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ORIGINAL ARTICLE

# Mitochondrial DNA control region diversity in a population from Parana state—increasing the Brazilian forensic database

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## Abstract

The entire mitochondrial DNA (mtDNA) control region (nucleotide position 16024-576) sequences were obtained through Sanger sequencing method for 122 individuals from Parana state, South of Brazil. We observed a total of 108 different haplotypes of which 97 were unique and 11 were shared by more than one individual. The haplogroups were classified according to the updated mtDNA phylogeny, by EMMA (estimating mitochondrial haplogroups using a maximum likelihood approach). Our results revealed the predominance of Amerindian haplogroups with a frequency of 49.2% of the population sample, followed by European lineages with 38.5% and 12.3% of African lineages. Parana population sample set presented a high haplotype diversity (0.9976) and the random match probability was 0.0106. The phylogenetical findings and the diversity indices confirm the high genetic heterogeneity of this population and suggest a high informativeness of mtDNA analyses in forensic cases. The population data will contribute to increase the Brazilian mtDNA database for forensic purposes and it is available through EMPOP (European DNA Profiling Group mitochondrial DNA population database) under the accession number EMP00714.

**Keywords** Mitochondrial DNA · Control region · Genetic population data · Forensic application · Parana · Brazil

## Introduction

Mitochondrial DNA (mtDNA) genome analysis has been a potent tool in forensic practice as result of its properties and certain advantages over nuclear DNA in some legal cases [1]. The increase of the mitochondrial population data, in the last 15 years, provided a better understanding of the mitochondrial phylogeny and it also motivated the publication of new forensic guidelines to ensure data quality and to establish standardization between laboratories around the world [2, 3].

For many years, the genetic diversity of the mtDNA sequence in different ethnic populations has been revealed through Sanger sequencing of the hypervariable 1 and 2 regions (HV1 and HV2) (from positions 16024 to 16365 and from 73 to 340, respectively) [4–7]. In the late 1990s, a third hypervariable region (HV3) (from positions 438 to 576) was also included in some population studies [8–11]. Nowadays, it recommended sequencing of the entire mitochondrial DNA control region (from positions 16024 to 576) in population database studies, to increase the power of discrimination and the haplogroup determination [3, 12].

In Brazil, previous studies based on the analysis of the HVs regions had been published [13–15]. Brazil is in South America and it has one of the most heterogeneous populations in the world, which is the result of five centuries of interethnic crosses of peoples from three continents: European colonizers, mainly represented by Portugueses, enslaved Africans and native Amerindians [16]. The mtDNA haplogroups frequencies are variable between Brazilian regions [17], as result of its large geographical extension and its highly mixed population [18]. Parana state is in the southern of Brazil and represents 2.34% of Brazilian territorial extension [19], and according to regional history data, the Parana population is also three-hybrid, with the contribution of Amerindians, Africans, and

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Europeans [20]. This is the first study to report the mitochondrial DNA diversity of Parana state. Therefore, the aim of this work is to generate high quality mtDNA forensic data to increase Brazilian mitochondrial genetic information, as well as to establish the predominant mtDNA haplogroups, in the Parana state population sample set.

## Materials and methods

### DNA samples

Genomic DNA, extracted from peripheral blood by Biopur Kit Extraction Mini Spin Plus (Mobius Life Science, Brazil) according to the manufacturer's instructions, was obtained from 94 Euro-Brazilians, 24 Brazilians of mixed ancestry, and 4 Afro-Brazilians healthy unrelated individuals. They were assigned to one of these three groups based on self-classification and the sample size selected for each ethnic group was guided to respect the proportional contribution of them for Parana population according to the last data published [19]. Informed consent was obtained from all participants. This study was performed according to Brazilian Federal laws and was approved by the Human Research Ethics Committee of the Federal University of Parana.

### PCR amplification

The entire mtDNA control region was amplified in a single amplicon (1418 bp), as recommended in [3], using the primer sets L15879 (5′ AAT GGG CCT GTC CTT GTA GT 3′) and H727 (5′ AGG GTG AAC TCA CTG GAA CG 3′) [13]. Amplification reactions were done with AmpliTaq Gold DNA polymerase (Applied Biosystems), following manufacturer's specifications, using 50 ng of genomic DNA, in a final volume of 50 ul. The reactions were performed under conditions of initial denaturation at 95 °C for 10 min followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and 40 s.

