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The study of genetic diversity of *Daemonorops draco* (Palmae) using ISSR markers

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ABSTRACT

Asra R, Syamsuardi, Mansyurdin, Witono JR. 2014. The study of genetic diversity of Daemonorops draco *(Palmae) using ISSR markers. Biodiversitas 15: 109-114.* The genetic diversity in five populations of *Daemonorops draco* (Willd.) Blume (*Jernang*: in Bahasa Indonesia) was analyzed using Inter Simple Sequence Repeat (ISSR) markers. The screening results from using 15 ISSR primers showed that only 5 of ISSR primers had clear and reproducible bands. Based on the data from the matrix binary analyzed using POPGENE version 3.2, the highest genetic diversity was found in the Sepintun population at 0.0969 average heterozygosis (H) and 0.146 average Shannon Index (I). The heterozygosis calculation of the total population (H_T) was 0.2571. The heterozygosis value within a population (H_S=0.0704) was smaller than that between populations (D_{ST}=0.1867). Using the clustering analysis program *Pastversion 32* on 43 individuals of *D. draco*, we found that there were three groups of *D. draco*. Group A consisted of 8 individuals in the Bengayoan population, group B consisted of 9 units in the Nunusan population and group C consisted of three populations; Tebo, Sepintun and Mandiangin consisted of 10, 8 and 8 individuals. The genetic similarity varied among all populations with the values between 0.07-0.93.

Key words: *Daemonorops draco*, genetic diversity, ISSR marker.

INTRODUCTION

Daemonorops is a genus of rattan palms. The genus consists of 115 species, but only 12 of which produce resin known as dragon's blood palm. Rustiami et al. (2004) states that the distribution of the species of the dragon's bloods is limited to Malaysia, Thailand and western Indonesia. *Daemonorops draco* (Willd.) Blume is a species that produces the most resin of high economic value, because of its larger fruits and longer in fructescences. *Daemonorops draco* is usually found in Sumatra (Jambi, Riau and Bengkulu) and Kalimantan (Purwanto et al. 2005). *Daemonorops draco* resin can be internally processed in the human body to improve blood circulation, improve tissue regeneration, cure sprains, ulcers, control bleeding, and relieve pain (Bensky and Gamble 1993; Thomson 2007).

The resin is a source of livelihood for the indigenous tribes (Anak Dalam, Talang Mamak and Melayu Tua) who live in the Bukit Tigapuluh National Park of Jambi and Riau Provinces, Indonesia. Cultivation of the species has not been done. Since local people usually harvest young fruit for high yields of resin, it is very difficult to find mature fruit to get seeds for cultivation. Planting seedlings is also less desirable, because usually less than50% of young individuals reach maturity. Habitat destruction of *D. draco* for oil palm and rubber plantations also causes population reduction of the species. According to the Forestry Department of Jambi Province (2009), the presence of *D. draco* in wild population is rare.

Efforts to overcome the scarcity of *D. draco* through both *in situ* and *ex situ* conservation are important to do. Because conservation deals with the species' adaptability in its environment, knowledge of the level of genetic diversity is the most important thing in the effort to conserve *D. draco*. Hamrick et al. (1990) argued that the data of genetic diversity and gene flow mechanisms must become a criterion to determine the effectiveness of conservation programs *in situ* and *ex situ*. Therefore, it is necessary to know the level of genetic diversity in the natural habitat, such as Bukit Tigapuluh National Park, secondary forest and the cultivation area of *D. draco*.

Molecular markers are usually used to analyze the genetic diversity within and among populations. ISSR (*Inter Simple Sequence Repeat*) markers are some of the most useful markers used in investigating the clonal diversity and population genetic structure (Rossetto et al. 1999). As a PCR-based marker, the ISSR marker has several advantages over others. The binding of ISSR primers is directed by *simple sequence repeat*, unlike the SSR marker that does not need prior knowledge of the target sequence on ISSRs (Godwin et al. 1997). In addition, the target sequences are very abundant throughout the ISSR eukaryotic genome and evolve rapidly. As a consequence,

ISSR markers generate a large number of polymorphic fragments per primer (Fang and Roose 1997). The study indicates that the ISSR markers produce both reliable and reproducible bands because the annealing temperature and sequence of ISSR primers are extended (Qian et al. 2001). The ISSR method has been used in several palm species such as *Pinanga* (Witono and Kondo 2006), *Calamus thwaitesii* (Ramesha et al. 2007) and *Phoenix dactylifera* (Younis et al. 2008).

