

Article

Haplogroup Distribution of 309 Thais from Admixed Populations across the Country by HVI and HVII Sanger-Type Sequencing

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Abstract: The mitochondrial DNA (mtDNA) control region sequences for the hypervariable regions I (HVI) and II (HVII) of 309 Thai citizens were investigated using Sanger-type sequencing to generate an mtDNA reference dataset for forensic casework, and the haplogroup distribution within geographically proximal Asian populations was analyzed. The population sample set contained 264 distinct haplotypes and showed high haplotype diversity, low matching probability, and high powers of discrimination, at 0.9985, 0.4744%, and 0.9953, respectively, compared with previous reports. Sub-haplogroup F1a showed the highest frequency in the Thai population, similar to Southeast Asian populations. The haplotype frequencies in the northern, northeastern, and southern populations of Thailand illustrate the relevance of social, religious, and historical factors in the biogeographical origin of the admixed Thai population as a whole. The HVI and HVII reference datasets will be useful for forensic casework applications, with improved genetic information content and discriminatory power compared to currently available techniques.

Keywords: control region; haplotype; forensics; mitochondrial DNA

1. Introduction

The analysis of human mitochondrial DNA (mtDNA) has been conducted globally in recent years, following the standard forensic casework guidelines using Sanger-type sequencing (STS) [1–5]. Compared with the nuclear genome, high copy numbers of mtDNA are observed within the cell, leading to an increased likelihood of recovering useable mtDNA data [3,6]. The mtDNA typing of hairs, saliva, and bone samples collected at crime scenes is frequently used as the DNA-based testing option [1,3,7–10]. The power of mtDNA typing to discriminate between individuals based on maternal inheritance is limited, with a lack of recombination, but mtDNA is frequently applied in routine forensic applications [7,11]. The noncoding control region is most suited for mtDNA typing and shows a high degree of genetic variability at the population level [8]. Historically, mtDNA typing has focused on the 342 bp (16,024–16,385) hypervariable region I (HVI), and the 268 bp (73–340) HVII, with reference to the human mtDNA genome sequence (GenBank accession number: NC_012920.1) [3,12–15]. The probability of previously reported mtDNA

from two random individuals matching in these regions is between 1 and 2% [8,16,17]. The Tsunami disaster on December 24, 2004 resulted in the deaths of nearly 5400 people in Southern Thailand [18]. Identification of the victims was conducted based on HVI and HVII sequences, with a success rate of 51% [18]. The HVI and HVII regions have also been used to identify the remains of Italian and Austrian soldiers killed during World War I, which are found occasionally during excavations [19]. mtDNA techniques have been adopted as the global standard forensic protocols by the International Society for Forensic Genetics [20], while an mtDNA dataset has been developed and applied to examine the compatibility between individuals as a reference database in several countries [8,16,21–27]. The polymorphisms of both HVI and HVII are also useful for addressing human evolution, migration, and the history of population descent by direct comparison with a haplogroup database [28,29].

Four macro haplogroups (L, M, N, and R), and 25 major haplogroups (L0, L1, L5, L2, L6, L4, L3*, M*, M7, M8, M9, G, D, N*, N1, N2, N9, A, X, R*, R0, JT, R9, B and U) comprise a total of more than 5400 haplogroups, with over 5000 subhaplogroups [30]. The distribution of macro haplogroup L, and the major haplogroups, L0, L1, L2 and L3, were reported in Africa [24]. The majority of the haplogroups in Asian populations are the macro haplogroups M and N, with subhaplogroups. F1a was found in Chinese and Southeast Asian populations, while M9a and D4 were predominantly detected in Chinese and Korean populations [31–33]. B4, B5a, and M9a were present in Southeast Asian populations [24,34–36].

