TECHNICAL NOTE

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Mitochondrial DNA Control Region Polymorphism in the Population of Alagoas State, North-Eastern Brazil

ABSTRACT: The sequences of the two hypervariable (HV) segments of the mitochondrial DNA (mtDNA) control region were determined in 167 randomly selected, unrelated individuals living in the state of Alagoas, north-eastern Brazil. One hundred and forty-five different haplotypes, associated with 139 variable positions, were determined. More than 95% of the mtDNA sequences could be allocated to specific mtDNA haplogroups according to the mutational motifs. Length heteroplasmy in the C-stretch HV1 and HV2 regions was observed in 22 and 11%, respectively, of the population sample. The genetic diversity was estimated to be 0.9975 and the probability of two random individuals presenting identical mtDNA haplotypes was 0.0084. The most frequent haplotype was shared by six individuals. All sequences showed high-quality values and phantom mutations were not detected. The diversity revealed in the mitochondrial control region indicates the importance of this locus for forensic casework and population studies within Alagoas, Brazil.

KEYWORDS: forensic science, DNA typing, mitochondrial DNA, polymorphism, hypervariable regions, haplotypes, haplogroups, Alagoas state, Brazil

The human mitochondrial DNA (mtDNA) genome is a 16,5 kb molecule which has been fully sequenced (1) and contains two highly polymorphic segments (the so-called hypervariable regions, HV1 and HV2) that are located in the control, non-coding or displacement loop (D-loop) region (2,3). In forensic and anthropological studies, analysis of mtDNA is of particular importance when only degraded DNA is available, as hundreds to thousands of copies of mtDNA may be present per cell in contrast to the single copy of nuclear DNA (4). Moreover, the strictly maternal inheritance, the lack of recombination, and the high mutational rate of mtDNA, provide further compelling reasons for the use of mitochondrial sequences in forensic science (5), population studies (6), molecular evolution (7), anthropology (8), and archaeology (9).

In forensic casework, determined sequences are frequently compared with those available in the literature or held in reference mtDNA databases. It is recognized, however, that such data are prone to errors deriving from a number of sources, and must be subjected to stringent quality assurance and control procedures (10–12). It has recently been demonstrated by Bandelt et al. (13–15) that erroneous haplotypes can be detected in data using phylogenetic methods and by comparison with closely related sequences from other databases.

The population of Brazil constitutes one of the most heterogeneous in the world, as it results from interethnic crosses between Europeans, Africans, Amerindians, and Asians. In the north-eastern region of the country, the majority of the inhabitants are of mixed ancestry (16). The objective of the present study was to analyze the sequence data of the two hypervariable regions of mtDNA derived from 167 unrelated individuals living in the north-eastern state of Alagoas in order to construct a reference database for the area. Such a database would be of specific value in regional forensic analyses and could contribute to our understanding of the matrilineal genetic contributions of ethnic groups to the gene pool of the present-day mixed population.

Methods

Blood samples were collected from 167 randomly selected, maternally unrelated, volunteers living in the state of Alagoas, Brazil. DNA was extracted from aliquots (30 μL) of blood using the Chelex extraction procedure (17), and two hypervariable segments (HV1 and HV2) of the mtDNA control region were amplified by PCR using the primers and conditions described by Imaizumi et al. (18).

The PCR products were precipitated with isopropanol and loaded on 2% agarose gel. Template concentration was estimated by comparison of their band intensities with those of an Invitrogen (Carlsbad, CA) low DNA mass ladder. Purified products (*c.* 10 ng) were sequenced by cycle sequencing using an ABI PRISM[TM] 310 Genetic Analyzer and BigDye[TM] Terminator kit version 3.1 (Applied Biosystems, Foster City, CA). The sequences of the PCR amplicons were verified from both forward and reverse sequence data and DNA sequences from position 16024 to 16365 in HV1, and from 73 to 340 in HV2, were determined. Individuals exhibiting length heteroplasmy in HV1 or HV2 were resequenced with internal primers (18) to confirm the sequences beyond the heteroplasmic region.

