PREHISTORIC HUMAN FORENSIC DNA ANALYSIS FROM LORE HIGHLANDS, CENTRAL SULAWESI WITH PRESENT-DAY HUMAN DNA

Analisis DNA Sampel Forensik Manusia Prasejarah dari Dataran Tinggi Lore, Sulawesi Tengah dengan DNA Manusia Kini

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Abstract. *Mitochondrial DNA or mtDNA(s) are inherited maternally, in other words they can only be inherited through females in a family, which makes them frequently used in forensic studies. In this research, the Hypervariable-I part (HV-1) in the D-Loop region of the ancient forensic samples acquired from Lore Highlands will be compared with present day human DNA from the same region. The steps conducted in this research includes DNA extraction, mtDNA amplification with specific primers, sequencing, and phylogenetic analysis. The analysis were done between 4 ancient forensic samples; A, B, G, and J, and 33 comparison sequences from GenBank, including ancient forensic sample acquired from Tadulako site, Lore Highlands, from previous research. The result of genetic distance analysis showed that the distance between 37 samples were very close; with difference ranging from 0,02% - 0,13%. The analysis also gives a clue about Austronesian relation with Australomelanesian. The result from phylogenetic tree reconstructions (maximum-likelihood and neighbor-joining) showed little differences. However, there is a small significant difference detected from the neighbour-joining tree construction result and will be discussed further in this paper.*

Keywords: *HV I, D-Loop Region, Prehistoric Sample, Lore Highland, Austronesia*

Abstrak .*Mitochondrial DNA* atau DNA mitokondria (mtDNA) pada umumnya bersifat *maternal inheritance* atau hanya dapat diturunkan dari ibu sehingga seringkali digunakan dalam studi forensik. Pada penelitian ini akan dibandingkan daerah HVR-I pada bagian D-Loop mtDNA antara sampel forensik prasejarah dengan manusia kini. Tahapan yang dilakukan dalam penelitian ini meliputi ekstraksi DNA prasejarah, amplifikasi mtDNA dengan primer spesifik , sekuensing, dan dilanjutkan analisis filogenetik. Analisis dilakukan pada 4 sampel forensik prasejarah A, B, G, dan J dengan 33 sekuen pembanding dari GenBank dan sampel prasejarah situs Tadulako dari penelitian sebelumnya. Hasil analisis filogenetik berupa jarak genetik menunjukkan bahwa jarak antar 37 sampel sangat berdekatan; dengan kisaran selisih pada sebesar 0,02% - 0,13%. Hasil tersebut tercerminkan pada rekonstruksi pohon filogenetik dengan metode *maximum-likelihood* dan *neighbor-joining* yang juga menunjukkan perbedaan signifikan.

Kata kunci: HV I, Daerah D-Loop, Sampel Prasejarah, Dataran Tinggi Lore, Austronesia

1. Introduction

Indonesia is an archipelagic country with various cultures and language dialects. If they are to be tracked down, those varieties came from immigrant merchant communities that came to Indonesia, one of them being the Austronesians. According to Peter Bellwood (2006), the emergence of Austronesian originated from Taiwan (Out of Taiwan), and reached Indonesia through the Philipines-Kalimantan-Sulawesi path, among others, and since then spread into other regions. The race of Austronesians is Mongoloid, which in their next migration stage they weeded out Australoomelanesids, the already existing race in Southeast Asia Archipelago and Oceania. The arrival of Austronesians in Indonesia occurred in a few waves. The first wave is estimated to have happened around 4000-2000 years ago (Neolithic age), carrying new cultures such as prehistoric technological innovation, domestication, and sedentarization (Simanjuntak 1992). The second wave happened around 2000-1500 years ago (Paleometalic or Early Metal Age) and was marked by a more advanced and complex way of life. At this age, the culture was characterized by a more advanced burial practice compared to the previous age in the form of utilizing burial jars and equipment or tools made out of metal that were influenced by the Dongson culture of Vietnam and Megalithic cultures. The wave after was the wave that happened around the transition into the Historical age (Hinduism, Buddhism, Islamism, and colonial) until now (Simanjuntak 1992 & 2015).