### Sequencing

PCR products were purified using 10 U Exonuclease I (EXO I) (United States Biochemical—USB, Staufen, Germany) and 2 U Shrimp Alkaline Phosphatase (SAP) and 10× SAP Buffer (United States Biochemical—USB, Staufen, Germany). The sequencing reactions were made using BigDye Terminator Cycle Sequencing Kit v3.1, according to manufacturer's protocol. To ensure high quality data and double coverage at all positions, sequencing reaction was performed in both, forward and reverse strands. The ten sequencing primers used are listed in Table 1. In cases of length heteroplasmy, the double coverage of some positions was obtained from the same strand which was sequenced at least twice with different primers, as recommended in [3]. The sequenced fragments were analyzed by ABI 3130 Genetic Analyzer (Applied Biosystems, CA, USA).

**Table 1** Sequencing Primers

| Primers | Sequence (5′- 3′) | References |
|---|---|---|
| L15879 | AAT GGG CCT GTC CTT GTA GT | [13] |
| L15971 | TTA ACT CCA CCA TTA GCA CC | [2] |
| L16413 | TGA AAT CAA TAT CCC GCA CA | [13] |
| H16548 | GGG AAC GTG TGG GCT ATT TA | [13] |
| L00015 | CAC CCT ATT AAC CAC TCA CG | [2] |
| H00159 | AAA TAA TAG GAT GAG GCA GGA ATC | [2] |
| L00314 | CCG CTT CTG GCC ACA GCA CT | [2] |
| H00484 | TGA GAT TAG TAG TAT GGG AG | [2] |
| H00649 | TTT GTT TAT GGG GTG ATG TGA | [21] |
| H00727 | AGG GTG AAC TCA CTG GAA CG | [13] |

### Data analyses

The mtDNA control region was aligned and compared with the revised Cambridge Reference Sequence [22, 23] using SeqScape Software v2.7 (Life Technologies, Foster City, CA, USA). To ensure high quality data, two independent evaluations of raw data were performed. The length heteroplasmy in homopolymeric sequence stretches was interpreted by reporting the dominant variant [3, 24]. Haplogroup affiliation was inferred according to Phylotree, build 17 [25], by EMMA software (estimating mitochondrial haplogroups using a maximum likelihood approach) provided by EMPOP ver.3 (http://www.empop.org) [26, 27]. The ARLEQUIN v3.5 software was used to calculate molecular diversity indices, such as the number of different haplotypes, number of polymorphic sites, sequence diversity, nucleotide diversity, and mean number of pairwise differences [28]. The random match probability was calculated as the sum of squared haplotype frequencies based on mtDNA control region sequences. For the statistical analysis, the C-stretch length variation at positions 16193, 309, and 573 was excluded, with a total of 1131 usable sites.

## Results and discussion

All haplotypes and haplogroups obtained from Parana samples are listed in table SM1. For the 122 individuals analyzed, 108 haplotypes were identified. Of them, 97 sequences were unique, 9 were observed twice, 1 was observed three times, and 1 was observed four times. The most frequent haplotype (16519C, 263G, 315.1C) was observed in 3.3% of the population sample.

In the 1131 analyzed positions, it was observed that 191 polymorphic sites (16.9%) distributed in 161 positions with only transition, 7 sites with only transversion, 6 positions with transition and transversion and 17 with indels. A previous study with higher sample size showed 6% of point heteroplasmy frequency [29]. In our data, we observed similar frequency (5.7%) of point heteroplasmy founded in seven samples, which presented five Y and two R transitions as followed: 16192Y, 16311Y, 16355Y, 185R, 271Y, 310Y, and 374R. All of them were reported before in [29], except the last one (374R).

The molecular diversity indices calculated for the entire control region and for HV1, HV2, and HV3 in Parana population sample are available in Table 2. The results reinforce the greater informativeness of the HV1 region when compared to the other two hypervariable regions and show the increased power of discrimination when the entire control region is analyzed.

The sequence diversity calculated for the entire control region was 0.9976 ± 0.0016 and the random match probability estimated between two unrelated individuals in Parana state population was 0.0106. High values from sequence diversity were also reported in other Brazilian population studies [13–15, 30–32], confirming the population heterogeneity in this country.