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TABLE 1—*mtDNA haplotypes and their frequencies in a sample population from Alagoas state.*

Sample	F	Hp	Variations in HV1 + 16,000	Variations in HV2
AL01	1	A	111, 124, 223, 319	73, 146, 150, 152, 263, 315.1C
AL02	1	A	111, 172, 223, 290, 319, 362	73, 146, 153, 263, 309.1C, 315.1C
AL03	1	A	223, 290, 319, 362	73, 146, 152, 153, 235, 263, 315.1C
AL04	1	A	111, 129, 183C, 189, 223, 290, 319, 362	73, 146, 152, 153, 235, 263, 309.1C, 309.2C, 315.1C
AL05	1	A	111, 126, 223, 259, 266, 290, 319, 327, 362	73, 146, 153, 235, 263, 315.1C
AL06	1	A	111, 126, 223, 259, 290, 319, 362	73, 146, 153, 235, 263, 315.1C
AL07	1	A	111, 209, 223, 290, 319, 362	73, 146, 153, 210, 235, 263, 309.1C, 315.1C
AL08	1	A	111, 192, 209, 223, 290, 319, 362	73, 143, 146, 152, 153, 235, 263, 309.1C, 315.1C
AL09	1	A	111, 223, 290, 319, 362	73, 146, 153, 185T, 235, 263, 315.1C
AL10	6	A	111, 223, 290, 319, 362	73, 146, 153, 235, 263, 309.1C, 315.1C
AL11	1	A	111, 188, 223, 290, 319, 362	73, 146, 153, 235, 263, 315.1C
AL12	1	A	111, 223, 290, 291, 319, 362	73, 146, 153, 235, 236, 263, 309.1C, 309.2C, 315.1C
AL13	1	A	111, 223, 290, 291, 319, 362	73, 146, 153, 235, 236, 263, 309.1C, 315.1C
AL14	1	B	182C, 183C, 189, 217	73, 263, 309.1C, 315.1C
AL15	1	B	182C, 183C, 189, 217, 260, 316	73, 151, 263, 315.1C
AL16	1	B	182C, 183C, 186, 189, 217	73, 263, 315.1C
AL17	1	B	173, 182C, 183C, 189, 217, 223, 357	73, 263, 306–309D, 315.1C
AL18	1	B	051, 183C, 189, 217	73, 152, 263, 309.1C, 309.2C, 315.1C
AL19	1	B	183C, 189, 217, 241	73, 103A, 189, 263, 309.1C, 315.1C
AL20	1	B	182C, 183C, 189, 217, 241	73, 103A, 152, 263, 309.1C, 315.1C
AL21	2	B	183C, 189, 217	73, 263, 315.1C
AL22	1	B	178, 183C, 189, 217	73, 263, 309.1C, 315.1C
AL23	2	B	178, 183C, 189, 217	73, 263, 315.1C
AL24	1	B	183C, 189, 217, 311	73, 185, 263, 309.1C, 309.2C, 315.1C
AL25	1	B	145, 183C, 189, 217, 316, 319	73, 192, 263, 309.1C, 309.2C, 315.1C
AL26	1	B	129, 183C, 189, 217	73, 150, 263, 315.1C
AL27	2	B	183C, 189, 217	73, 150, 263, 315.1C
AL28	1	C	223, 292, 298, 325, 327, 362	73, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL29	1	C	223, 292, 298, 325, 327, 362	73, 249D, 263, 290D, 291D, 315.1C
AL30	1	C	223, 292, 298, 325, 327, 362	73, 151, 152, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL31	1	C	223, 292, 298, 325, 327, 362	73, 151, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL32	1	C	126, 223, 270, 298, 325, 327	73, 249D, 263, 290D, 291D, 309.1C, 309.2C, 315.1C
AL33	2	C	126, 223, 270, 298, 325, 327	73, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL34	1	C	126, 183C, 189, 223, 298, 325, 327	73, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL35	1	C	147, 223, 298, 325, 327	73, 249D, 263, 290D, 291D, 315.1C
AL36	1	C	051, 172, 223, 298, 325, 327	73, 194, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL37	1	C	051, 223, 298, 325, 327	73, 194, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL38	1	C	051, 223, 298, 311, 325, 327	73, 194, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL39	2	C	051, 184, 223, 287, 298, 311, 325, 327	73, 146, 194, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL40	1	C	051, 184, 223, 287, 298, 311, 325, 327	73, 146, 194, 249D, 263, 290D, 291D, 315.1C