The Austronesian relics that was developed in early Metal ages (Paleometalic, nearing early AD) which was dated quiet old in Indonesia, among others, are megalithic cultures that are located in Poso, Central Sulawesi Province, in Lore Highlands to be exact. Lore Highlands are divided into three valleys: Napu Valley, Bada Valley, and Behoa Valley. In the archaeological sites that are located in those valleys, there are relics such as stone vats and stone mortars**,** including burial jars containing human bones and teeth remains. The three valleys all use the Lore language with different dialects; Behoa Valley uses the Lore Behoa dialect, and Bada Valley uses the Lore Bada dialect (Yuniawati 2016 & Yuniawati-Umar 2020).

Along with the integrated knowledge and technological advancement, there needs to be a genetic analysis to support archaeological evidence to uncover the migration origin of Austronesians. In 2003, Sentausa did a DNA sequence analysis and gender identification from five ancient human bones found in the Tadulako site, Behoa Valley, Central Sulawesi, that were found during research that was done by the National Research Center for Archaeology in 1999 (Yuniawati 2000). The results showed that from the prehistoric bone sample analysis in the Tadulako site, compared with modern-day human samples from several regions in Central Sulawesi, were related to a few populations such as Kaili (North Sulawesi), Toraja (South Sulawesi), Kajang (South Sulawesi), and Mandar (West Sulawesi). In 2016, the Eijkman Institute for Molecular Biology researched modern-day human genetic population analysis in Bada Valley and Behoa Valley. The results are then compared with those from the Tadulako samples analysis by Sentausa. The results showed that both sides of the samples were not closely related regarding their phylogenetic relationship (Apriyana *et al*. 2019).

In this research, sequence analysis was done on several ancient human forensic samples taken from Bada Valley and Behoa Valley, which were then compared to DNA sequences of modern-day humans from the GenBank. The hypervariable region in mtDNA (Mitochondrial DNA) is chosen as the site for analysis because it is the non-coding region which has a high mutation rate. Moreover, mtDNA has uniparental maternal inheritance, thus, it is generally used to analyze the phylogenetic relationship.

2. Method

Four ancient human bone samples from stone vats in the Wineki site and burial jars from the Petawua site owned by the National Archaeology Research Institute were used in this research (Yuniawati *et al*. 2012 & Yuniawati *et al*. 2013). DNA extraction from the ancient bone samples was done in the Human Genetics Laboratorium, Institute of Tropical Disease, Airlangga University. DNA extraction was done to the bones that were already crushed with modified DNAzol Invitrogen

reagent protocol. Samples are then amplified using midi-plex primer sets (Kim *et al*. 2013). Separate amplification according to the primer sets was done to produce better amplicons. Two PCR-ready mixes were used; MyTaq HS Red Mix PCR Kit (Bioline) and Phusion U Green Multiplex PCR Master Mix (Thermo Fisher Scientific). The Red master mix contains 3 µL DNA, 25 µL MyTaq HS Red Mix, 2,5 µL reverse and forward primer, and 14 µL ddH2O. The Phusion U Green master mix contains 4 µL DNA, 20 µL Phusion U Green Mix, 2,5 µL reverse and forward primer, and 11 µL ddH2O. Samples were amplified using Thermal Cycler Bio-Rad T100, and the cycle was as follows; predenaturation at 95°C for 11 minutes, then 95°C for 20 seconds, annealing at 55°C for 60 seconds, followed by 72°C for 30 seconds, repeated by 42 cycles, and lastly extension in 72°C for 7 minutes. Amplified samples went through electrophoresis in 20 mL 2% agarose gel. The setting was in 100 volts and 400 mA. The results were visualized using a UV Transiluminator, documented with GelDoc, and sent to First Base for sequencing.
Sequenced samples will be analyzed for their genetic distance, gene variation, and phylogenetics. Genetic distances were analyzed using MEGA 11 with Kimura-2-Parameter (K2P) with 1000 bootstrap. Gene variations were analyzed using DnaSP6. Phylogenetic tree reconstruction was done using the neighbour-joining and maximum likelihood methods in MEGA11 using the K2P model and 1000 bootstrap.

3. Results and Discussion

Analysis was done on four ancient bone samples from the Wineki Site and Petawua Anditu Site (Table 1) against 33 sequences taken from NCBI GenBank and sequences from Sentausa's research (2003) for comparison. Other sequences were included from the researcher of this paper as a contamination control (Table 1).

