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الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
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Original Article

Genetic Diversity of Five Native Populations (Dusun, Rungus, Sonsogon, Murut and Sungai-Lingkabau Paitan) of North Borneo, East Malaysia based on 17 Y-chromosomal Short-Tandem Repeats Polymorphism

التنوع الوراثي لخمسة من السكان الأصليين (دوسون، رونغوس، سونسوغون، موروت وسونغاي تومبونو بايتان) من بورنيو الشمالية، شرق ماليزيا تبعاً للتتابعات القصيرة المتكررة في 17 موقع وراثي على الكرموسوم الذكورة Y.

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Abstract

In this study, we typed 51 male individuals from North Borneo using 17 Y-chromosome STRs contained in the AmpFLSTR® Yfiler® kit (Applied Biosystems). These individuals constitute five indigenous ethnic populations representing the three major linguistic groups (Dusunic, Murutic and Paitanic): the Dusun (*n*=7), Rungus (*n*=12), Sonsogon (*n*=12), Murut Paluan (*n*=12), and Sungai Lingkabau Paitan (*n*=8). A total of 37 haplotypes were identified, of which 30 individuals were represented by a single haplotype.

The mean ± S.D. haplotype diversity was 0.600±0.181 and the discrimination capacity was 0.725. The results also showed that the haplotype H33 was the most frequent haplotype observed in the sampled male populations occurring exclusively in the Murut population. Comparative analysis between Y-haplotype populations of North Borneo and the ethnic populations (Bidayuh, Iban, and Melanau) of neighbouring Sarawak (East Malaysia) i.e. indicated that the Sungai Lingkabau Paitan was more closely associated with the Melanau with respect to Y-haplotype descent (RST=-0.0023). In addition, the Multidimensional Scaling (MSD) analysis managed to clearly differentiate the eight groups from Borneo.

We concluded that the 17 Y-chromosome STRs data of North Bornean populations are valuable resources in the applications of forensic and population genetics of the ethnic groups.

Keywords: *Forensic Sciences, Y-chromosomal Short Tandem Repeats (Y-STRs), Haplotype, Sabah Populations, Diversity, Pairwise Analysis*

المستخلص

في هذه الدراسة، تم تحديد ملف السمات الوراثية لـ 51 من الذكور من منطقة بورنيو الشمالية باستخدام التتابعات القصيرة المتكررة لـ 17 موقع وراثي على كروموسوم الذكورة Y الواردة في مجموعة كيماويات التكثير AmpFLSTR® Yfiler® kit، المصنعة بواسطة شركة (النظم البيولوجية التطبيقية).

وهؤلاء الأفراد يشكلون خمسة أعراق من السكان الأصليين الذين يمثلون المجموعات اللغوية الثلاثة الرئيسية (دوسونيك، موروتيك وبيتانيك)، حيث كان تمثيلهم في الدراسة كالتالي: دوسون (عددهم = 7)، رونغوس (عددهم = 12)، سونسوغون (عددهم = 12)، موروت بالوان (عددهم = 12) ، و سونغاي تومبونو بايتانيك (عددهم = 8). وقد تم حساب التنوع للسمات الفردية (Hd) والقدرة على التمييز (DC)، وتمت مقارنة تركيبة السمات الفردية في سكان بورنيو الشمالية الثلاثة مع السكان من ساراواك المجاورة (شرق ماليزيا)، والمتمثلة في المجموعات العرقية في بدايوه و إيبان و ميلاناو.