AL41	1	C	051, 184, 223, 287, 298, 311, 325, 327, 357	73, 146, 194, 249D, 263, 290D, 291D, 309.1C, 315.1C
AL42	1	D	223, 325, 362	73, 263, 315.1C
AL43	1	D	142, 179, 189, 223, 295, 325, 362	73, 192, 198, 263, 309.1C, 309.2C, 315.1C
AL44	1	D	042, 145, 223, 325, 362	73, 195, 263, 315.1C
AL45	1	D	223, 278, 291, 325, 362	73, 195, 263, 315.1C
AL46	1	L1a	093, 129, 148, 168, 172, 187, 188G, 189, 223, 230, 278, 293, 311, 320	93, 95C, 152, 185, 189, 236, 247, 263, 315.1C
AL47	1	L1a	129, 148, 168, 172, 187, 188G, 189, 223, 230, 278, 293, 311, 320	93, 95C, 150, 185, 189, 236, 247, 263, 309.1C, 315.1C
AL48	1	L1b	126, 187, 189, 223, 264, 270, 278, 311	73, 152, 182, 185T, 195, 247, 263, 309.1C, 315.1C
AL49	1	L1b	126, 187, 189, 223, 264, 270, 278, 311	73, 152, 182, 185T, 195, 247, 263, 315.1C
AL50	1	L1b	126, 187, 189, 223, 264, 270, 278, 293, 311	73, 114, 152, 182, 185T, 189, 195, 247, 263, 315.1C
AL51	1	L1b	126, 187, 189, 223, 264, 270, 278, 293, 311	73, 152, 182, 185T, 195, 247, 263, 315.1C
AL52	1	L1c	093, 129, 183C, 189, 215, 223, 278, 294, 311, 355, 360	73, 151, 152, 182, 186A, 189, 192, 247, 263, 309.1C, 315.1C, 316
AL53	1	L1c	093, 129, 183C, 189, 215, 223, 278, 294, 311, 360	73, 151, 152, 182, 186A, 189, 247, 263, 309.1C, 315.1C, 316
AL54	1	L1c	129, 182C, 183C, 189, 215, 223, 278, 294, 311, 360	73, 152, 182, 186A, 189, 247, 263, 309.1C, 315.1C, 316
AL55	2	L1c	093, 184, 187, 189, 223, 278, 294, 301, 311, 360	73, 94.1G, 152, 182, 186A, 189, 195, 247, 263, 297, 309.1C, 315.1C, 316
AL56	1	L1c	093, 129, 184, 187, 189, 223, 278, 294, 301, 311, 360	73, 94.1G, 152, 182, 186A, 189, 195, 247, 263, 297, 309.1C, 315.1C, 316
AL57	3	L1c	129, 189, 223, 274, 278, 293, 294, 311, 360	73, 89, 93, 95C, 152, 182, 186A, 189, 236, 247, 263, 297, 315.1C, 316
AL58	1	L1c	129, 187, 189, 223, 278, 293, 294, 311, 360	73, 151, 152, 182, 186A, 189, 247, 263, 265, 315.1C, 316
AL59	1	L1c	129, 187, 189, 223, 278, 293, 294, 311, 360	73, 93, 151, 152, 182, 186A, 189, 195, 247, 248, 263, 309.1C, 315.1C, 316
AL60	1	L1c	093, 129, 187, 189, 223, 263, 278, 293, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 297, 315.1C 316
AL61	1	L1c	086, 129, 187, 189, 223, 241, 278, 293, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 297, 315.1C, 316
AL62	1	L1c	129, 187, 189, 270, 278, 293, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 247, 263, 297, 315.1C, 316
AL63	1	L1c	129, 187, 189, 223, 265C, 278, 286G, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 247, 263, 297, 315.1C, 316
AL64	1	L1c	129, 169, 187, 189, 223, 265C, 278, 286G, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 297, 309.1C, 315.1C, 316
AL65	1	L1c	093, 129, 187, 189, 223, 265C, 278, 286G, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 264, 297, 315.1C, 316
AL66	1	L1c	129, 134, 145, 187, 189, 213, 223, 265T, 274, 278, 286G, 294, 311, 360	73, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 297, 315.1C, 316