MATERIALS AND METHODS

Plant materials

Forty three individuals of *D. draco* were obtained from five locations: three locations in Bukit Tiga Puluh National Park (BTNP) in the Province of Jambi and Riau, Indonesia, another location in the secondary forest in the Province of Jambi and the other location in a rubber cultivation area in the Province of Jambi, Indonesia (Figure 1).

Genomic DNA extraction

All of genomic DNA were extracted from silica gel-dried leaf tissues. First, the leaves were ground into a fine powder in liquid nitrogen using a mortar following the modification of CTAB (*cetyl trimethyl ammonium bromide*) protocol described by Doyle and Doyle (1987) with additional modifications described by del Castillo (2006). The DNA template samples were diluted with TE buffer to create the working solution of 10 ng μL^{-1} for PCR analysis.

PCR (Polymerase Chain Reaction)

The DNA amplification was carried out following ISSR technique using kits Go Taq® Green Master Mix, Promega Madison WI USA's product, which contained PCR amplification components: enzyme Taq DNA polymerase, dNTPs, $MgCl_2$ and PCR buffer. To create the cocktail needed, we used 12.5 µL Go Taq® Green Kit, 3 µL ISSR primer (20 pmol mL^{-1} concentration), 3 µL of DNA template (20 ng μL^{-1} concentration) and 6.5 µL of nuclease-free water. The DNA amplification was done on a PCR machine (MultiGEN labnet), for about 35 cycles. PCR procedures were carried out in the following order: (1) one cycle of denaturation at 94°C for 5 min: (2) 35 cycles of 94°C for 60 s (denaturation), 50°C for 45 s (annealing), 72°C for 120 s (extension); and (3) final extension 72°C for 5 minutes and followed by soaking at 4°C.

Electrophoresis

The amplified result of DNA fragments by ISSR marker was processed using electrophoresis with 5 µL of the standard DNA, 1 kb DNA ladder (100 ng μL^{-1}) in the first slot of 1.5% agarose gel in TBE 1X as the buffer solution. Then, agarose gel was run using electrophoresis technique with 60 volt for 90 minutes at room temperature. The resulting amplified bands were observed using gel documentation (Alpha Innotech) and recorded onto a CD. Each reaction was repeated 2 times to get the reproducible bands.