تم تحديد 37 ســـمة فردية، من بينها 30 شـــخصاً تم تمثيلهم فقط من خلال ســمة فردية واحدة. وكان المتوسط الحسابي (± S.D.) للتنوع الفردي (0.181 ± 0.600) وكانت القدرة على التمييز 0.725. وأظهرت النتائج أيضا أن تكوين السمات الفردية (H33) كانت السمات الفردية الأكثر شيوعا والتي لوحظت في عينة من السكان الذكور، التي ظهرت حصرا في سكان منطقة موروت. وأظهر التحليل المقارن بين سكان نورنيو بورنيو وساراواك في السمات الفردية Y ، أن مجموعة البيتاني في السابق ترتبط ارتباطا وثيقا بمجموعة ميلاناو من هذا الأخير فيما يتعلق بالانحدار من السمات الفردية Y (RST=-0.0023). وبالإضافة إلى ذلك، تمكن تحليل التحجيم المتعدد الأبعاد (MSD) التمييز بوضوح بين المجموعات الثماني من بورنيو.

في الخلاصة، وجدنا أن مواقع التتابعات القصيرة المتكررة الـ 17 في الكروموسوم Y ذات قيمة للتطبيقات الوراثية في الأدلة الجنائية لبناء قاعدة بيانات محددة وكذلك لتقييم التركيبة الوراثية للسكان من المجموعات العرقية في منطقة بورنيان.

الكلمات المفتاحية: علوم الأدلة الجنائية،التتابعات القصيرة المتكررة على كروموسوم الذكورة Y، السمات الفردية، سكان صباح، التنوع، مقارنة التحليل.

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1. Introduction

Borneo is the third largest island in the world. Three countries, Malaysia, Brunei and Indonesia, have sovereignty over the island. The Malaysian section is comprised of the states of Sabah (which is also known as North Borneo) and Sarawak, which is located south-westerly in the island of Borneo. North Borneo comprises of heterogeneous and culturally diverse populations with more than 30 different ethnicities that converse in over 80 local dialects [1]. These 30 different major ethnics are further divided into several sub-ethnic groups, each with a unique set of cultural characteristics [2]. The highly heterogeneous ethnicities of the Sabah native populations are grouped into four broadly defined linguistic-based clusters: the Dusunic, Murutic, Paitanic, and Ida'an family groups [3]. The Kadazan-Dusun natives of the Dusunic-speaking group are the largest ethnic category in North Borneo. According to traditional folklore, the ancestors of the Kadazan-Dusuns originally resided in an area known as "Nunuk Ragang" (a Kadazan-Dusun word meaning red-coloured Banyan tree) before dispersing throughout the state [4,5]. Meanwhile, population migration pattern studies have shown that the other ethnic groups have migrated from Brunei, the Philippines, Indonesia, and from the neighbouring state of Sarawak [3]. The migrations of the ancestral populations and cross-ethnic marriages between different ethnic groups have led to admixture in genetic diversity, which is essential for adaptation [6]. In this study, we examined the haplotype diversity (based on the patrilineal Y-chromosome) of representative sub-populations belonging to five ethnic groups, each representing the three linguistic clusters namely, the Murutic-, Dusunic-, and Paitanic-speaking natives found in the north-west region of North Borneo. The Ida'an-speaking ethnic groups are predominantly found in the East coast and were not included in this study [3].

2. Materials and Methods

2.1 Study Sites and Collection of Samples

Collection of samples was carried out at five districts in North Borneo (Figure-1) from June 2010 to October 2012. These populations comprised of the Murut Paluan (residing in Nabawan District), Dusun (Ranau District), Sonsogon (interior Ranau District), Rungus (Kudat District), and Sungai Lingkabau Paitan (Kota Marudu District) (Table-1). Blood samples were obtained from 51 male individuals between the ages of 18 and 65 with informed consent and under the supervision of a medical doctor. The ethnicities of the blood donors were self-declared and priority was given to healthy persons with no recent admixtures (mixed marriages) in their families for at least three generations.

2.2 DNA Extraction and PCR Amplification

Genomic DNA was isolated using the conventional phenol-chloroform extraction and ethanol precipitation [7]. Seventeen Y-chromosome STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, YGATAH4) were subsequently amplified using the AmpFLSTR® Yfiler® PCR Amplification Kit (Applied Biosystems) according to the manufacturer's protocol [8,9].