TABLE 1—*Continued*

Sample	F	Hp	Variations in HV1 + 16,000	Variations in HV2
AL67	1	L1c	071, 129, 145, 187, 189, 213, 223, 234, 265C, 278, 286G, 294, 304, 311, 360	73, 146, 151, 152, 182, 186A, 189, 195, 198, 247, 263, 297, 309.1C, 315.1C, 316
AL68	1	L2a	071, 189, 192, 223, 278, 294, 309	73, 146, 152, 195, 263, 309.1C, 315.1C
AL69	1	L2a	189, 192, 223, 278, 294, 309	73, 146, 152, 195, 263, 309.1C, 315.1C
AL70	1	L2a	189, 192, 223, 245, 278, 294, 309	73, 143, 146, 152, 263, 309.1C, 315.1C
AL71	1	L2a	189, 223, 278, 294, 309	73, 146, 152, 192, 198, 263, 315.1C
AL72	1	L2a	189, 192, 223, 278, 294, 309	73, 146, 152, 192, 263, 309.1C, 315.1C
AL73	1	L2a	182C, 183C, 189, 223, 278, 290, 294, 309	73, 146, 152, 192, 263, 315.1C
AL74	1	L2a	131, 183C, 189, 223, 225, 234, 278, 294, 309	73, 146, 152, 192, 263, 315.1C
AL75	1	L2a	131, 183C, 189, 223, 225, 234, 278, 294	73, 146, 152, 192, 263, 315.1C
AL76	1	L2a	126, 223, 278, 286, 294, 309	73, 146, 152, 195, 263, 315.1C
AL77	1	L2a	192, 223, 278, 294	73, 143, 146, 152, 195, 263, 309.1C, 315.1C
AL78	1	L2a	223, 278, 294, 309	73, 143, 146, 152, 195, 263, 315.1C
AL79	1	L2a	037, 093, 223, 278, 294, 309, 357	73, 143, 146, 152, 195, 263, 315.1C
AL80	1	L2b	114A, 129, 213, 223, 278, 354	73, 146, 150, 152, 182, 195, 198, 204, 263, 309.1C, 315.1C
AL81	1	L2b	114A, 129, 213, 223, 274, 278	73, 146, 150, 152, 182, 183, 195, 198, 204, 263, 309.1C, 315.1C
AL82	1	L2b	114A, 129, 213, 223, 278	73, 146, 150, 152, 182, 195, 198, 204, 207, 263, 309.1C, 309.2C, 315.1C
AL83	1	L2b	114A, 129, 213, 223, 278, 325, 355	73, 150, 152, 182, 195, 198, 204, 263, 309.1C, 315.1C
AL84	1	L2b	114A, 129, 213, 223, 278, 355, 362	73, 150, 152, 182, 195, 198, 204, 249D, 263, 315.1C
AL85	1	L2c	223, 278	73, 89, 93, 146, 150, 182, 195, 198, 263, 315.1C, 325
AL86	1	L2c	223, 264, 278	73, 93, 146, 150, 152, 182, 195, 198, 263, 315.1C, 325
AL87	1	L2c	114, 223, 264, 278, 292, 362	73, 146, 150, 152, 182, 195, 198, 263, 309.1C, 315.1C, 325
AL88	2	L3b	124, 183C, 189, 223, 278, 304, 311	73, 152, 263, 315.1C
AL89	1	L3e1	189, 223, 311, 327	73, 150, 189, 200, 204, 214, 263, 309.1, 315.1C
AL90	1	L3e1	189, 223, 311, 327	73, 150, 189, 200, 204, 263, 309.1C, 315.1C
AL91	1	L3e1	185, 189, 223, 311, 327	73, 150, 185, 189, 200, 263, 315.1C
AL92	1	L3e1	185, 223, 311, 327	73, 150, 185, 189, 200, 263, 315.1C
AL93	1	L3e1	223, 287, 311, 327	73, 150, 189, 200, 263, 309.1C, 315.1C