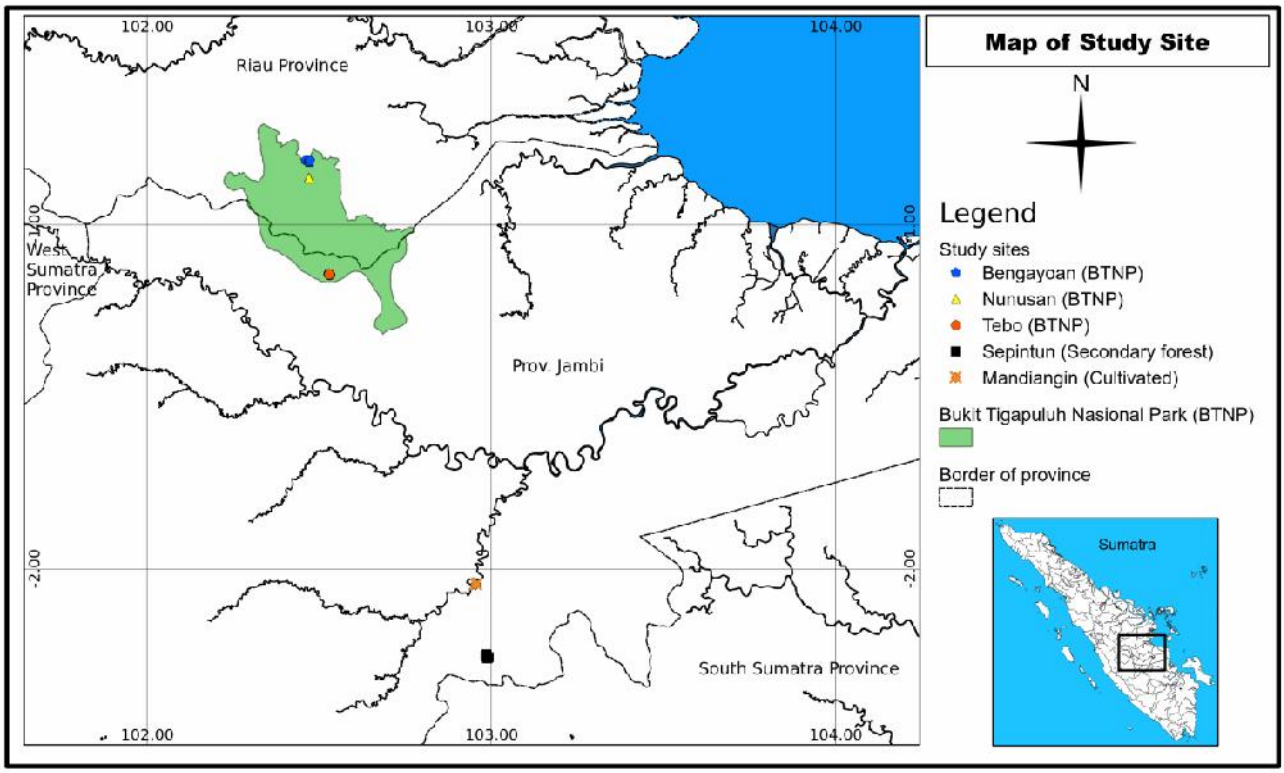

Figure 1. *Daemonorops draco* leaf sampling sites for genetic diversity research.

Data analysis

The results of the binary data matrix were analyzed using the population genetics software POPGENE version 3.2 (Yeh et al. 1997). A dendrogram was constructed based on the genetic distance matrix introduced by Nei (1978) using the program *Past*.

RESULTS AND DISCUSSION

There were five useful primers (HB 8, UBC807, UBC808, UBC810 and UBC834) found from the 15 primers that were screened. These five primers were used for PCR amplification. These five primers produced various sizes of bands. The band profiles produced from the five primers were used to study the genetic variation within and among populations of *D. draco*, the resulting bands were clear, polymorphic and reproducible (Figure 2).

The five primers used in this research produced 175 bands that were scored and used for further analysis. Of the total number of bands produced using the five primers on the five different populations of jernang, there were 47 bands in the Mandiangin population, 51 bands in the Bengayoan population, 53 bands in the Tebo population, 57 and 64 bands in the Sepintun and the Nunusan populations. These polymorphic bands from every primer varied from 11to 15 and the scores varied from 30 to 37 (Table 1). The percentage of polymorphic bands was calculated on the profile DNA bands different in different individuals (Klug et al. 2012).

In this research, the UBC810 produced the greatest number of polymorphic bands (15) and the total bands scored (37). On the other hand, HB8 produced the minimum number of polymorphic bands (10) and total bands scored (30). As a result, the HB8 primer contained fewer repeated DNA (GA)6) in their DNA sequences than the other 4 primers, which had DNA sequences repeated for about eight times. According to the research of Witono and Kondo (2006) on *Pinanga* genetic analysis using ISSR markers, the minimum number of polymorphic bands (10) was found with the UBC primer 881,which had fewer repeated DNA sequences (GGGTG)3) than the 8 other primers that had repeating DNA sequences t repeated for about eight times. Some of the other primers used in this study were: UBC813 (CT)8T), UBC815(CT)8G), UBC844 (CT)8RC and UBC845(CT)8RG).

The POPGENE analysis program version 3.2 was used to analyze the genetic diversity of *D. draco* in each population (Bengayoan, Nunusan, Tebo, Sepintun and Mandiangin). Table 2 shows that the highest percentage of polymorphic loci was found in the *D. draco* population in Sepintun (29.14% with 51 polymorphic loci), followed by the population in Bengayoan (20.57% with 36 polymorphic loci), Tebo (17.71% with 31), Nunusan (16.00% with 28) and finally Mandiangin (9.71% with 17). The percentage of polymorphism and the number of polymorphic loci demonstrate that the percentage of polymorphism is influenced by the population and the research sample. The Mandiangin population was the population with the fewest individuals (20 individuals).