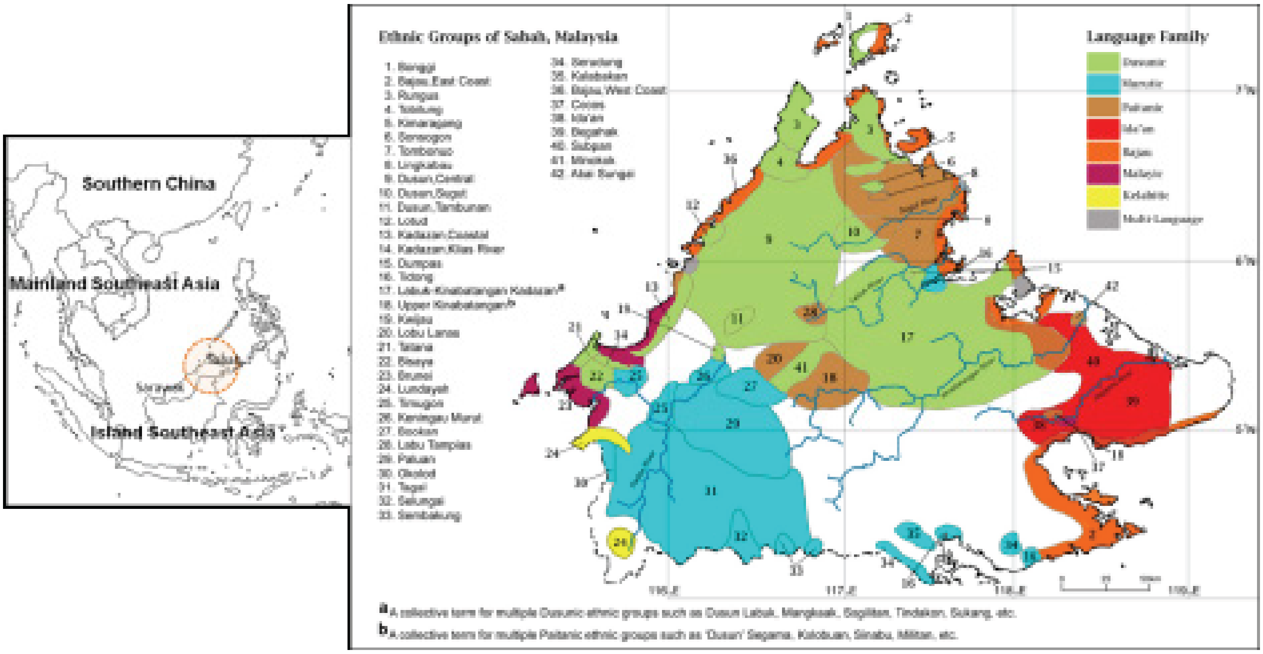

Figure 1- *Locations of the districts of the studied populations and linguistic groups predominantly spoken in them: Murutic in Nabawan (1), Dusunic in Ranau (2), Kudat (3), and Pitas (4), and Paitanic in Beluran (5). This figure was adopted and modified from Yew et al. [21].*

Table 1- *Number of samples typed in the five ethnic populations representing the Dusunic, Murutic and Paitanic linguistic group.*

Ethnic Population	Number of sample (n=51)	Linguistic Group
Dusun	7	Dusunic
Rungus	12	Dusunic
Sonsogon	12	Dusunic
Murut Paluan	12	Murutic
Sungai Lingkabau	8	Paitanic

2.3 Fragment Analysis and Typing

PCR-amplified products were detected and separated by capillary electrophoresis on a 3130 Genetic Analyzer (Applied Biosystems). The STR profiling protocol in GeneMapper ID-X Version 1.4 (Applied Biosystems) was employed in allele calling. Allele nomenclature was reported following the guidelines of the Y-Chromosome Haplotype Reference Database (YHRD) [10] and the DNA Commission of the International Society of Forensic Genetics (ISFG). According to recommendations by the ISFG, alleles for the YGATAH4 locus were renamed by a nine-repetition addition [11].