Table 1. Sample Type and Code

Ancient Sample Type	Quantity	Sample Code
Petauwa Anditu Site		
Long bone	2*	J (J1 – J6)
Wineki Site		
Sinistrous Petrous Bone	5*	A (A1 – A6)
Petrous Bone (Juvenile)	3*	B (B1 – B6)
Fibula Bone (Indet)	2*	G (G1 – G6)
Contamination Control Sample Type	**Quantity**	**Sample Code**
Blood	1	N
Buccal Swab	1	S

* In fragments

Source: Author 2022

The sequences that were taken from GenBank are *Homo sapiens* rCRS (NC012920), *Homo sapiens* Aes9 from Switzerland (MT079025), *Homo sapiens* from Papua (AB490084, AB490158, and AB495206), *Homo sapiens* from Bugis tribe (KC114979, KC114980, KC114981, and KC114982), *Homo sapiens* from Kajang tribe (KC115029, KC115030, KC115031, and KC115032), *Homo sapiens* from Mandar tribe (KC115075, KC115076, KC115077, and KC115078), *Homo sapiens* from Toraja tribe (KC115129, KC115130, KC115131, and KC115132), *Homo sapiens* from Bajo tribe (KM591166, KM591167, KM591168, and KM591169), and two outgroup sequences from *Homo sapiens neanderthalensis* (NC011137) and *Homo heidelbergensis* (NC023100). Sequences from the previous research by Sentausa were indicated as Tadulako 1, Tadulako 2, Tadulako 3, and Tadulako 5.

Clean consensus fragment length from samples A, B, G, and J (see Table 1 for sample origin) are 500 bp after indels. The indels referred, occurrs when compared with the rCRS (revised Cambridge Reference Sequence) sequence. The rCRS is the mitochondrial gene reference for *Homo sapiens* (Andrews *et al*., 1999).

Genetic Distance

Analysis showed that sample A, B, G, and J with insertion has a genetic distance of 0.02% to 0.13% compared to the other 33 sequences (Table 2). Genetic distance numbers show the nucleotide difference between individuals, the smaller the number means the more closely related each individual is with each other (Trisyani and Rahayu 2020). From the result, the distance between each

sample and their comparison is very small although there are samples that came from different regions and races as well.

The results are supported by the nucleotide composition analysis of all samples. The difference in nucleotide T is around 21.22% - 22.65%, nucleotide C around 32.65% - 34.49%, nucleotide A around 30.14 – 31.33%, and in nucleotide G around 13.67% - 14.54% (Table 3). Besides the relatively small differences, some sequences also have almost similar percentages of nucleotide composition, and this explains the relatively small genetic distance as well.

Genetic Variation

Genetic variation analysis was done with the DnaSP6 program. The analysis shows 61 polymorphism sites and 14 indel sites (insertion/deletion). From 61 polymorphic sites, there are 19 base transitions and 32 base transversions (Table 4). Generally, more often than not, transition mutation happens because of natural selection, and there is a bias that the mutation, particularly in the coding region, happens more frequently because it does not actually affect the nucleotide structure, biochemistry, and protein changes or amino acid of a DNA. However, the result from the ancient samples showed that transversion occurred more than transition and takes place in the non-coding region. This signifies that nucleotide evolution in that region occurred more rapidly than in the coding region. However, the amino acid shift does not affect the fenotype change (silent mutation) because the region is not coding proteins. This is backed by the mtDNA characteristic that does not have a DNA repair mechanism and little polymerase mtDNA that, in the end, increases mutation (Stoltzfus and Norris 2016; Lyons and Lauring 2017).

Phylogenetic Tree Reconstruction

Evolutionary relationships between organisms are visualized with the help of a phylogenetic tree. This tree represents the evolutionary relationship between taxons in the form of a diagram consisting of nodes and branches. In this research, the reconstruction was done with the Neighbor Joining (NJ) method and Maximum Likelihood (ML) method using Kimura-2-Parameter (K2P) and 1000 bootstrap numbers. The neighbour-joining is a distance-based method that is mostly used because of its simplicity, efficiency, and algorithm speed in analyzing data (Yang and Rannala 2012). This method will reconstruct a tree with a total branch length as minimal as possible. On the other hand, the Maximum Likelihood method searches for the best possible trees to produce an evolution hypotheses model according to the data. Maximum likelihood algorithms are more complex, so they consume more time to run than its counterpart, thus producing a more accurate result (Ludwig *et al*. 2011; Bleidorn 2017). The K2P is a nucleotide substitution model that estimates the genetic distance and phylogenetic relationship. Bootstrap number comes into play to estimate the level of confidence of the phylogenetic tree reconstruction. Bootstrap number that can be considered to be reliable ranges above 70% or 80% (Dharmayanti 2011; Trisyani and Rahayu 2020).