Heterozygosity (H) or gene diversity is used to show diversity, since it has a few correlations to the population size and the polymorphic loci number. The highest number in genetic diversity was found in the Sepintun population with an average heterozygosity (H) of 0.0969 and an average Shannon index (I) of 0.146. The values of Bengayoan population were 0.0806 (H) and 0.1175 (I), Tebo population 0.0756 (H) and 0.1088 (I), the Nunusan population 0.0620 (H) and 0.0910 (I.) The lowest genetic diversity was found in the Mandiangin population with H = 0.0542 and I = 0.0369.

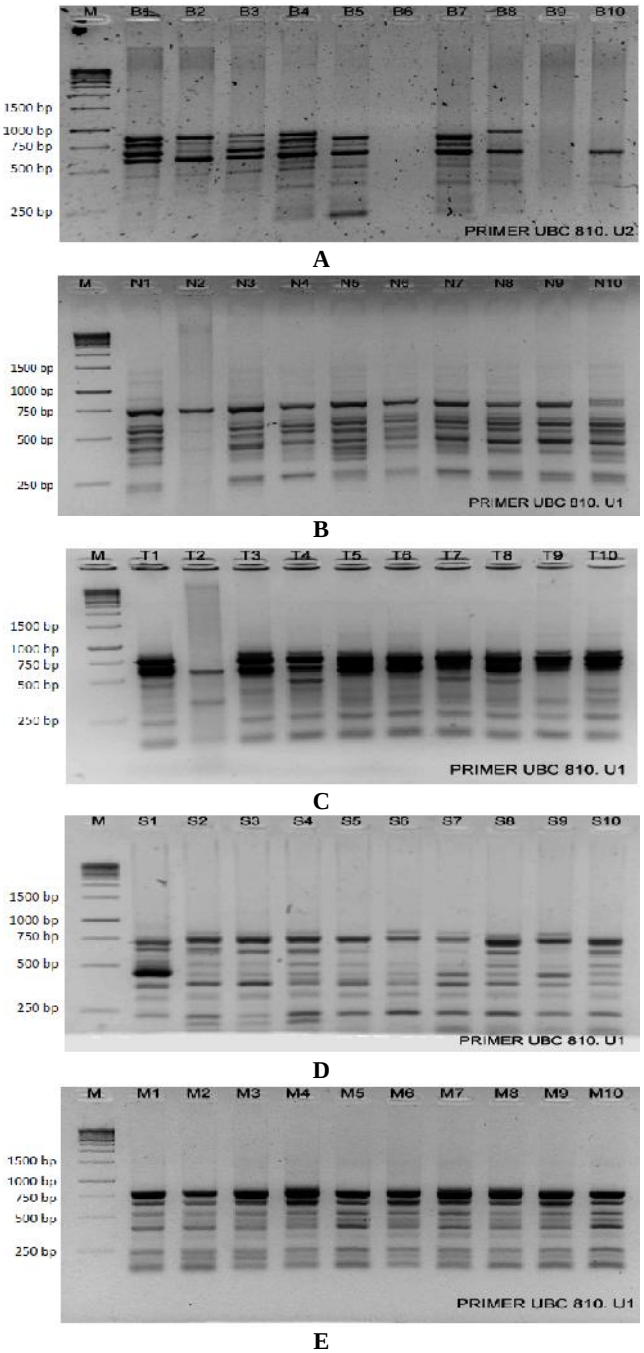

Figure 2. ISSR profile from *D. draco* in the five populations: (a) Bengayoan (BTNP), (b) Nunusan (BTNP), (c) Tebo (BTNP), (d) Sepintun (secondary forest) and (e) Mandiangin (plantation).

Table 1. Primer sequences and amplification numbers of polymorphic bands

Primer	Sequences	Repeated DNA	No. of amplification bands	No. of polymorphic bands	Polymorphic bands (%)	No. of bands scored
HB 8	5'-GAGAGAGAGAGAGG-3'	(GA)6 GG	12	11	96.67	30
UBC 807	5'-AGAGAGAGAGAGAGAGT-3'	(AG)8 T	13	13	100	37
UBC 808	5'- AGAGAGAGAGAGAGAGC-3'	(AG)8 C	13	13	100	37
UBC 810	5'-GAGAGAGAGAGAGAGAT-3'	(GA)8 T	16	15	93.75	36
UBC 834	5'-AGAGAGAGAGAGAGAGYT-3'	(AG)8 YT	13	13	100	35
	Total					175

Table 2. The results of the analysis of *Daemonorops draco* genetic diversity with ISSR markers in each population: Bengayoan, Nunusan, Tebo, Mandiangin and Sepintun.