## mtDNA haplogroups composition

The haplogroups identified in the examined sample showed the coexistence of matrilineal lineages with different phylogeographic origins. The importance of the indigenous women into the formation of Parana population was revealed by the prevalence of 49.2% of Amerindian mitochondrial lineages. It was observed that the coexistence of haplogroups B and C (15.6% each) with the highest frequencies, followed by A (13.9%) and D (4.1%), as showed in Table 3. The haplogroups A, C, and D were identified in Guarani tribes, and the haplogroups A, B, and C in Kaingang tribes, the two major Native Amerindian groups who lived in Parana state [33]. The high frequency of Amerindian matrilineal lineages in Parana state population reinforces the suggestion of a directional mating involving European males and Amerindian females in Brazil, as it was reported in [34] and also in agreement with historical data. In the XVI century, the mating between European men and indigenous women was encouraged as a strategy for population growth and colonial occupation of the country [35].

Regarding the European component, represented by 38.5% of the haplotypes in our study (haplogroups H, U3, U5, R0, T, K, J, V, W, and X2), as shown in Table 3, the prevalence of haplogroup H agrees with previous descriptions of European lineages in Brazil [31]. The haplogroup H was the most representative (11.5%), as occurs in most European populations [36]. The haplogroup U (except U6) was the second most frequent European haplogroup (7.4%) in the sample set, followed by haplogroup R0 with 6.5%. All these three haplogroups are also the most frequent haplogroups found in Portugal population [37], which agrees with the Brazilian colonization history. In the second half of the nineteenth century, many immigrants from Europe settled in Parana such as Italians, Germans, Ukrainians, and some English, French, and Swiss people [20]. Accordingly, with this populational diversity, we also observed different European haplogroups (T, K, J, V, W, and X2) with small frequencies (Table 3).

Considering the 12.3% of African lineages (L2, L1, L3, L0, and U6), the African sub-Saharan haplogroups (L2, L1, L3, and L0) were the most frequent (10.7%) in this study (Table 3). The subhaplogroup L3e is known to have high frequencies in West-Central Africa, which was the main source of Africans brought to Brazil during the colonial period [38]. But with the abolition of slavery (1888), the proportion of Africans in the population of the state of Parana has decreased substantially [13]. The North African contribution in this sample set, represented by the haplogroup U6 (1.6%), showed components of Maghreb origin, which has a high frequency in Portuguese, the main European colonizers of Brazil [39].

Comparisons between different states and regions of Brazil emphasize the differences between haplogroups compositions in each Brazilian region [13–15, 30–32]. However, the precise haplogroup assignment for some haplotypes is difficult even with entire control region analyzed. In this situation, the analysis of mtDNA coding region SNPs is recommended to refine the haplogroup classification, as it was done for H European

**Table 2** Genetic diversity in 122 samples from Parana state population, Brazil

| Molecular diversity indices | HV1 | HV2 | HV3 | HV1 + 2 | HV1 + 2 + 3 | Control region |
|---|---|---|---|---|---|---|
| Number of haplotypes | 95 | 52 | 27 | 103 | 105 | 108 |
| Number of polymorphic sites | 96 | 51 | 23 | 147 | 170 | 191 |
| Sequence diversity | 0.9917 ± 0.0035 | 0.9453 ± 0.0117 | 0.8592 ± 0.0180 | 0.9958 ± 0.0021 | 0.9966 ± 0.0018 | 0.9976 ± 0.0016 |
| Random match probability | 0.0164 | 0.0624 | 0.1478 | 0.0124 | 0.0116 | 0.0106 |
| Mean number of pairwise differences | 7.6602 ± 3.5961 | 4.5768 ± 2.2639 | 2.4820 ± 1.3496 | 12.2370 ± 5.5660 | 14.7190 ± 6.6330 | 16.1710 ± 7.2570 |
| Nucleotide diversity | 0.0223 ± 0.0116 | 0.0170 ± 0.0093 | 0.0174 ± 0.0105 | 0.0200 ± 0.0101 | 0.0195 ± 0.0097 | 0.0143 ± 0.0071 |