The geographic location of the Kingdom of Thailand, incorporating the upland and lowland areas in the center of mainland Southeast Asia (or Indochina), is a critical factor in facilitating multicultural diversity. The Thai census recorded 66.5 million people in 2020 (<http://www.moi.go.th/>, accessed on 15 June 2020). Recent analyses of HVI and HVII revealed three macro haplogroups—M (D4 and M7b), N (A), and R (B4, B5a, F1a, and R9b)—in mainland Southeast Asia [23,34,36–42]. The majority of residents in this region use languages that belong to the Tai linguistic group, including Yuan, Lue, Yong, Tai Yai (Shan), and Khuen, from northern Thailand, associated with the subhaplogroups F1a, B6a, B5a, M7b, and R9b, Isan and Phuthai from the northeast with F1 and B5, and Malayu from the south with B5a, F1a, and M [38,43–45]. This haplogroup information correlates with classification in accordance with linguistic family distributions and is important for understanding the results of archaeological studies [43]. However, according to historical records, several foreign migrations of the Daic, Khmuic, Lao, Shan, Yuan, and Malayu peoples occurred and they intermixed with local Thai groups [16,44,46], resulting in genetic admixture in the current Thai population [16,45,47]. Multidirectional cross-cultural marriages are guided by social, religious, and historical conditions that vary significantly for each distinct geographical region [28,29,48]. Given this scenario, we hypothesize that the modern Thai population shows a high degree of genetic admixture, resulting from historically interracial marriages [38,45,47,49]. However, no reference mtDNA haplogroup data are available for the current Thai population. Such data would be advantageous for forensic casework and human migration mtDNA analysis, which require relevant and reliable databases to estimate the probability of a given haplotype in a population. This study estimated HVI and HVII diversity, using the STS approach, for 309 randomly selected Thai individuals. This report aims to establish a reference mtDNA dataset of modern Thai individuals to calculate statistical values that will benefit forensic casework investigation in the Kingdom of Thailand.

2. Materials and Methods

2.1. DNA Samples and Extraction

Blood samples were collected from 309 unrelated individuals across Thailand. The families of each individual had lived in the respective area for at least three generations (Table 1). Subjects were recruited from the northern (N) (17°10'0" N, 20°27'0" N, 97°10'0" N, 101°10'0" E) ($n = 93$), northeastern (NE) (14°8'18" N, 18°26'33" N) ($n = 61$), central (C)

(13°15'0" N, 17°45'0" N, 98°55'0" E, 101°35'0" E) ($n = 92$), and southern (S) (5°37'0" N, 11°32'0" N, 98°13'0" E, 102°52'0" E) ($n = 63$) populations. The study focus is concentrated on single-source reference specimens for the Thai population. This research was approved by the Ethical Clearance Committee on Human Rights related to research involving human subjects, and the Faculty of Medicine, Ramathibodi Hospital, Mahidol University (COA. MURA2020/825). Blood samples were collected as a source of DNA, from which genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). DNA quantity was determined using the spectrophotometry approach (NanoDrop, Thermo Fisher Scientific, Waltham, MA, USA).

Table 1. Summary statistics for the Thai population for two mtDNA regions historically targeted for forensic typing.

Population Statistics	HVI and HVII Sequences
Thai populations ($n = 309$)	
Haplotype	264
Unique haplotype	231
Diversity	0.9985
Matching probability (%)	0.4744
Power of discrimination	0.9953

2.2. Amplification and Sequencing of HVI and HVII by STS

The nucleotide sequences of HVI (16,024–16,365) and HVII (73–340) [13] of the mtDNA control region were amplified using primers (HV1F, 5'-CTCCACCATTAGCACCCAA-3'; HV1R, 5'-ATTTCACGGAGGATGGTG-3'; HV2F, 5'-CACCTATTAACCACTCACG-3'; and HV2R, 5'-CTGTAAAAGTGCATACCGC-3') specifically designed for the study. PCR amplification total volumes were performed using 25 μ L Master Mix, consisting of 10X Taq buffer, containing 1.5 mM MgCl₂, 0.2 mM dNTPs, 5.0 μ M primers, 0.5 U AmpliTaq Gold DNA Polymerase (Life Technologies, Foster City, CA, USA), and 25 ng genomic DNA. In the PCR reaction tube, each primer pair was amplified individually. The amplification conditions of both primer pairs were: initial denaturation at 95 °C for 10 min, followed by 35 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis in 1% agarose gel and treated with ExoSAP-IT (USB, Cleveland, OH, USA) to eliminate the primers and unused dNTPs. Sequencing reactions were performed with BigDye Terminator version 1.1 (Applied Biosystems, Foster City, CA, USA), in accordance with the manufacturer's instructions, and purified using the DyeEx 2.0 Spin Kit (Qiagen). All reactions were sequenced using a 3130 Genetic Analyzer (Applied Biosystems), and the results were analyzed using SeqScape version 2.5 software (Applied Biosystems). The consensus sequences were aligned and compared with the revised Cambridge Reference Sequence (rCRS, NC_012920.1) following the nomenclature guidelines for mtDNA typing [13,50,51].

2.3. Data Analysis of mtDNA HVI and HVII

The summary statistics (number of haplotypes, haplotype diversity, matching probability, and power of discrimination) for combinations of HVI and HVII were based on pairwise comparisons of Thai populations. The matching probability (P) was calculated using the equation $P = \sum x^2$. The power discrimination (PD) was determined using the equation $PD = 1 - \sum x^2$. The haplotype diversity HD was determined using the equation $HD = \frac{(1 - \sum x^2)n}{n-1}$, where x is the frequency of each mtDNA haplotype, and n is the total number of samples [52–54].