Population	Total samples	Na	Ne	*H*	*I*	Pp (%)	N
Bengayoan (BTNP)	8	1.2057 ± 0.4054	1.1449 ± 0.3147	0.0806 ± 0.1691	0.1175 ±0.2417	20.57	36
Nunusan (BTNP)	9	1.16 ± 0.3677	1.1083 ± 0.2685	0.062 ± 0.149	0.091 ± 0.2156	16.00	28
Tebo (BTNP)	10	1.1771 ± 0.3829	1.1373 ± 0.3095	0.0756 ± 0.1673	0.1088 ±0.2389	17.71	31
Sepintun (Secondary forest)	8	1.2914 ± 0.4557	1.1661 ± 0.3148	0.0969 ± 0.1713	0.146 ± 0.2477	29.14	51
Mandiangin (Plantation)	8	1.0971 ± 0.297	1.0655 ± 0.2201	0.0369 ± 0.1201	0.0542 ± 0.1728	9.71	17

Note:Pp (%): Percentage of polymorphic loci, N: Number of polymorphic loci, Na: The average number of alleles observed, Ne: The average number of effective alleles, H: The average heterozygosity/Nei's genetic diversity, I: Mean Shannon index (Lewontin 1972)

Genetic diversity of *D. draco* in the Bukit Tigapuluh National Park (BTNP) should be higher than other populations. Based on the study, genetic diversity of three populations of *D. draco* in BTNP (Bengayoan H = 0.0806, Nunusan H = 0.062 and Tebo H = 0.0756) was lower than that of the populations of *D. draco* in Sepintun Secondary Forest (H = 0.0969). This is caused by the habit of indigenous tribes (Anak Dalam Tribe, Talang Mamak Tribe and Melayu Tua Tribe) who always cut down male individuals of *D. draco*. According to the indigenous tribe, *D. draco* male is considered useless, since it does not produce fruits. The reduced or absence of male individuals lead to low probability of sexual reproduction of *D. draco* females, so that they will reproduce asexually (apomixis) (Asra 2012). This does not occur in Sepintun Secondary Forest because people in the Sepintun do not cut down male individuals.

Based on interviews with the owner of the jernang population in Mandiangin, we discovered that the *D. draco* population in Mandiangin originally came from seeds or plants collected from Sepintun. As comparison, the populations of *D. draco* from Sepintun had the highest genetic diversity while the lowest genetic diversity was recorded in the populations of *D. draco* from Mandiangin. This is probably due to the selection of seeds from single or few parents which leads to a more uniform seeds. Another factor that causes the low genetic diversity in Mandiangin population is the limited number of individuals in the population.

The heterozygosity calculation of the total population (H_T) was equal to 0.2571 (Table 3). It seems that there was a very large genetic variation between the *D. draco* populations in Bengayoan, Nunusan, Tebo, Mandiangin and Sepintun. The heterozygosity of the total population (H_T) of *D. draco* genetic variation was 72.6% between populations and 27.4% among the populations.

Table 3. The analysis of *D. draco*'s genetic diversity of 43 individuals (*D. draco*) using ISSR markers and the gene flow program (POPGENE Program 3.20)

Samples	H_T	H_S	D_{ST}	G_{ST}	N_M
43	0.2571	0.0704	0.1867	0.7262	0.1885

Note:
H_T : The total population heterozygosity ($H_S + D_{ST}$)
H_S : The value of heterozygosity in the population
D_{ST} : The value of heterozygosity between populations
GST : Genetic differentiation between populations
NM : The flow of genes (gene flow)

Table 6 shows that the value of heterozygosity within the population (H_S=0.0704) was smaller than the value of heterozygosity between populations (D_{ST} = 0.1867). This result is contrary to the popular opinion of Nybom and Bartish (2000) which states that outcrossing species have the lowest genetic diversities between populations. Besides outcrossing, *D. draco* also reproduces by apomixis (Asra 2012). Apomixis will produce individuals similar to their parents. That means that within population they will produce lower genetic diversities and between populations they will produce higher genetic diversities.