2.4 Quality Control

The fragment analysis and typing protocols were performed in compliance with the quality assurance standards as stipulated by the Scientific Working Group on DNA Analysis Methods (SWGDAM) [12]. The Biotechnology Research Insititute of Universiti Malaysia Sabah, where the analyses were carried out, participated in the Y-STR haplotype reference database (YHRD) quality assurance exercise by typing blind samples with the 17 Yfiler® loci (certificate dated May 21, 2013).

2.5 Data Analysis

Haplotype and allele frequencies were determined by using the gene counting method. The discrimination capacity (DC) was calculated as the proportion of different haplotypes in the sample [13]. Genetic diversity was presented as haplotype diversity (H_d) and calculated according to the study of other authors [14–16]. In addition to these parameters, the haplotype data for the Dusunic (Dusun, Rungus and Sonsogon), Murutic (Murut), and Paitanic (Sungai-Lingkabau) groups of North Borneo was also compared with similar haplotype data for the Melanau, Bidayuh, and Iban ethnic groups from Sarawak that were previously deposited in the YHRD. Inter-population AMOVA (R_{ST}) for each North Borneo ethnic with the three Sarawak ethnics was computed with the application software provided in the YHRD website (https://yhrd.org/amova) according to the algorithm by Reynolds et al. [17]. In addition, we performed a Multidimensional Scaling (MSD) analyses on the five ethic populations of North Borneo and included the Melanaus, Bidayuhs and Ibans (data obtained from YHRD) from Sarawak, which is situated in the western region of Borneo [18]. The MDS calculation is based on Kruskal's non-metric MDS algorithm [19, 20].

3. Results and Discussion

All 17 Y-chromosome loci were polymorphic across the five ethnic populations. The number of alleles ranged between 2 to 10 (Table-2). Meanwhile, the haplotype diversity of each locus ranged from 0.079 (Locus DYS438) to 0.881 (Locus DYS385b), averaging at 0.600 ± 0.181. The discrimination capacity (DC) was 76.5%, which was calculated from the number of haplotypes identified in the samples. The data set for all the 17 Y-STR markers has been deposited YHRD with the accession number YA003927-1 [23].

Within the five North Borneo ethnic populations, a total of 37 Y-chromosome haplotypes were identified of which 30 occurred only once while another 7 were shared among more than one individual (Table-3). The most frequent haplotype, H33, was exclusive to the Murut Paluan with 58.3% carrying the haplotype (n = 7). Meanwhile, the next common haplotype arrangement of 15-12-28-24-10-13-13-16-12-17-10-12-15-19-15-16-21-12 (H12) was shared between the Dusun and Paitanic population.

The Y-STR data indicated that the native ethnic groups of North Borneo are uniquely distinct from the Iban, Bidayuh, and Melanau from Sarawak. Interestingly, the Y-STR haplotype indicate that the Paitanic-speaking group is nearer to the Melanau in Sarawak with respect to haplotype descent as the degree of genetic differentiation between the two groups was the smallest (R_{ST}=-0.0023; Table-4). Meanwhile, the largest degree of genetic differentiation was observed between the Paitanic and the Bidayuh population (R_{ST}=0.3240; Table-4). The MDS analysis clearly separated the Dusun, Rungus, Sonsogon, Murut and Paitan, Melanau, Bidayuh and Iban populations (Figure-2). Furthermore, it was observed that the Dusun and Rungus, which are part of the Dusunic family, are closely grouped together. The

Table 2- *Alleles identified in the 17 Y-chromosomal STR loci in the North Borneo population, relative frequency of each allele, and haplotype diversity of each locus. Numerical designation for each allele is based on the ISFG nomenclature. n= number of observed alleles.*