We analyzed the differentiation among Thai populations based on the administrative regions (N, NE, C, and S), using a pairwise F_{ST} with the "stamppFst" function in the StAMPP package in R [55], following the method proposed by Wright [56], and updated by Weir and Cockerham [57]. Empirical p -values were obtained by comparing the observed value of F_{st} with 1000 bootstrapping pseudoreplicates. The principal component analysis

(PCA) was also performed on the dataset, using the function “prcomp” in the “stats” package in R [58]. The results from the PCA were visualized using the function “fviz_pac_ind” in the package “factoextra” in R [59].

2.4. Classification of mtDNA Haplogroup and Data Analysis

The MtDNA haplotypes were affiliated to the haplogroups based on the patterns of the shared haplogroup-specific nucleotide polymorphisms, using HaploGrep2 with PhyloTree 17 [60], and the MITOMAP (<https://www.mitomap.org>, accessed on 15 March 2020) and EMPOP (<https://www.empop.online>, accessed on 15 March 2020) [61] online databases, based on HVI and HVII sequences. All generated data were deposited in the EMPOP database as rCRS variations, under reference number EMP00840 [62]. Haplogroup frequencies were visualized using the “ggplot” function [63] and analyzed for significant differentiation among the regions using the chi-square test in the “stats” package in R [58]. The workflow graphic from the 309 sampling processes for data evaluation are shown in Figure S1.

3. Results

A total of 264 different haplotypes were observed, comprising 231 unique haplotypes (74.76%), and 33 haplotypes that shared more than one individual. One shared haplotype also resulted from the varied numbers of 2–5 individuals in 78 unrelated individuals (25.24%) (Table 1).

The number of haplotypes was estimated in relation to four continental Thai groups, namely, the northern, northeastern, central, and southern populations (Table S1). The haplotype diversity of the HVI/HVII combinations was 0.9985, and the matching probability and power of discrimination were 0.4744% and 0.9953, respectively (Table 1). The first two dimensions of the principal component analysis (PCA) of the SNP data accounted for 22.4% of the variation. The PCA showed slight differentiation among the populations of the four regions (Figure 1).

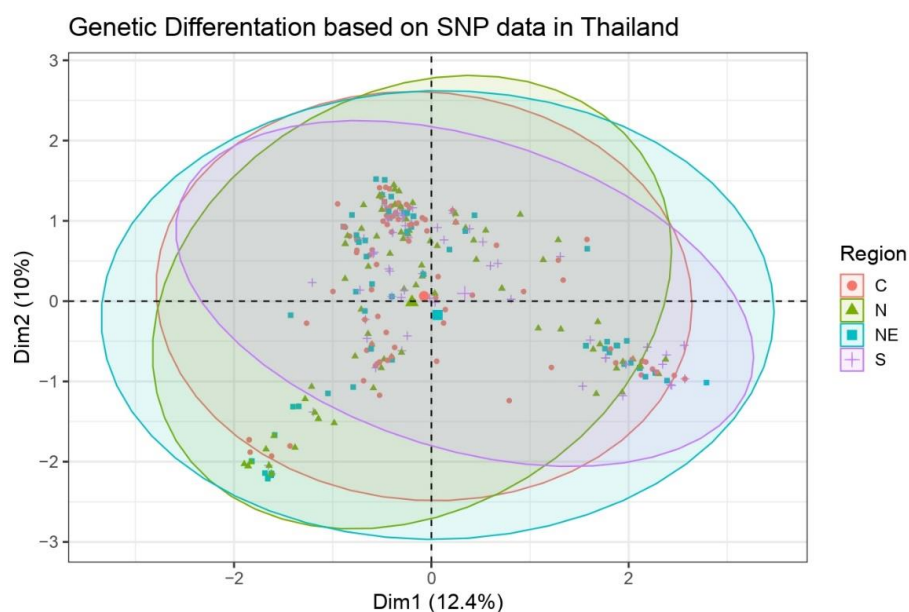


Figure 1. Genetic differentiation based on SNP data in Thailand.

We analyzed differentiation among the Thai population based on the administrative regions (N, NE, C and S). The pairwise F_{ST} values among the populations were approximately zero and did not differ significantly from the random (p -value ≥ 0.715), suggesting little differentiation among the populations. (Table 2).

Table 2. Pairwise F_{ST} among regional Thai populations.