Geographical isolation is also a factor that probably causes high genetic diversity between populations. The geographical distance between the five populations of *D. draco* (several kilometers) prevents cross pollination with individuals in the other populations. Hamrick and Godt

(1996) state that the combination of the breeding systems and geographic distance causes low genetic variation within a population, however the genetic variation between our populations was high.

The coefficient of genetic differentiation (GST) on *D. draco* was equal to 0.7262. That number is higher than the standard genetic differentiation number proposed by Nybom and Bartish (2000) which is equal to 0.23 (for an outcrossing breeding system) and 0.19 (for endemic plants). Geographical isolation and apomixis are the major factors that have caused the high genetic differentiation in *D. draco*. The geographical isolation has inhibited gene flow. This condition was proved by the low number of NM (0.1885). Fischer and Matthies (1998) states that the greater geographical isolation is, the less gene flow there will be.

Cluster analysis was used to calculate the genetic distances between the 43 individuals (Figure 3). The dendrogram shows the individuals grouping according to their genetic similarities. The grouping by genetic similarities also demonstrates the genetic differentiation among *D. draco* populations.

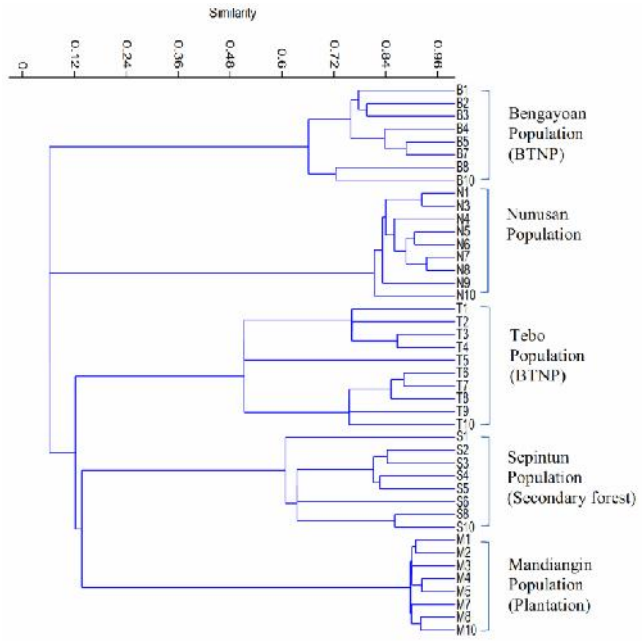

Figure 3. The results of the dendrogram's cluster analysis of genetic distance between individuals from the 5 populations using the ISSR markers.

The results of the cluster analysis program using *Pastversion 32* on the 43 *D. draco* individuals show that they were divided into three similar groups. Group A consisted of 8 individuals from the Bengayoan population, group B 9 individuals from the Nunusan population, and group C three populations; Tebo, Sepintun and Mandiangin. According to the similarity coefficient, the genetic similarities among all populations varied between 0.07 and 0.93. The genetic similarity between groups A, B, and C was 0.07. The differences or dissimilarities between the groups correlate with the geographical distances separating them from each other. Group C was divided into two subgroups: subgroup Tebo and subgroup Sepintun and Mandiangin. We learned that the origin of the Mandiangin population was the relocation of a portion of the Sepintun population. In other words, Sepintun's population is the ancestor of the entire Mandiangin's population. This was proven with dendrogram which shows that the Sepintun and Mandiangin populations were related and contained within the same group separated by a small genetic distance of only 0.14.

CONCLUSION

Five of the15 ISSR primers showed clear and reproducible bands. Genetic diversity of the *D. draco* populations in the conservation area of Bukit Tigapuluh National Park (BTNP) (Bengayoan H= 0.0806, Nunusan H= 0.062 and Tebo H= 0.0756) was lower than that of the Sepintun Secondary Forest (H=0.0969). Genetic diversity of the *D. draco* plantation (Mandiangin H=0.0369) was lower than that of the natural population (BTNP and Sepintun Secondary Forest). The heterozygosity within populations (H_S=0.0704) was smaller than that between populations (D_{ST}=0.1867). The cluster analysis program of 43 individuals of *D. draco* found that there were three similar groups: group A consisted of 8 individuals from the Bengayoan population, group B 9 individuals from the Nunusan population and group C three populations; the Tebo (10), the Sepintun (8), and the Mandiangin (8) individuals. The genetic similarities among all populations varied between 0.07-0.93.

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