**Table 3** Frequencies of the mtDNA haplogroups and subhaplogroups in Parana sample set

| Haplogroup | Subhaplogroup | Number of samples | % |
|---|---|---|---|
| A | | 17 | 13.9 |
| | A | 1 | 0.8 |
| | A2 | 13 | 10.7 |
| | A2a | 1 | 0.8 |
| | A2i | 1 | 0.8 |
| | A2l | 1 | 0.8 |
| B | | 19 | 15.6 |
| | B2 | 3 | 2.5 |
| | B2b | 2 | 1.6 |
| | B2h | 3 | 2.5 |
| | B4b | 11 | 9.0 |
| C | | 19 | 15.6 |
| | C1a | 1 | 0.8 |
| | C1b | 15 | 12.4 |
| | C1c | 2 | 1.6 |
| | C1d | 1 | 0.8 |
| D | | 5 | 4.1 |
| | D1e | 4 | 3.3 |
| | D1f | 1 | 0.8 |
| H | | 14 | 11.5 |
| | H | 5 | 4.1 |
| | H1a | 1 | 0.8 |
| | H1b | 1 | 0.8 |
| | H1c | 1 | 0.8 |
| | H1q | 1 | 0.8 |
| | H2a | 1 | 0.8 |
| | H2c | 1 | 0.8 |
| | H5a | 1 | 0.8 |
| | H10 | 1 | 0.8 |
| | H15 | 1 | 0.8 |
| R | R0 | 8 | 6.5 |
| U | | 11 | 9.0 |
| | U3 | 1 | 0.8 |
| | U5a | 7 | 5.8 |
| | U5b | 1 | 0.8 |
| | U6a | 2 | 1.6 |
| K | K1a | 4 | 3.3 |
| T | | 4 | 3.3 |
| | T1a | 2 | 1.6 |
| | T2b | 1 | 0.8 |
| | T2e | 1 | 0.8 |
| J | | 3 | 2.5 |
| | J1 | 1 | 0.8 |
| | J1c | 1 | 0.8 |
| | J2 | 1 | 0.8 |
| V | V18 | 2 | 1.6 |
| W | W6 | 2 | 1.6 |
| X | X2b | 1 | 0.8 |
| L | | 13 | 10.7 |
| | L0a | 2 | 1.6 |
| | L1c | 3 | 2.5 |
| | L2a | 2 | 1.6 |
| | L2b | 1 | 0.8 |
| | L2c | 2 | 1.6 |
| | L3d | 1 | 0.8 |
| | L3e | 2 | 1.6 |

haplogroup subdivided into many lineages [40, 41], and in some Native American clades, such as B2, which is incompletely classified by control region motifs [42, 43]. The analysis of SNPs in the coding region is also a tool to increase the discrimination power of common haplotypes in forensic cases [44].

Finally, the high sequence diversity and the relatively low random match probability calculated from Parana state population sample set imply in a high probability of differentiating between two given maternal lineages and reinforce the mtDNA analyses informativeness in forensic cases. The haplotypes reported in the present study are available for forensic purposes via EMPOP (www.empop.org), under the accession number, EMP00714.

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## Compliance with ethical standards

This study was performed according to Brazilian Federal laws and was approved by the Human Research Ethics Committee of the Federal University of Parana.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Carracedo A, Bär W, Lincoln P, Mayr W, Morling N, Olaisen B, Schneider P, Budowle B, Brinkmann B, Gill P, Holland M, Tully G, Wilson M (2000) DNA commission of the International Society of Forensic Genetics: guidelines for mitochondrial DNA typing. Forensic Sci Int 110:79–85
2. Parson W, Bandelt HJ (2007) Extended guidelines for mtDNA typing of population data in forensic science. Forensic Sci Int Genet 1:13–19
3. Parson W, Gusmao L, Hares DR, Irwin JA, Mayr WR, Morling N, Pokorak E, Prinz M, Salas A, Schneider PM, Parsons TJ (2014) DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. Forensic Sci Int Genet 13:134–142
4. Budowle B, Wilson MR, Dizinno JA, Stauffer C, Fasano MA, Holland MM, Monson KL (1999) Mitochondrial DNA regions HVI and HVII population data. Forensic Sci Int 103:23–35
5. Imaizumi K, Parsons TJ, Yoshino M, Holland MM (2002) A new database of mitochondrial DNA hypervariable regions I and II sequences from 162 Japanese individuals. Int J Legal Med 116:68–73
6. Budowle B, Allard MW, Fisher CL, Isenberg AR, Monson KL, Stewart JE, Wilson MR, Miller KW (2002) HVI and HVII mitochondrial DNA data in apaches and Navajos. Int J Legal Med 116: 212–215
7. Tetzlaff S, Brandstätter A, Wegener R, Parson W, Weirich V (2007) Mitochondrial DNA population data of HVS-I and HVS-II sequences from a northeast German sample. Forensic Sci Int 172: 218–224
8. Lutz S, Weisser HJ, Heizmann J, Pollak S (1997) A third hypervariable region in the human mitochondrial D-loop. Hum Genet 101:384