Table 2. Genetic Distance

Sampel	Aes9	Tadulako 1	Tadulako 2	Tadulako 3	Tadulako 5	NC011 137	NC012 920	NC023 100	KC114 979	KC114 980	KC114 981	KC114 982	KC115 029	KC115 030	KC115 031	KC115 032	KC1 07
Aes9																	
Tadulako-1	0,008																
Tadulako-2	0,01	0,01															
Tadulako-3	0,01	0,00	0,01														
Tadulako-5	0,01	0,01	0,02	0,01													
NC_011137	0,06	0,05	0,05	0,05	0,05												
NC_012920	0,00	0,00	0,01	0,01	0,01	0,05											
NC_023100	0,02	0,01	0,02	0,02	0,02	0,03	0,01										
KC114979	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02									
KC114980	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,01								
KC114981	0,01	0,01	0,02	0,02	0,02	0,06	0,01	0,02	0,02	0,02							
KC114982	0,01	0,00	0,01	0,01	0,01	0,05	0,01	0,02	0,01	0,01	0,01						
KC115029	0,02	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,01	0,02	0,02	0,01					
KC115030	0,02	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,02	0,02	0,02	0,01	0,01				
KC115031	0,02	0,01	0,02	0,01	0,02	0,05	0,01	0,02	0,01	0,02	0,02	0,01	0,02	0,02			
KC115032	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,01	0,01	0,02	0,01	0,01	0,01	0,02		
KC115075	0,02	0,02	0,02	0,01	0,01	0,05	0,02	0,02	0,02	0,02	0,03	0,02	0,02	0,01	0,03	0,01	
KC115076	0,01	0,01	0,02	0,01	0,01	0,04	0,01	0,01	0,01	0,01	0,02	0,01	0,02	0,02	0,01	0,01	0,0
KC115077	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,00	0,01	0,02	0,01	0,01	0,02	0,01	0,01	0,0
KC115078	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,02	0,01	0,02	0,01	0,01	0,01	0,02	0,01	0,0
KC115129	0,02	0,02	0,02	0,01	0,01	0,05	0,02	0,02	0,02	0,02	0,03	0,02	0,02	0,01	0,03	0,01	0,0
KC115130	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,00	0,01	0,02	0,01	0,01	0,02	0,01	0,01	0,0
KC115131	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,01	0,01	0,02	0,01	0,02	0,02	0,01	0,01	0,0
KC115132	0,01	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,00	0,01	0,02	0,01	0,01	0,02	0,01	0,01	0,0
Sample B	**0,04**	**0,04**	**0,05**	**0,04**	**0,04**	**0,09**	**0,04**	**0,04**	**0,04**	**0,04**	**0,05**	**0,04**	**0,05**	**0,05**	**0,05**	**0,04**	**0,0**
Sample J	**0,05**	**0,05**	**0,05**	**0,05**	**0,05**	**0,09**	**0,04**	**0,05**	**0,05**	**0,05**	**0,05**	**0,05**	**0,06**	**0,06**	**0,06**	**0,05**	**0,0**
Sample G	**0,06**	**0,06**	**0,06**	**0,06**	**0,06**	**0,10**	**0,05**	**0,07**	**0,06**	**0,06**	**0,06**	**0,06**	**0,06**	**0,06**	**0,06**	**0,06**	**0,0**
KM591166	0,02	0,01	0,02	0,01	0,01	0,05	0,01	0,02	0,02	0,02	0,02	0,02	0,01	0,00	0,02	0,00	0,0
KM591171	0,02	0,01	0,03	0,02	0,01	0,05	0,01	0,02	0,02	0,02	0,02	0,02	0,01	0,01	0,02	0,01	0,0
KM591179	0,02	0,01	0,02	0,01	0,02	0,05	0,01	0,02	0,01	0,01	0,02	0,01	0,02	0,02	0,01	0,02	0,0
KM591191	0,01	0,01	0,01	0,01	0,01	0,06	0,01	0,02	0,01	0,01	0,01	0,01	0,02	0,02	0,02	0,01	0,0
N Control	0,02	0,01	0,02	0,01	0,02	0,05	0,01	0,02	0,01	0,02	0,02	0,01	0,02	0,02	0,01	0,01	0,0
S Control	0,02	0,01	0,03	0,02	0,01	0,05	0,01	0,02	0,02	0,02	0,02	0,02	0,01	0,01	0,02	0,01	0,0
Sample A	**0,08**	**0,08**	**0,08**	**0,08**	**0,09**	**0,13**	**0,08**	**0,10**	**0,09**	**0,09**	**0,09**	**0,09**	**0,09**	**0,09**	**0,09**	**0,09**	**0,1**
AB490084	0,03	0,02	0,03	0,03	0,03	0,07	0,02	0,04	0,03	0,03	0,03	0,02	0,03	0,03	0,03	0,03	0,0
AB490158	0,03	0,02	0,03	0,02	0,03	0,06	0,02	0,03	0,02	0,03	0,03	0,01	0,03	0,03	0,03	0,03	0,0
AB495206	0,03	0,02	0,03	0,02	0,03	0,06	0,02	0,03	0,02	0,03	0,03	0,02	0,02	0,03	0,03	0,03	0,0