Locus	Alleles	Allele Frequency	Haplotype diversity
DYS19 (*n* = 4)	12	0.059	0.573
	13	0.098	
	15	0.608	
	16	0.235	
DYS389I (*n* = 4)	12	0.510	0.622
	13	0.333	
	14	0.137	
	15	0.020	
DYS389II (*n* = 5)	27	0.059	0.706
	28	0.451	
	29	0.275	
	30	0.137	
	31	0.078	
DYS390 (*n* = 4)	21	0.039	0.687
	23	0.353	
	24	0.216	
	25	0.392	
DYS391 (*n* = 3)	9	0.059	0.558
	10	0.529	
	11	0.412	
DYS392 (*n* = 5)	11	0.039	0.583
	12	0.020	
	13	0.490	
	14	0.431	
	15	0.020	
DYS393 (*n* = 5)	12	0.039	0.560
	13	0.627	
	14	0.216	
	15	0.098	
	16	0.020	
DYS385a (*n* = 6)	11	0.078	0.787
	12	0.353	
	13	0.157	
	14	0.118	
	15	0.059	
	16	0.235	

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Table 2- *(continued)*.

Locus	Allele	Allele Frequency	Haplotype diversity
DYS385b (*n* = 10)	12	0.039	0.881
	13	0.157	
	14	0.176	
	15	0.020	
	16	0.078	
	17	0.078	
	19	0.118	
	20	0.196	
	21	0.020	
	22	0.118	
DYS438 (*n* = 3)	9	0.020	0.079
	10	0.960	
	11	0.020	
DYS439 (*n* = 3)	11	0.294	0.501
	12	0.647	
	13	0.059	
DYS437 (*n* = 2)	14	0.745	0.388
	15	0.255	
DYS448 (*n* = 5)	16	0.176	0.702
	18	0.490	
	19	0.157	
	20	0.078	
	21	0.098	
DYS456 (*n* = 6)	14	0.039	0.730
	15	0.255	
	16	0.431	
	17	0.059	
	18	0.157	
	19	0.059	
DYS458 (*n* = 5)	14	0.039	0.760
	15	0.275	
	16	0.255	
	17	0.314	
	18	0.118	
DYS635 (*n* = 3)	21	0.529	0.543
	22	0.432	
	24	0.039	
YGATAH4 (*n* = 4)	10	0.020	0.547
	11	0.510	
	12	0.450	
	13	0.020	

Table 3- *Frequency of Y-Chromosome STR haplotypes in the North Borneo population (n= 51).*

Hap.	Freq.	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS458	DYS385	DYS438	DYS439	DYS437	DYS448	DYS456	DYS458	DYS635	YGATAH4
H1	1	15	14	30	25	11	13	14	15	16,20	10	12	14	18	16	15	22	11
H2	1	15	13	29	25	11	13	15	15	16,20	10	11	14	18	16	15	21	11
H3	1	15	15	31	25	11	13	14	15	16,20	10	12	14	18	16	15	22	11
H4	1	13	12	28	23	10	14	13	15	12,13	10	12	14	18	18	15	21	12
H5	1	15	14	31	25	11	13	14	15	16,20	10	12	14	18	16	15	22	11
H6	1	15	13	29	25	11	13	15	15	16,20	10	12	14	18	16	15	22	11
H7	1	15	13	29	25	11	13	14	17	15,19	10	12	14	18	16	17	22	11
H8	1	15	12	28	23	10	14	13	15	12,13	10	12	14	18	18	15	21	12
H9	1	15	12	28	23	10	14	13	14	12,14	10	12	14	18	18	14	21	12
H10	1	15	12	28	24	10	14	13	15	13,13	10	13	14	18	15	15	21	11
H11	1	16	12	28	23	10	14	13	17	14,14	10	11	14	17	16	17	21	12
H12	4	15	12	28	24	10	13	13	16	12,17	10	12	15	19	15	16	21	12
H13	1	15	13	28	25	11	15	16	17	15,20	10	12	14	18	16	17	22	11
H14	1	15	14	31	24	9	13	13	17	11,12	10	11	14	19	14	17	22	11
H15	1	16	12	28	24	10	14	13	15	12,13	10	13	14	18	16	15	21	11
H16	2	16	12	28	23	10	14	13	15	12,13	10	12	14	18	18	15	21	12
H17	1	15	12	28	24	10	14	13	15	12,13	10	12	14	18	18	15	22	12
H18	1	16	12	28	23	11	14	13	17	14,14	10	11	14	17	16	17	21	12
H19	1	13	14	30	25	11	14	13	17	12,19	10	12	15	20	15	17	22	11