9. Lutz S, Wittig H, Weisser HJ, Heizmann J, Junge A, Dimo-Simonin N, Parson W, Edelmann J, Anslinger K, Jung S, Augustin C (2000) Is it possible to differentiate mtDNA by means of HVIII in samples that cannot be distinguished by sequencing the HVI and HVII regions? Forensic Sci Int 113:97–101
10. Bini C, Ceccardi S, Luiselli D, Ferri G, Pelotti S, Colalongo C, Falconi M, Pappalardo G (2003) Different informativeness of the three hypervariable mitochondrial DNA regions in the population of bologna (Italy). Forensic Sci Int 135:48–52
11. Zhang YJ, Xu QS, Zheng ZJ, Lin HY, Lee JB (2005) Haplotype diversity in mitochondrial DNA hypervariable region I, II and III in northeast China Han. Forensic Sci Int 149:267–269
12. Carracedo A, Butler JM, Gusmão L, Parson W, Roewer L, Schneider PM (2010) Publication of population data for forensic purposes. Forensic Sci Int Genet 4:145–147
13. Fridman C, Gonzalez RS, Pereira AC, Cardena MM (2014) Haplotype diversity in mitochondrial DNA hypervariable region in a population of southeastern Brazil. Int J Legal Med 128:589–593
14. Ribeiro-dos-Santos AK, Carvalho BM, Feio-dos-Santos AC, dos Santos SE (2007) Nucleotide variability of HV-I in afro-descendents populations of the Brazilian Amazon region. Forensic Sci Int 167:77–80
15. Bernardo S, Hermida R, Desidério M, Silva DA, de Carvalho EF (2014) MtDNA ancestry of Rio de Janeiro population, Brazil. Mol Biol Rep 41:1945–1950
16. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD (2003) Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 100:177–182
17. Alves-Silva J, da Silva Santos M, Guimarães PE, Ferreira AC, Bandelt HJ, Pena SD, Prado VF (2000) The ancestry of Brazilian mtDNA lineages. Am J Hum Genet 67:444–461
18. Salzano FM, Freire-Maia N (1967) Populações Brasileiras, Aspectos Demográficos, Genéticos e Antropológicos. Companhia Editora Nacional, São Paulo
19. IBGE, Censo demográfico (2010) Fundação Instituto Brasileiro de Geografia e Estatística. www.ibge.gov.br. Accessed 25 May 2017
20. Wachowicz RC (1995) História do Paraná. Gráfica Vicentina, Curitiba
21. Brandstätter A, Peterson CT, Irwin JA, Mpoke S, Koech DK, Parson W, Parsons TJ (2004) Mitochondrial DNA control region sequences from Nairobi (Kenya): inferring phylogenetic parameters for the establishment of a forensic database. Int J Legal Med 118:294–306
22. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465
23. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147
24. Berger C, Hatzer-Grubwieser P, Hohoff C, Parson W (2011) Evaluating sequence derived mtDNA length heteroplasmy by amplicon size analysis. Forensic Sci Int Genet 5:142–145
25. van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:E386–E394
26. Röck AW, Dür A, van Oven M, Parson W (2013) Concept for estimating mitochondrial DNA haplogroups using a maximum likelihood approach (EMMA). Forensic Sci Int Genet 7:601–609
27. Parson W, Dür A. EMPOP – a forensic mtDNA database (2007) Forensic Sci Int Genet1:88–92
28. Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and windows. Mol Ecol Resour 10:564–567
29. Irwin JA, Saunier JL, Niederstätter H, Strouss KM, Sturk KA, Diegoli TM, Brandstätter A, Parson W, Parsons TJ (2009) Investigation of heteroplasmy in the human mitochondrial DNA control region: a synthesis of observations from more than 5000 global population samples. J Mol Evol 68:516–527