(Continuation of **Table 2**)

Sampel	KC115077	KC115078	KC115129	KC115130	KC115131	KC115132	Sample B	Sample J	Sample G	KM591166	KM591171	KM591179	KM591191	N Control	S Control	Sample A	AB490084	AB490158	AB495206
KC115078	0,02																		
KC115129	0,02	0,01																	
KC115130	0,00	0,02	0,02																
KC115131	0,01	0,02	0,02	0,01															
KC115132	0,00	0,02	0,02	0,00	0,01														
Sample B	**0,04**	**0,05**	**0,05**	**0,04**	**0,04**	**0,04**													
Sample J	**0,05**	**0,05**	**0,06**	**0,05**	**0,05**	**0,05**	**0,02**												
Sample G	**0,06**	**0,06**	**0,07**	**0,06**	**0,06**	**0,06**	**0,06**	**0,05**											
KM591166	0,01	0,01	0,01	0,02	0,02	0,02	**0,05**	**0,06**	**0,06**										
KM591171	0,02	0,01	0,00	0,02	0,02	0,02	**0,05**	**0,06**	**0,07**	0,01									
KM591179	0,01	0,02	0,02	0,01	0,01	0,01	**0,05**	**0,06**	**0,06**	0,02	0,02								
KM591191	0,01	0,01	0,02	0,01	0,01	0,01	**0,04**	**0,05**	**0,06**	0,02	0,02	0,02							
Control N	0,00	0,02	0,03	0,01	0,01	0,01	**0,05**	**0,06**	**0,06**	0,02	0,02	0,01	0,02						
Control S	0,02	0,01	0,01	0,02	0,02	0,02	**0,05**	**0,06**	**0,07**	0,01	0,01	0,02	0,02	0,02					
Sample A	**0,09**	**0,08**	**0,10**	**0,09**	**0,09**	**0,09**	**0,07**	**0,07**	**0,09**	**0,09**	**0,09**	**0,09**	**0,08**	**0,09**	**0,09**				
AB490084	0,03	0,03	0,03	0,03	0,03	0,03	**0,05**	**0,06**	**0,06**	0,03	0,03	0,03	0,03	0,03	0,03	**0,10**			
AB490158	0,02	0,03	0,03	0,02	0,03	0,02	**0,05**	**0,06**	**0,06**	0,03	0,03	0,03	0,03	0,02	0,03	**0,10**	0,02		
AB495206	0,02	0,03	0,03	0,02	0,02	0,02	**0,05**	**0,05**	**0,06**	0,03	0,03	0,03	0,03	0,02	0,03	**0,09**	0,03	0,02	