AL94	2	L3e1	185, 223, 327	73, 150, 189, 200, 263, 309.1C, 315.1C
AL95	1	L3e1	185, 209, 223, 327	73, 150, 152, 189, 195, 200, 207, 263, 309.1C, 315.1C
AL96	1	L3e1	129, 185, 209, 223, 327	73, 150, 152, 189, 195, 200, 207, 263, 309.1C, 315.1C
AL97	1	L3e1	186, 223, 327, 355	73, 150, 189, 200, 263, 309.1C, 315.1C
AL98	1	L3e1	223, 327	73, 150, 189, 200, 263, 309.1C, 315.1C
AL99	1	L3e1	223, 327	73, 150, 189, 200, 263, 315.1C
AL100	1	L3e1	223, 325D, 327	73, 150, 185, 189, 263, 309.1C, 315.1C
AL101	1	L3e2	223, 254, 320	73, 150, 195, 198, 263, 315.1C
AL102	1	L3e2	223, 320	73, 150, 195, 198, 263, 309.1C, 315.1C
AL103	1	L3e2	223, 320	73, 150, 195, 263, 315.1C
AL104	1	L3e2	223, 258T, 320	73, 150, 189, 195, 263, 315.1C
AL105	1	L3e2b	126, 172, 182C, 183C, 189, 223, 320	73, 150, 192, 263, 315.1C
AL106	1	L3e2b	172, 183C, 189, 223, 320	73, 150, 263, 309.1C, 315.1C
AL107	1	L3e3	093, 223, 265T, 316	73, 150, 195, 263, 315.1C
AL108	1	L3f	209, 223, 311	73, 150, 189, 200, 263, 315.1C
AL109	1	L3f	209, 218, 223, 256, 292, 311	73, 150, 189, 263, 315.1C
AL110	1	L3f	093, 129, 209, 223, 292, 295, 311	73, 189, 195, 200, 263, 272, 309.1C, 315.1C
AL111	2	L3f	129, 209, 223, 292, 295, 311	73, 189, 200, 263, 309.1C, 309.2C, 315.1C
AL112	1	L3f	129, 209, 223, 292, 295, 311	73, 189, 200, 263, 309.1C, 315.1C
AL113	1	H	192	73, 263, 309.1C, 315.1C
AL114	1	H	240	199, 263, 315.1C
AL115	1	H	362	239, 263, 309.1C, 309.2C, 315.1C
AL116	2	H	362	239, 263, 309.1C, 315.1C
AL117	1	H	311, 362	239, 263, 309.1C, 315.1C
AL118	1	H	311	263, 309.1C, 309.2C, 315.1C
AL119	2	H		152, 263, 315.1C
AL120	1	H	129	263, 315.1C
AL121	1	H		194, 263, 315.1C
AL122	3	H		263, 315.1C
AL123	2	H		263, 309.1C, 315.1C
AL124	1	H	293	263, 309.1C, 315.1C
AL125	1	J	069, 126, 193, 319, 360	73, 150, 152, 263, 295, 309.1C, 315.1C
AL126	1	J	069, 126	73, 185, 188, 263, 295, 315.1C
AL127	1	T	126, 294, 296, 304, 318C	263, 315.1C
AL128	1	T	126, 294, 296, 304	73, 195, 263, 309.1C, 315.1C
AL129	1	T	126, 292, 294, 296	73, 146, 263, 309.1C, 315.1C
AL130	1	T	126, 163, 186, 189D, 294	73, 263, 309.1C, 315.1C
AL131	2	U3	343, 356	73, 150, 263, 315.1C
AL132	1	U3	343	73, 150, 263, 309.1C, 315.1C
AL133	1	U5	167, 192, 270, 311, 318, 356	73, 150, 263, 309.1C, 315.1C
AL134	1	U5	189, 270	73, 150, 263, 315.1C
AL135	1	U6	172, 219, 235, 278, 355	73, 146, 263, 309.1C, 315.1C
AL136	1	U6	172, 184, 219, 234, 278	73, 199, 263, 309.1C, 315.1C
AL137	1	Pre V	187, 298, 311	150, 186, 263, 309.1C, 315.1C