Region	North	Northeast	Central	South
North	-	0.999	1.000	0.669
Northeast	−0.0069569	-	1.000	0.715
Central	−0.0115752	−0.0051491	-	1.000
South	−0.0011671	−0.0023083	−0.0101708	-

Lower left half pairwise F_{ST} . Bold text indicates F_{ST} p -value.

All 264 haplotypes were subsequently classified into 82 haplogroups using Haplo-Grep2 with PhyloTree 17 and EMPOP [54]. The most frequent haplogroups were: F1a (13.92%, $n = 43$); B5a (8.41%, $n = 26$); M7b (6.47%, $n = 20$); R9b (5.83%, $n = 18$); and B4c (3.56%, $n = 11$) (Table 3, Figure 2).

Table 3. Haplogroup frequencies in Thailand (based on classification of HVI and HVII sequence data) ($n = 309$).

Haplogroup (Macrohaplogroup M)	<i>n</i>	Frequency (%)	Haplogroup (Macrohaplogroup N)	<i>n</i>	Frequency (%)
C4a	3	0.9709	A	1	0.3236
C4c	1	0.3236	A4	5	1.6181
C7	2	0.6472	A4d	1	0.3236
C7a	8	2.5890	A5b	1	0.3236
D4	4	1.2945	B2g	1	0.3236
D4a	1	0.3236	B4	1	0.3236
D4b	2	0.6472	B4a	9	2.9126
D4j	1	0.3236	B4b	3	0.9709
D4g	1	0.3236	B4c	11	3.5599
D5	1	0.3236	B4g	4	1.2945
D5b	2	0.6472	B5a	26	8.4142
D6c	1	0.3236	B5b	2	0.6472
G1a	3	0.9709	B6a	1	0.3236
G2a	7	2.2654	F1a	43	13.916
M	7	2.2654	F1b	1	0.3236
M3a	1	0.3236	F1c	1	0.3236
M3c	1	0.3236	F1d	1	0.3236
M5c	2	0.6472	F1e	1	0.3236
M7a	1	0.3236	F2d	1	0.3236
M7B	20	6.4725	F3a	5	1.6181
M7c	9	2.9126	F4b	1	0.3236
M8a	2	0.6472	N7a	1	0.3236
M9a	4	1.2945	N9a	8	2.5890
M9b	2	0.6472	N10a	5	1.6181
M10	2	0.6472	N10b	1	0.3236
M10a	11	3.5599	N11a	1	0.3236
M11	1	0.3236	N21	1	0.3236
M12a	3	0.9709	N22	2	0.6472
M12b	4	1.2945	R2	1	0.3236
M17c	1	0.3236	R5a	1	0.3236
M20	4	1.2945	R9b	18	5.8252
M21d	4	1.2945	R9c	8	2.5890
M26	2	0.6472	R11	1	0.3236
M34a	1	0.3236	R22	4	1.2945
M38	2	0.6472	U7a	1	0.3236
M50	1	0.3236	Y2	1	0.3236
M50a	1	0.3236			
M51a	1	0.3236			
M68a	3	0.9709			
M71	1	0.3236			
M71a	3	0.9709			
M71b	1	0.3236			
M72	1	0.3236			
M91a	1	0.3236			
Z	1	0.3236			
M26	2	0.6472			