Source: Author 2022

Table 3. Nucleotide Composition Percentage

Samples	T(U)	C	A	G
Aes9	22,45	32,86	30,82	13,88
Tadulako-1	22,04	33,27	30,82	13,88
Tadulako-2	21,43	33,88	30,82	13,88
Tadulako-3	21,84	33,47	30,82	13,88
Tadulako-5	21,84	33,67	30,61	13,88
NC 011137	22,61	32,99	30,14	14,26
NC 012920	22,04	33,27	30,82	13,88
NC 023100	22,03	33,04	30,40	14,54
KC114979	22,04	33,27	31,02	13,67
KC114980	21,43	33,88	31,02	13,67
KC114981	21,84	33,27	30,82	14,08
KC114982	22,04	33,27	30,82	13,88
KC115029	21,43	33,88	30,61	14,08
KC115030	21,22	34,49	30,41	13,88
KC115031	22,45	32,86	30,82	13,88
KC115032	21,63	33,67	30,82	13,88
KC115075	21,22	34,49	30,41	13,88
KC115076	22,04	33,06	31,02	13,88
KC115077	22,04	33,27	31,02	13,67
KC115078	21,22	34,29	30,61	13,88
KC115129	21,22	34,49	30,41	13,88
KC115130	22,04	33,27	31,02	13,67
KC115131	22,45	32,65	31,02	13,88
KC115132	22,04	33,27	31,02	13,67
Sample B	22,22	33,13	30,71	13,94
Sample J	21,98	33,27	30,65	14,11
Sample G	22,09	32,73	31,33	13,86
KM591166	21,63	34,08	30,41	13,88
KM591171	21,43	34,29	30,41	13,88
KM591179	21,84	33,27	31,02	13,88
KM591191	21,63	33,67	30,82	13,88
(Control) N	21,84	33,47	31,02	13,67
(Control) S	21,63	33,67	30,61	14,08
Sample A	21,77	33,06	31,05	14,11
AB490084	22,04	33,06	30,82	14,08
AB490158	22,65	32,86	30,20	14,29
AB495206	22,45	33,06	30,20	14,29

Source: Author 2022

Samples	*Polymorphic Sites*																															
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**																								
rCRS	T	T	C	T	T	C	A	T	G	A	G	C	A	G	A	T	G	-	G	T	A	C	A	T	A	C	T	C	-	A	G	
SAMPLE A	C	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	-	.	.	.
SAMPLE B	C	.	T	C	A	T	G	G	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.
SAMPLE G	.	.	.	.	C	A	T	G	A	G	C	A	G	A	T	G	A	G	T	A	C	A	T	A	C	T	A	A	G	C	C	A
SAMPLE J	.	C	T	.	C	A	T	G	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.
TADULAKO-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.
TADULAKO-2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.
TADULAKO-3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.
TADULAKO-5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.

Table 4. Polymorphic Sites (557 bp)

Samples	***Polymorphic Sites***																															
rCRS	A	-	C	C	T	T	C	-	-	T	C	A	T	C	-	T	C	C	T	-	T	T	T	A		T					T	-
SAMPLE A	.	G	.	.	.	.	.	-	-	.	.	.	.	.	A	.	.	.	.	-	.	.	.	.	.		T	A	A	.		G
SAMPLE B	.	-	.	.	.	.	.	T	A	.	.	.	.	.	-	.	.	T	.	-	.	.	.	.	.	.	.	.	.	.		T
SAMPLE G	C	C	A	T	A	.	.	T	C	A	-	.	.	.	-	.	.	T	.	T	A	.	.	T	C		T	A	.	.		G
SAMPLE J	.	G	.	.	.	.	.	C	A	.	.	.	.	.	-	.	.	T	.	-	.	.	C	T	C		T	A	.	-		G
TADULAKO-1	.	-	.	.	.	.	.	-	-	.	.	.	.	T	-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-
TADULAKO-2	.	-	.	.	.	C	.	-	-	.	.	.	.	.	-	C	T	.	C	-	.	C	.	.	.	.	.	.	.	.	.	-
TADULAKO-3	.	-	.	.	.	C	.	-	-	.	.	.	.	T	-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-
TADULAKO-5	.	-	.	.	.		T	-	-	.	.	C	C		-	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	-

(Continuation of **Table 4**)

Sampel	*Polymorphic Sites*										
rCRS	-	-	T	A	G	-	C	A	C	A	C
SAMPEL A	T	C	.	.	.	C	A	C	A	T	.
SAMPEL B	C	C	.	T	A	G	.	.	.	.	.
SAMPEL G	G	T	.	.	.	C	A	C	A	C	-
SAMPEL J	G	T	.	T	A	G	.	.	.	.	.
TADULAKO-1	-	-	C	.	.	-	.	.	.	.	.
TADULAKO-2	-	-		.	.	-	.	.	.	.	.
TADULAKO-3	-	-	C	.	.	-	.	.	.	.	.
TADULAKO-5	-	-	C	.	.	-	.	.	.	.	.