Continued on the next page

Table 3- *(continued)*

Hap.	Freq.	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS458	DYS385	DYS438	DYS439	DYS437	DYS448	DYS456	DYS458	DYS635	YGATAH4
H20	2	15	12	28	23	10	14	13	18	12,14	10	11	14	17	16	18	21	12
H21	2	12	13	30	23	10	13	13	17	13,19	10	12	15	21	17	17	21	12
H22	1	16	12	28	23	10	14	13	17	14,14	10	11	14	17	18	17	21	12
H23	2	15	12	28	21	10	11	12	17	11,16	10	11	14	21	15	17	24	11
H24	1	16	12	28	23	10	14	13	18	14,14	10	11	14	17	18	18	21	12
H25	2	13	14	30	25	11	14	13	16	12,20	10	12	15	20	15	16	21	11
H26	1	15	13	29	25	11	13	15	15	16,19	10	12	14	18	16	15	21	11
H27	1	13	12	28	24	11	14	13	17	12,20	10	12	15	20	15	17	22	11
H28	1	15	12	28	23	10	14	13	14	13,13	10	12	14	18	15	14	22	12
H29	1	16	12	27	23	10	14	13	18	13,14	10	11	14	17	18	18	22	12
H30	1	15	12	29	24	9	13	13	17	12,16	9	13	15	19	15	17	21	12
H31	1	16	12	27	23	10	14	13	18	13,14	10	11	14	17	18	18	21	12
H32	1	16	13	29	24	10	13	15	18	14,16	10	11	14	18	18	18	22	10
H33	7	15	13	29	25	11	13	14	16	16,22	10	12	14	18	16	16	22	11
H34	1	15	13	29	25	11	13	14	16	15,21	10	12	14	18	16	16	22	11
H35	1	12	14	31	23	10	13	13	17	13,20	10	12	15	21	16	17	21	12
H36	1	16	13	30	25	9	12	13	17	11,12	11	11	15	19	14	17	22	11
H37	1	16	12	27	23	10	14	13	17	14,15	10	11	14	17	17	17	21	13

Note: Two values are presented for DYS385, each representing DYS385i and DYS3

Table 4- *ANOVA pairwise distance (R_{ST}) values (below diagonal) of the Dusunic, Murutic and Paitanic populations from North Borneo and three other ethnics (Bidayuh, Iban, and Melanau) from Sarawak. Associated P-values are shown in the above diagonal.*

Population	Bidayuh	Iban	Melanau	Dusunic
Bidayuh	-	0.0000	0.0000	0.0000
Iban	0.1440	-	0.1121	0.0000
Melanau	0.1792	0.0080	-	0.0000
Dusunic	0.2841	0.2139	0.2311	-
Population	**Bidayuh**	**Iban**	**Melanau**	**Murutic**
Bidayuh	-	0.0000	0.0000	0.0000
Iban	0.1440	-	0.1066	0.0007
Melanau	0.1792	0.0080	-	0.0000
Murutic	0.2299	0.1800	0.2393	-
Population	**Bidayuh**	**Iban**	**Melanau**	**Paitanic**
Bidayuh	-	0.0000	0.0000	0.0000
Iban	0.1440	-	0.1072	0.2463
Melanau	0.1792	0.0080	-	0.3923
Paitanic	0.3240	0.0176	-0.0023	-