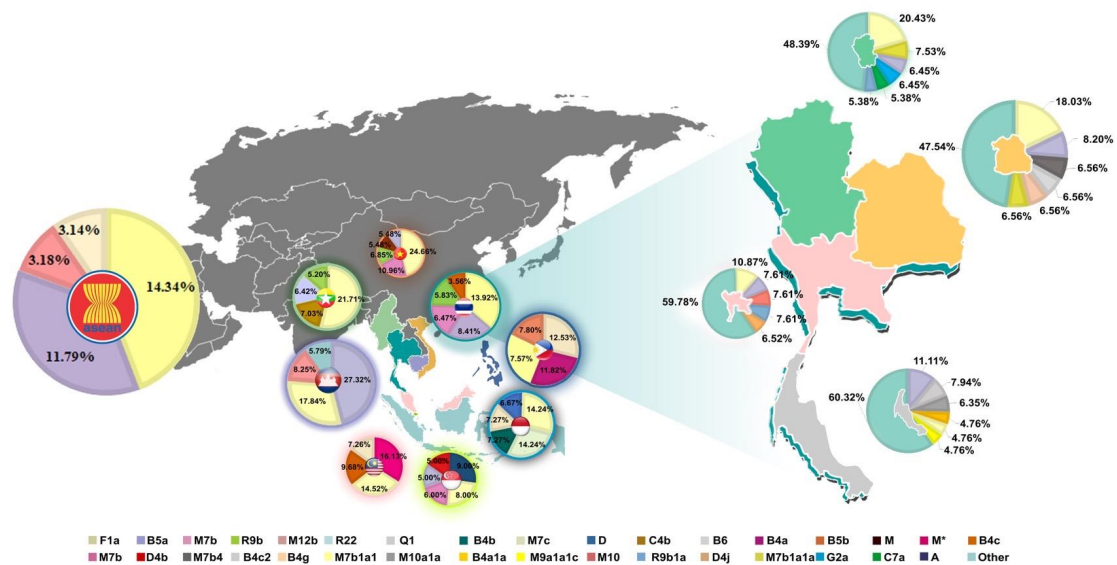


Figure 2. Haplogroup diversity observed in the Thai population ($n = 309$) compared with Asian populations. Haplotype frequency of each Asian population was derived from Maruyama et al. [11], Li et al. [16], Yao et al. [21], Lee et al. [23], Summerer et al. [36], Tabbada et al. [39], Maruyama et al. [40], Zhang et al. [41], Shee et al. [42], Fornarino et al. [64], and Mona et al. [65].

The haplogroup frequencies based on the HVI and HVII SNP data of the Thai population showed some degree of separation among the populations of different regions (Figure 3).

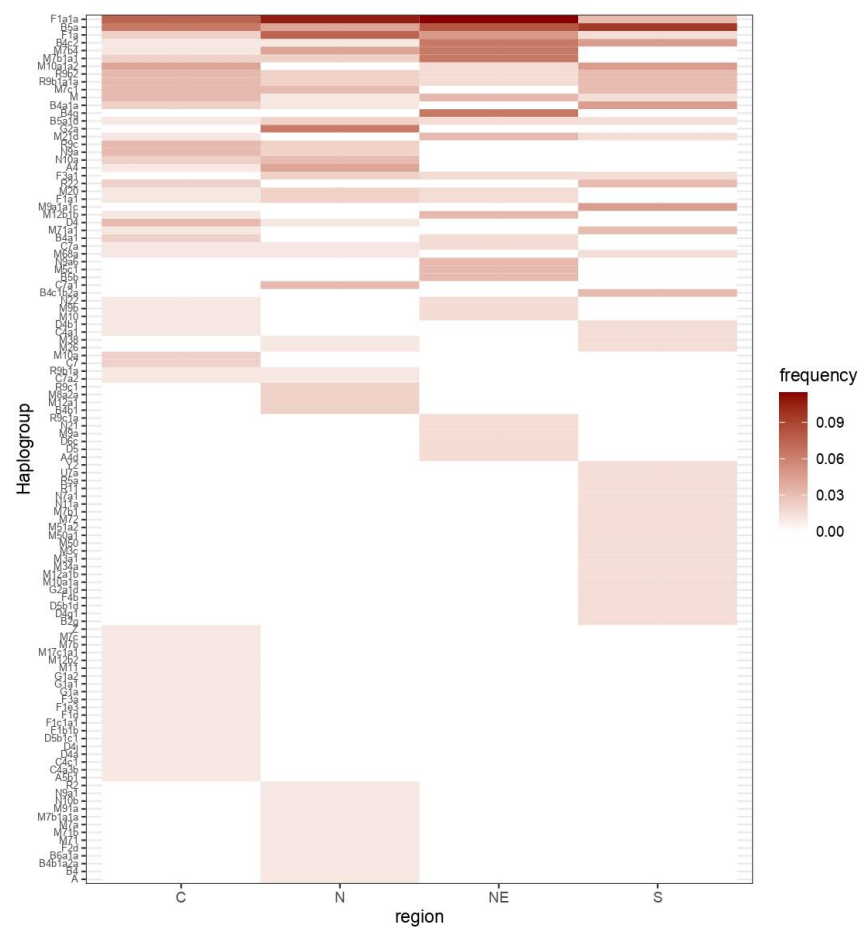


Figure 3. Heatmap of haplogroup frequencies among Thai populations in the four regions. Darker color indicates higher frequencies.

However, the proportion of haplogroup frequencies did not differ significantly from the random (chi-square test, $p = 1.00$). In this study, haplogroup frequencies were compared with the 12 neighboring countries in Southeast Asia, South Asia, and East Asia (Figure 4). The results show that the frequencies of haplogroups in Thailand are similar to the data from Southeast Asia, while the Thai population shows a slight differentiation from other Asian countries (Figure 4; chi-square test, $p = 1.00$). However, almost all of the Southeast Asian countries had higher frequencies of the F1a haplogroup compared to the other regions (Figure 4).