(Continuation of **Table 4**)

Source: Author 2022

The reconstruction from Maximum Likelihood and neighbour-joining method showed a different result. From the ML reconstruction, the A, B, G and J samples are seen to group into one clade with one sample from the Bugis tribe (KC114980), Tadulako-2 sample, the ancient DNA sample from Switzerland (Aes9), and the rCRS. Meanwhile, from the NJ reconstruction, the A, B, G and J samples are seen to group into one clade only with samples from Papua (AB490084, AB490158, AB495206). This difference is notably significant because from the ML reconstruction, the A, B, G, and J samples are still grouped with samples from one cluster, which are from Sulawesi that generally has mongoloid characteristics. However, from the NJ reconstruction, those samples have the smallest distance from the population from Papua, which are Australomelanesoid.

These differences can be explained as a possibility that A, B, G, and J samples were Australomelanesoid because, according to the evidence in Behoa Valley, there were not only sculptures of Mongoloid fenotypes, but sculptures with Australomelanesoid characteristics as well, that was estimated to have settled in Lore Highlands in earlier times. The sculptures that showed Mongo oid characteristics can be seen from its head, which does not have a wavy feature (exhibiting straight hair) and slit slanted elliptical eyes (Figure 2). The sculptures that showed Australomelanesoid characteristics can be seen to have a wavy feature on its head, which exhibits curly hair and big rounded eyes. Those characteristic matches with the fenotype of the modern day human of each race (Figure 1). In Central and West Sulawesi there actually is an existing ethnic population with the described characteristics of Australomelanesian, specifically in Palu Highlands and Pasang Kayu (Yuniawati 2016; Yuniawati-Umar 2020).

Figure 1. (A) Individuals with Mongoloid Characteristics from Lore Highlands and (B) Individuals with Australomelanesoid characteristics from Palu Highlands and Pasang Kayu (Da'a Ethnic Tribe)
(Source: Yuniawati-Umar, 2016)

Figure 2. Sculptures with Mongoloid Characteristics (Austronesian) in Behoa and Bada Valley
(Source: Yuniawati-Umar, 2020)

Figure 3. Sculptures with Australomelanesoid Characteristics in Behoa and Rampi Valley
(Source: Yuniawati-Umar, 2020)

The ethnic population with the Australomelanesoid fenotype are called the Da'a ethnic tribe (Central and West Sulawesi), and they used to reside in tall tree houses (Figure 4), similar to Korowai people from Papua (Yuniawati-Umar 2020). This opens the possibility of a union through marriage between Austronesians and Australomelanesoids in Lore Highlands.

From the two phylogenetic tree reconstruction that has been done, the results from the Maximum Likelihood method presents that the prehistoric A, B, G, and J samples are closely related to samples of modern-day human from Sulawesi, particularly Bugis and Bajo tribe (Figure 5). The Tadulako-2 bone samples are also in one clade with the prehistoric samples. The bootstrap of the prehistoric cluster (A, B, G, and J) shows a number of 99%, indicating a good inference value (Figure 5). Besides all points, the possibility that the prehistoric samples are of Australomelanesoid descent is rather small. The arrival chronology of Australomelanesoid can explain it and their age are much older than the prehistoric samples used.

Figure 4. Tree House from Da'a Ethnic Tribe in Palu Valley, Central Sulawesi (Source: Yuniawati-Umar, 2016)

The modern day human that currently resides in Behoa Valley and Bada Valley of Lore Highlands has mongoloid fenotype and is in the D6a haplogroup. D6a haplogroup is the haplotype of Austronesian descent that is spread throughout Lesser Sunda Island (Nusa Tenggara) and the Philipines (Tabadda *et al*. 2010; Gomes *et al*. 2017). The acquired archaeological, anthropological, and genetic data attributes that the prehistoric A, B, G, and J samples were possibly of Austronesian descent. Even so, the obtained phylogenetic tree analysis result can not be a stand-alone reference because most of the bootstrap numbers are below 88%, thus it has to be observed alongside the genetic distance analysis as well.

