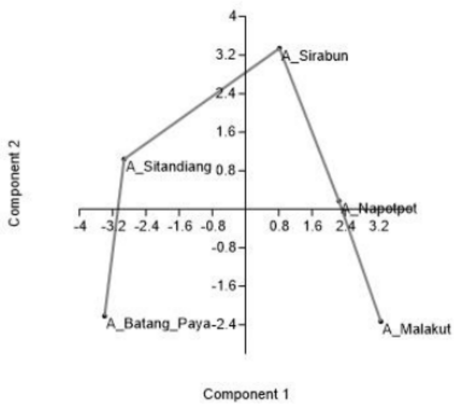

Figure 2. PCA based in two ordinate components

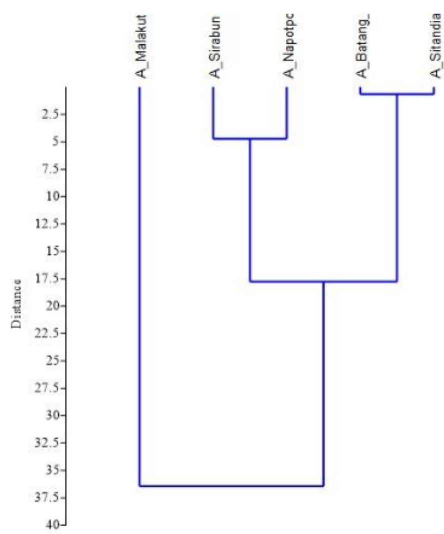

Figure 3. UPGMA that showed the distance between populations

In conclusion, there are five characters significantly different among all population examined of *N. sumatranus*, they are Body Depth (BD), Body Width (BW), Eye Diameter (ED), Length of the Anal Base (LAB) and Length of Middle Caudal (LMCR). The character also detected showed significant between two different populations, the most typical character found are Body Depth (BD), Body Width (BW), Eye Diameter (ED). Those characters normally related to the water conditions. Therefore we can predict the environmental effect to each population and vice versa.The population of *N. sumatranus* at AekMalakut diverged and standalone from other groups which consist of the populations in AekBatangPaya and AekSitandiang (as group 1) and AekSirabun and Aek Na Pot Pot (as group 2) respectively.

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