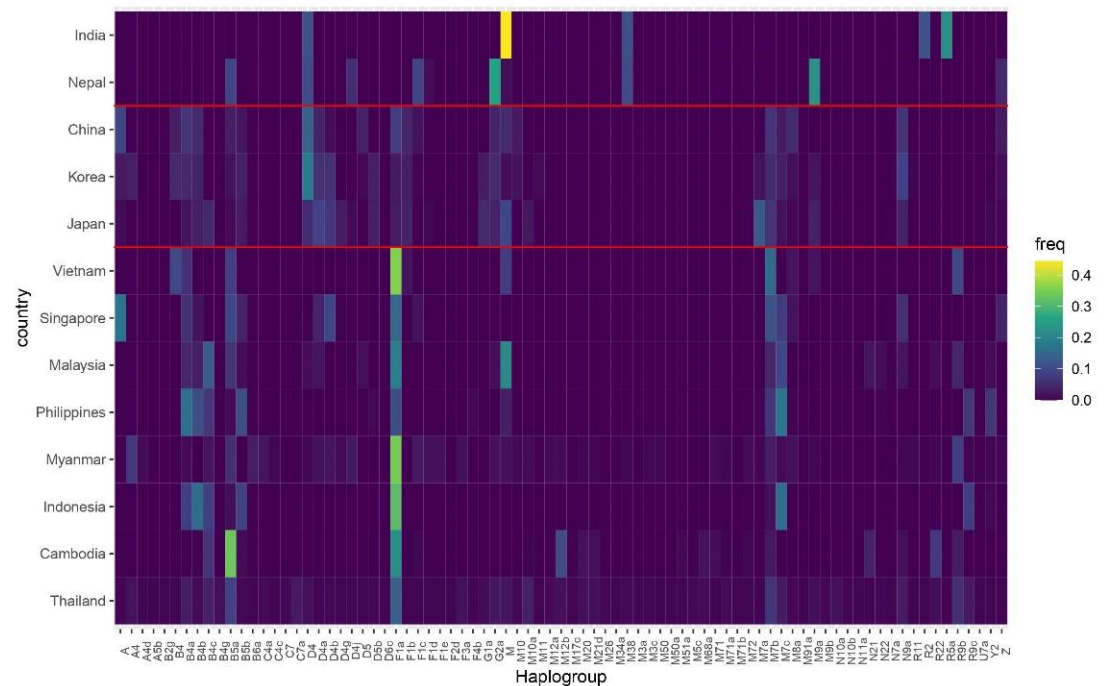


Figure 4. Heatmap of haplogroup frequencies of some Asian populations based on SNP data.

The first two PCAs of the haplogroup frequencies accounted for 55.3% of the variation and show that Southeast Asian populations appear more similar to each other than to countries from the other regions (Figure 5).

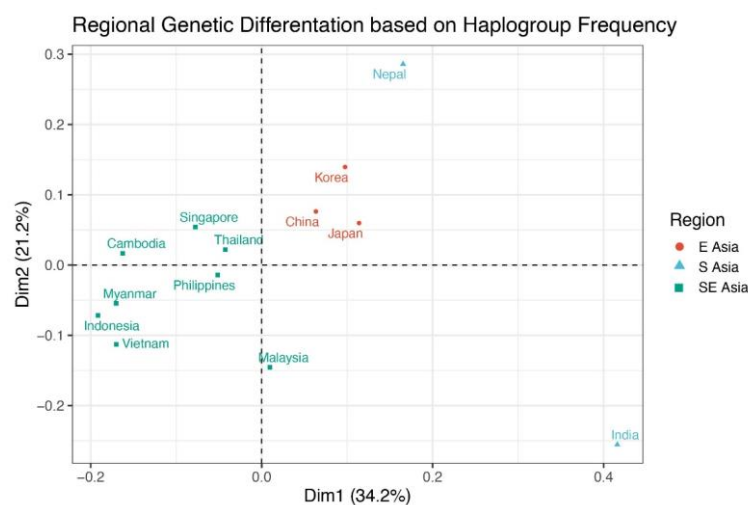


Figure 5. Principal component analysis based on haplogroup frequencies.