TABLE 1—*Continued*

Sample	F	Hp	Variations in HV1 + 16,000	Variations in HV2
AL138	1	V	124, 298, 319	263, 309.1C, 315.1C
AL139	1	V	298	263, 315.1C
AL140	1	W	223, 292	73, 189, 194, 199, 204, 207, 263, 315.1C
AL141	1	W	223, 292	73, 189, 194, 199, 204, 207, 263, 309.1C, 315.1C
AL142	1	?	124, 223, 362	73, 152, 195, 263, 309.1C, 315.1C
AL143	1	?	124, 223, 311	73, 152, 263, 309.1C, 315.1C
AL144	1	?	124, 223, 256	73, 146, 152, 263, 309.1C, 315.1C
AL145	1	?	129, 145, 176G, 223, 291	73, 152, 185, 188, 263, 315.1C

F: frequency; Hp: haplogroups. ?: haplotype not classified.

Sequences were aligned and compared with the revised Cambridge Reference Sequence (rCRS) (19) using Applied Biosystems SeqScape software (version 2.5). The mtDNAs were initially classified into haplogroups on the basis of search and directed comparison with the published HV1 motifs of haplogroup-specific sequences (16,20–22). In order to further characterize the mtDNA lineage assigned, the corresponding HV2 motifs were also compared. The filtering process described by Bandelt et al. (14) for HV1 data was applied to the experimental data set to ensure that its accuracy had not been compromised through the production of phantom mutations. This analysis filters out the speedy mutations leaving only the weighty variations. When the data are potentially free of phantom mutations, the resulting weighty network exhibits a perfect tree pattern. All suspicious sequences indicated following application of the filter were resequenced according to the guidelines of Bandelt et al. (13). The Mitomap database (http://www.mitomap.org/) was used as vehicle to search for single mutational variants described in the literature.

Genetic diversity (h) was calculated from the formula $h = (1 - \sum x^2)n/(n - 1)$, where $\sum x^2$ is the sum of squares of the haplotype frequencies and n is the sample size (23). Estimates of genetic diversity, haplotype frequencies, nucleotide diversity, and pair-wise differences were computed using the program Arlequin version 3.0 (24). The probability (p) of a random match was established from the simple identity $p = \sum x^2$ according to Stoneking et al. (25).

Results and Discussion

The sequences of the mtDNA hypervariable regions were determined in 167 randomly selected, unrelated individuals living in the state of Alagoas, Brazil. A total of 145 different haplotypes were revealed and these were associated with 139 variable positions between nucleotide positions 16024–16365 in HV1 and 73–340 in HV2.

The HV1/HV2 segments were then compared with closely related sequences from other databases to improve data quality. More than 95% of mtDNA samples could be allocated to specific mtDNA haplogroups on the basis of HV1/2 motifs without recourse to extra information concerning the coding region sequences. Some 2% of the haplotypes present in the data set could not, however, be classified into haplogroups. The overall results, arranged according to haplogroups, are presented in Table 1.