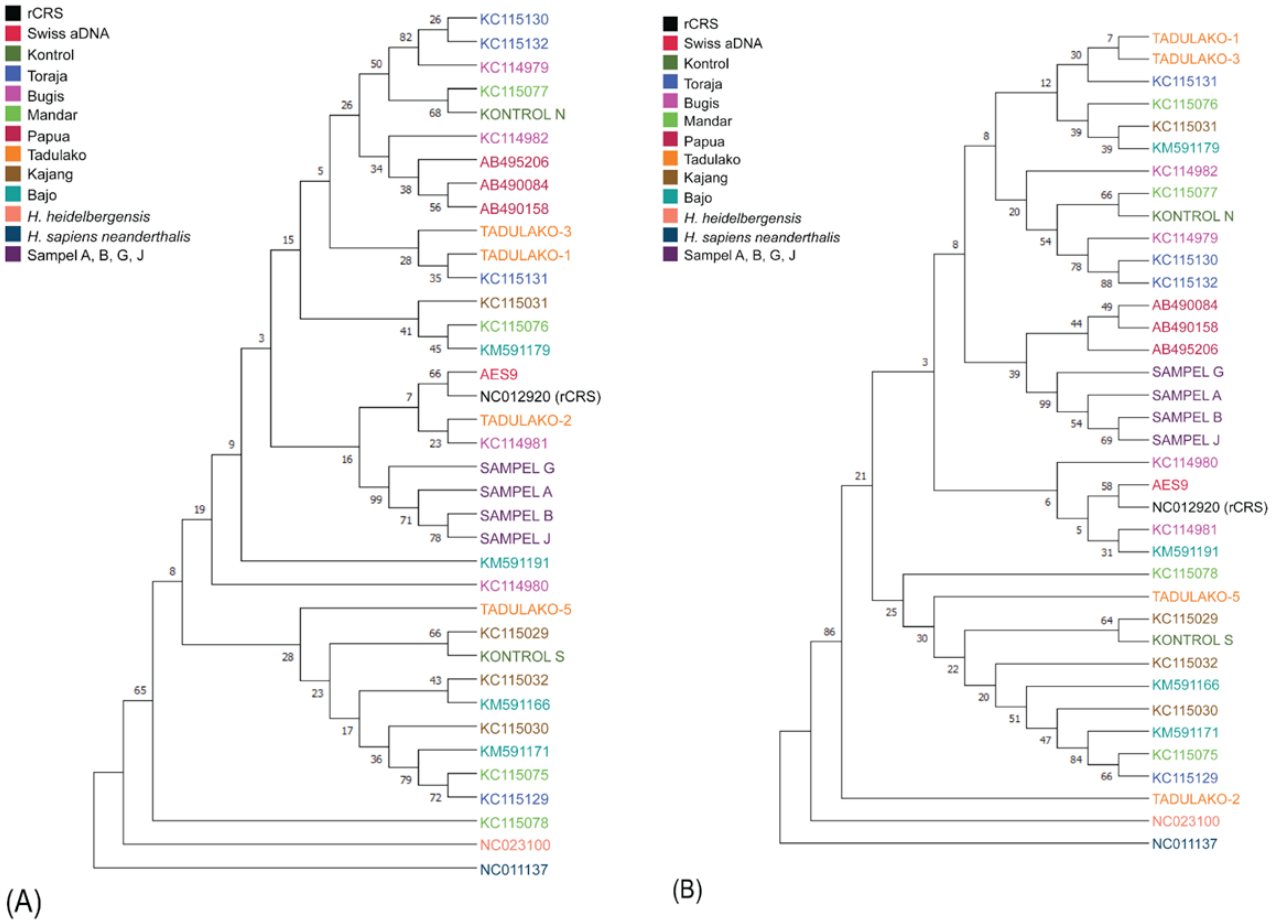

Figure 5. Phylogenetic Tree Reconstruction Result; (A) Maximum Likelihood Method and (B) Neighbor Joining Method

4. Conclusion

Based on this research, several things can be concluded; among other things, the result of the genetic variation analysis between the A, B, G, and J prehistoric samples and the prehistoric Tadulako samples with rCRS shows the presence of polymorphism in the form of SNP and MNP, also the presence of transversion, transition, and indels. Subsequently, the phylogenetic analysis of genetic distance shows that the distance between 37 samples is extremely close, with differences ranging from 0.02% to 0.13%. The reconstruction result shows that the A, B, G, and J samples are close to Sulawesi samples, particularly the Bajo and Bugis tribes, which are tribes of Austronesian descent.

Additional prehistoric samples and comparison samples from other regions, such as Taiwan and whole mitochondrial genome analysis, need to be included for further research to obtain a more conclusive result.

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