4. Discussion

Forensic genetic analyses provide useful information, as biological evidence found at crime scenes, for the identification of missing people after mass disasters, or for inferring the cause and manner of death. The STS approach is accepted as the standard method for mtDNA typing using HVI and HVII [2,3]. However, this approach is labor-intensive, time-consuming, and costly as an analytical method. An alternative choice is required for practical mtDNA sequencing. Accurate human identification analysis is extremely important and a reference database for an individual country can be used by forensic laboratories to report casework precisely. To establish a reference mtDNA database in forensic casework for Thailand, we compiled a high-quality HVI and HVII sequence dataset for 309 Thai individuals. A total of 264 haplotypes showed 0.9985 diversity. This diversity was higher than recorded in the datasets of Thai populations ($n = 190$, 0.989; $n = 124$, 0.9958; $n = 100$, 0.9859) reported in previous studies [28,61,62,66], as well as in datasets for the Lao ($n = 214$, 0.790), Vietnam ($n = 187$, 0.991), and Singapore ($n = 205$, 0.9961) populations [15,23,67–69]. Previous studies of the random matching probability and the power of discrimination in a unique ethnic group from Chiang Mai, located in the north of Thailand, (Tai, Mon-Khmer), were 0.9980 and 0.9906, respectively [28,54]. Modern Thai individuals, comprising an admixed population, demonstrated random matching probability, and the powers of discrimination were 0.4744% and 0.9985, respectively. These findings suggest that the present mtDNA dataset exhibited high haplotype variation and was suitable for human identification as a reference dataset.

In this study, information on the single-nucleotide polymorphisms (SNPs) of HVI and HVII was classified into haplogroups. These groups may provide valuable information for determining the patterns of variation and population structures of maternal lineages to support human identification [36]. In Thailand, human identification is commonly performed using autosomal STR (short tandem repeat) markers, but this method is sometimes unsuccessful in skeletal-remains identification. Therefore, to improve the results, we applied mtDNA haplogroups for additional supporting evidence. The majority of haplotypes detected in Thai populations belong to the M and N macro haplogroups, and comprise three major haplogroups (M7, R9, and B), as also observed in Asian populations [15,21,36]. The subhaplogroup F1a showed the highest prevalence in Thai populations (13.92%), consistent with other Southeast Asian populations (Table S2) [16,36,68–70] (Figures 2 and 5). This finding concurs with historical and geographical details, given that Thailand has a long tradition of granting political asylum to refugees from neighboring countries who have been persecuted as a result of their religion or ethnicity. Thailand is the only Southeast Asian country that has never been colonized by a European power [71,72]. For centuries, Vietnamese Christians, the Mon ethnic group from Myanmar, and political dissidents from Cambodia, have all sought and received shelter in Thailand [71]. Many ethnic groups are resident in Thailand, including Malays, Mon, Khmer, and various hill tribes, as a result of the historic migrations of those fleeing civil wars and economic crises [15,71]. By contrast, Chinese, and other East Asian populations, contain a high proportion of subhaplogroup D4, whereas the majority of populations in South Asian countries, such as Nepal and India, contain subhaplogroup G2a [73,74] (Table S2). These results collectively suggest that subhaplogroup proportions can be used to determine the geographic origin of human populations.

In order to shed more light on the genetic admixture of haplogroups in Thailand, we compared the current samples, organized into four distinct geographic groups, with various Asian populations (Figure 3). For the migration of various ethnic groups, originating from distinct geopolitical regions, into the same geographical area, dynamic gene flow was observed in India, Indonesia, Australia, Sweden, Macedonia, Brazil, and the USA [75–79]. Although F1a was the predominant subhaplogroup detected in Thailand, distinctively high proportions of F1a, and other subhaplogroups, were observed in the northern and northeastern populations compared with the central population. The Kingdom of Lan Na captures the cultural development of the northern Thai people, which originated

more than 650 years ago, coincident with successive Kingdoms of Thailand [80]. The northern population of hill tribes was also impacted by sex-specific migration and cultural factors [46]. The predominant subhaplogroup of the northern population is F1a, which differs substantially from the other subhaplogroups in the population. Over 200 years ago, Burma expanded its influence to the Lan Na Kingdom, and Burmese populations contain a high proportion of F1a [36]. This suggests the possibility of genetic exchange between the two kingdoms, in accordance with the Lan Na territorial occupation dynamic. The Lan Na Kingdom merged with the Kingdom of Thailand 88 years ago [80], and the distinctively high proportion of F1a remains. These results concur with the general distribution of maternal lineages in the northern population of Thailand, observed by Kampuansai et al., and Kriengchutima et al. [47,54], whereas in the northeastern population, the frequencies of F1a and B5a were consistent with the proportion of subhaplogroups in Lao, in accordance with historic migration, as determined by Bodner et al. [16]. By contrast, the subhaplogroups in the southern population became isolated, as exemplified by the absence of B5a and B4 from the majority of the Thai population. This result agrees with the most frequent subhaplogroup in populations on the Malay Peninsula, as recorded by Tabbada et al. [39], and Maruyama et al. [40]. Religion possibly influenced marriage because, in southern areas, the majority of residents are Muslims, similar to Malay populations [40]. It is likely that multidirectional cross-cultural marriages were influenced by social and historical conditions that varied significantly for each distinct geographical area. The modern Thai population reflects a high degree of genetic admixture as a result of recurrent historically interracial marriage events, as also observed in similar proportions in the subhaplogroups of the central population (Figure 1) [45,47].