Length heteroplasmy in the C-stretch region was observed in mtDNA HV1 segments of 22% (37/167) of the individuals studied. Length heteroplasmy, which occurred exclusively in HV1 variants exhibiting T16189C polymorphism, was easily identified because of the dramatic decrease in sequence quality (caused by out-of-register templates) beyond the heteroplasmic region (26). The discrimination of length heteroplasmy in HV2 is significantly more difficult as heteroplasmic ratios vary considerably between individuals (27). However, amongst the 167 mtDNA samples sequenced, 11% (19/167) were considered to be heteroplasmic for HV2.

The genetic diversity was estimated to be 0.9975 and the probability of two random individuals showing identical mtDNA haplotypes was determined at 0.84% (Table 2). The most frequent haplotypes revealed in this study were 16111–16223–16290, 16319–16362–73–146–153–235–263–309.1C-315.1C (haplogroup A) found in 6 individuals and 16129–16189–16223–16274–16278–16293–16294–16311–16360–73–89–93–95C–152–182–186A–189–236–247–263–297–315.1C–316 (haplogroup L1C) and 263–315.1C (haplogroup H) found both in three individuals. The 13 haplotypes that were each shared by two individuals belonged to haplogroups B, C, L1c, L3b, L3e1, L3f, H, and U (Table 1). In the sample population studied, single nucleotide polymorphism occurred most frequently at nucleotide positions 263, 315.1, 73, 16223, and 309.1.

The weighty network displayed little reticulation (data not shown). In accordance with accepted guidelines, however, the doubtful transversions, namely, 16188G (AL46) and 16318C (AL127), were resequenced and exhibited high-quality values (QV) of 47 and 48, respectively. The suspicious sequences AL135, 136 and AL39, 40 were also resequenced and confirmed the transitions 16219 and 16184 in both strands with QVs of 39 and 41, respectively. Another reticulation involving the transition 16215 and the transversion

TABLE 2—*Genetic diversity and statistical parameters in a sample of 123 individuals from Alagoas state.*

Parameter	HV1	HV2	HV1 + HV2
Number of haplotypes	124	99	145
Number of polymorphic sites	90	49	139
Transitions	85	38	123
Transversions	10	4	14
Insertions	0	4	4
Deletions	2	7	9
Genetic diversity	0.9934 ± 0.0020	0.9902 ± 0.0019	0.9975 ± 0.0012
Random match probability	0.012534	0.015761	0.008437
Nucleotide diversity	0.024291 ± 0.012517	0.024475 ± 0.012849	0.024373 ± 0.012124
Mean number of pair-wise differences	8.307409 ± 3.867848	6.657312 ± 3.157779	14.964721 ± 6.726241

16182 in the sequences AL52, AL54, and AL16 was also checked. These mutations have already been recorded in the Mitomap database, indicating that these may be real phenomena rather than artefacts introduced in the course of the sequencing process.

One substitution in HV2, 265 (AL58), had not been previously recorded in the Mitomap database but resequencing of the sequence confirmed this site. Mutations at the HV1/HV2 positions recognized as being the most vulnerable to artefact formation (28) were not found in our data, with the single exception of site 16320. This mutation was checked thoroughly in the electropherograms and there were no indications of ambiguity or of weak-base calling.

A comparison of the distribution of haplogroups in the studied population from the state of Alagoas with published data for a population from the north-eastern state of Pernambuco (16) revealed some differences, particularly associated with European lineage. Thus, the lineage values associated with African, Native American, and European ancestries for the general Alagoas sample were 44%, 33%, and 21%, respectively, in contrast to those of 44%, 22%, and 34%, respectively, for a sample from Pernambuco. However, these differences were expected as Alves-Silva et al. (16) analyzed individuals classified as "white" and belonging to the middle class in north-eastern Brazil.

In conclusion, the results obtained confirm that sequence polymorphism of the control region of human mtDNA could be highly informative in forensic casework. Moreover, the application of phylogenetic methods to our data and comparison with closely related sequences will provide valuable information for forensic and population genetic analyses in the mixed population of Alagoas.

Electronic-Database Information

All the sequences and electropherograms are available on the site http://www.labdnaforense.org/sequencias.php

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