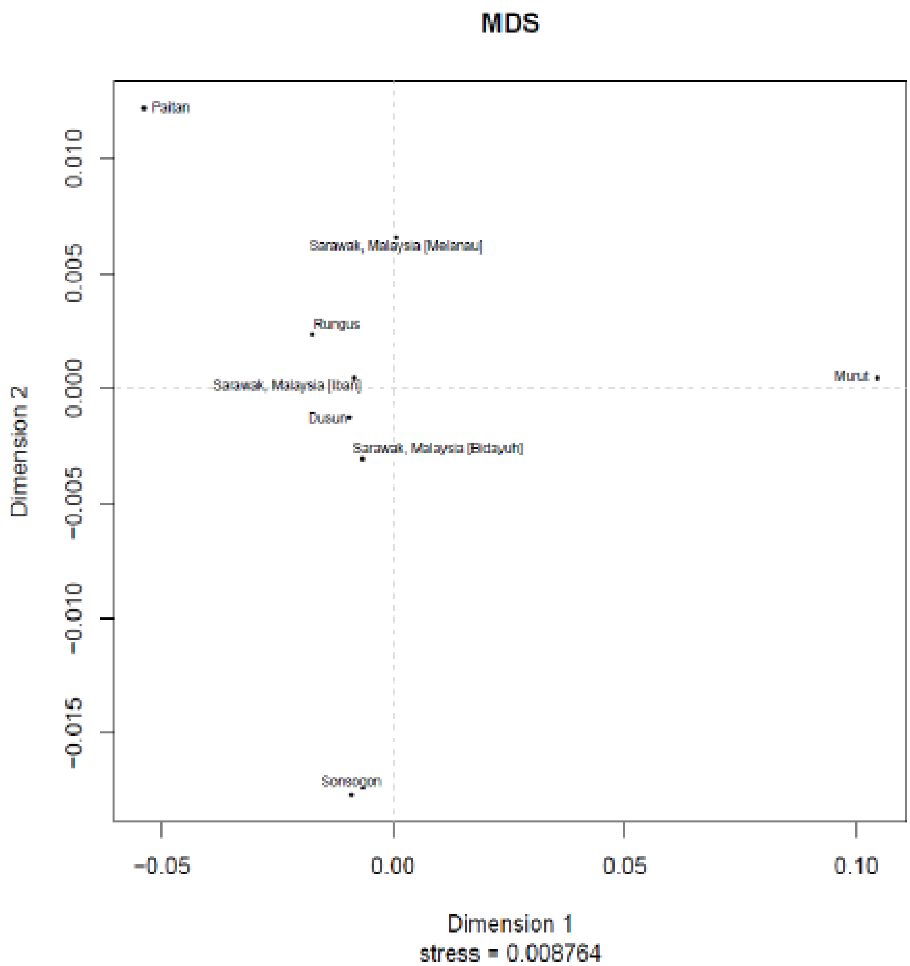

Figure 2- *A Multidimensional Scaling (MSD) visualisation of the five ethnic groups (Dusun, Rungus, Murut, Paitan and Sonsogon) from North Borneo. Three ethnic groups from Sarawak (Iban, Bidayuh and Melanau) were included for comparison. The Paitan, Murut and Sonsogon showed distinct identities.*

Songoson, although also part of the Dusunic family, show distinct separation from the other members of the family probably due to their long history of isolation [21]. We see a similar differentiation of the populations using whole genome genotyping (with Single Nucleotide Polymorphisms) and whole genome sequencing data [21,22]. The Bornean populations have indicated the presence of genetic drift due to the differences in ancestral components even with the small number of samples used.

The present study included a limited number of individuals; studies including large number of individuals are needed to generalize specifically the application of 17 Y-chromosome STRs in the North Borneo populations.

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Conflict of interest

None.

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