During the twelfth and thirteenth centuries, Thai people were subjugated under the Khmers (in the area now known as Cambodia), leading to mtDNA exchange between the Tai-Kadai and Khmer groups throughout the central part of Thailand [29]. This might also reflect the proportion of haplogroups in the central and northeastern regions of Thailand, which show high frequencies of the subhaplogroups F1a and B5a, similar to the subhaplogroup distribution in Cambodia (Figure 4) [41]. One subhaplogroup M7b lineage was also detected several times in the northern and northeastern populations of the dataset and is also observed in southern China [21]. The Chinese colonization of Thailand occurred during the nineteenth and twentieth centuries, with the migration from South China into Southeast Asia of those fleeing national economic and political unrest [16,81]. This suggests that the emergence of subhaplogroup M7b was caused by Chinese migration to Southeast Asia (Figure 4). However, a high proportion of the population retains haplogroup F1a. This result is consistent with a significant gender bias and contribution imbalance reported for parental lineages in the Chinese-Thai population caused by traditional ethnicity, together with maternal contribution [16]. Alternatively, Chinese colonization had a notable gender bias toward masculine contributions. Such migrant men interbred with local Thai residents, especially during the initial colonization phases [46]. mtDNA markers display a more asymmetrical distribution of ancestral population contributions to the current genetic pool [12]. The historical and cultural factors that motivated the admixture mechanisms in Thailand have resulted in the current admixed population. Such diverse biogeographical groups shaped the current Thai population, forging a genetically and culturally diverse, highly admixed country [70].

This study evaluated the utility of mtDNA HVI and HVII in the Thai population to develop a national reference database for real forensic casework using 309 specimens collected across the country. Diversity indices and other relevant forensic statistical parameters indicated a high degree of polymorphism suitable for human identification purposes. Workflow automation should also be considered when casework volumes are high or a backlog exists. The construction of a representative forensic national database is also essential, with the addition of a substantial number of specimens to evaluate possible genetic regional or ethnic stratification in the Thai population. However, in particular situations, HVI and HVII data do not provide sufficient discriminatory information to resolve distinct

maternal lineages. A recent study examining individuals from across the USA determined HVI and HVII information, with a discrimination of 0.9942, and a matching probability of 0.96% for specimen haplotypes [79]. Complete mtGenome (mitogenome) data allowed for identification at almost full resolution, with higher discrimination at 0.9999, and matching probability at 0.39%, for the US population [79]. Therefore, mitogenomic data are required to improve human identification by mtDNA testing in Thailand. A reference mtDNA database is also needed to develop the entire mitogenome reference population data suitable for forensic comparison. Such a database would supply additional information, with special consideration for the high ethnic diversity and admixed populations in Thailand. The investigatory unit might seek to resolve missing person cases, in which more than one reference family share the same HVI and HVII haplotype, using the mitogenome, thereby increasing statistical support when exclusionary references are unavailable [81–83]. This approach can lead to the correction of haplotype classification and the identification of undescribed subclades. STS processing is labor-intensive, costly, and time-consuming; however, routine work still follows this technique in local units across Thailand. These days, parallel sequencing technology is an efficient high-throughput tool, and further reports of mitogenomic sequencing should be challenged in Thai mtDNA datasets.

5. Conclusions

The HVI and HVII sequences analyzed in this study were generated in accordance with high-quality laboratory standards. The frequencies of the identified haplogroups were characterized for the admixed Thai population. The F1a, B5a, M7b, and R9b sub-haplogroups showed high frequencies in the population database. The establishment of regional databases is necessary, especially for forensic database searches, in order to obtain reliable frequency estimates. Additional sequences from such haplotypes must be collected and genotyped in order for the mitogenome to expand the Thai population database. The haplotypes reported in this study will be available from the EMPOP database (<https://www.empop.org>, accessed on 21 December 2020) upon publication.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13100496/s1>. Figure S1: Workflow graphic from 309 sampling processes for data evaluation; Table S1: Haplogroup diversity in the Thai population (based on classification of HVI and HVII sequence data); Table S2: Haplogroup frequencies in Thailand and other Asian populations.

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