30. Barbosa AB, da Silva LA, Azevedo DA, Balbino VQ, Mauricio-da-Silva L (2008) Mitochondrial DNA control region polymorphism in the population of Alagoas state, north-eastern Brazil. J Forensic Sci 53:142–146
31. Palencia L, Valverde L, Alvarez A, Cainé LM, Cardoso S, Alfonso-Sánchez MA, Pinheiro MF, de Pancorbo MM (2010) Mitochondrial DNA diversity in a population from Santa Catarina (Brazil): predominance of the European input. Int J Legal Med 124:331–336
32. Sanches NM, Paneto GG, Figueiredo RF, de Mello AO, Cicarelli RM (2014) Mitochondrial DNA control region diversity in a population from Espirito Santo state, Brazil. Mol Biol Rep 41:6645–6648
33. Marrero AR, Silva-Junior WA, Bravi CM, Hutz MH, Petzl-Erler ML, Ruiz-Linares A, Salzano FM, Bortolini MC (2007) Demographic and evolutionary trajectories of the Guarani and Kaingang natives of Brazil. Am J Phys Anthropol 132:301–310
34. Pena SD, Carvalho-Silva DR, Alves-Silva J, Prado VF, Santos FR (2000) Retrato Molecular do Brasil. Ciência hoje 27:16–25
35. Mörner, M (1967) Race mixture in the history of Latin America. Little Brown & Company, Boston
36. Achilli A, Rengo C, Magri C, Battaglia V, Olivieri A, Scozzari R, Cruciani F, Zeviani M, Briem E, Carelli V, Moral P, Dugoujon JM, Roostalu U, Loogväli EL, Kivisild T, Bandelt HJ, Richards M, Villems R, Santachiara-Benerecetti AS, Semino O, Torroni A (2004) The molecular dissection of mtDNAhaplogroup H confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene pool. Am J Hum Genet 75:910–918
37. Marques SL, Goios A, Rocha AM, Prata MJ, Amorim A, Gusmão L, Alves C, Alvarez L (2015) Portuguese mitochondrial DNA genetic diversity—an update and a phylogenetic revision. Forensic Sci Int Genet 15:27–32
38. Bandelt HJ, Alves-Silva J, Guimaraes PE, Santos MS, Brehm A, Pereira L, Coppa A, Larruga JM, Rengo C, Scozzari R, Torroni A, Prata MJ, Amorim A, Prado VF, Pena SD (2001) Phylogeography of the human mitochondrial haplogroup L3e: a snapshot of African prehistory and Atlantic slave trade. Ann Hum Genet 65:549–563
39. Secher B, Fregel R, Larruga JM, Cabrera VM, Endicott P, Pestano JJ, González AM (2014) The history of the north African mitochondrial DNA haplogroup U6 gene flow into the African, Eurasian and American continents. BMC Evol Biol 14:109. https://doi.org/10.1186/1471-2148-14-109
40. Grignani P, Peloso G, Achilli A, Turchi C, Tagliabracci A, Alù M, Beduschi G, Ricci U, Giunti L, Robino C, Gino S, Previderè C (2006) Subtyping mtDNAhaplogroup H by SNaPshot minisequencing and its application in forensic individual identification. Int J Legal Med 120:151–156
41. Köhnemann S, Sibbing U, Pfeiffer H, Hohoff C (2008) A rapid mtDNA assay of 22 SNPs in one multiplex reaction increases the power of forensic testing in European Caucasians. Int J Legal Med 122:517–523
42. Achilli A, Perego UA, Bravi CM, Coble MD, Kong QP, Woodward SR, Salas A, Torroni A, Bandelt HJ (2008) The phylogeny of the four pan-American MtDNAhaplogroups: implications for evolutionary and disease studies. PLoS One 3:e1764
43. Bobillo MC, Zimmermann B, Sala A, Huber G, Röck A, Bandelt HJ, Corach D, Parson W (2010) Amerindian mitochondrial DNA haplogroups predominate in the population of Argentina: towards a first nationwide forensic mitochondrial DNA sequence database. Int J Legal Med 124:263–268
44. Goncalves FT, Cardena MMSG, Gonzalez RS, Krieger JE, Pereira AC, Fridman C (2011) The discrimination power of the hypervariable regions HV1, HV2 and HV3 of mitochondrial DNA in the Brazilian population. FSI: Genetics Supplement Series 3:e311–e312