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Forensic Science International 151 (2005) 307-313

www.elsevier.com/locate/forsciint

# Announcement of population data

# Population data of Y-STR minimal haplotypes in a sample of Caucasian-Mestizo and African descent individuals of Colombia

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Received 23 October 2004; accepted 2 February 2005 Available online 5 March 2005

Keywords: Y chromosome; STR; Haplotypes; Colombia; Forensic genetics; Population genetics

#### 1. Material and methods

Population: One hundred and thirty four (134) unrelated Afro-Colombian individuals collected in four different towns (Quibdo, the state capital, Condoto, Istmina, and Tado) of the Chocó department in the Pacific region of Colombia and 137 unrelated Caucasian-Mestizo individuals from the East-central Andean region of Colombia (Cundinamarca, Boyacá, and Santander departments as well as Bogotá, the capital city).

Extraction: Genomic DNA was extracted by the Quick light DNA isolation kit (Lifecodes Corporation) following manufacturers recommendations or by Chelex 100 as described previously [1].

PCR: The tetranucleotide STR DYS19, DYS385, DYS389-I, DYS389-II, DYS390, DYS391 and DYS393 and the trinucleotide DYS392 were analyzed as follow: primers sequences for DYS19, DYS390, DYS391 and DYS392 were those described previously (see tables of refs. [2,3]). PCR amplifications were carried out as single loci for DYS19, and DYS392 with identical conditions as described

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by Kayser et al. [3]. A multiplex reaction was performed for DYS390, DYS391 and DYS393 as follow: Initial denaturing step at 94 °C for 3 min; five cycles of 94 °C for 15 s, 58 °C for 20 s, 72 °C for 20 s, and 30 cycles of 94 °C for 15 s; 54 °C for 20 s; 72 °C for 20 s. For DYS389-I and DYS389-II, Fluorescent labeled primers (FITC) and amplification conditions were identical as described by Schultes et al. [4]. For DYS385, Fluorescent labeled primers (TMR) and amplification conditions were identical as described by Schneider et al. [5].

Typing: The PCR products were resolved in 4% Acrylamide/bis-acrylamide denaturing gels for DYS19, DYS390, DYS391, DYS392 and DYS393 with silver nitrate staining. For DYS389 and DYS385, typing was carried out in 4% acrylamide-bis-acrylamide denaturing gels and analyzed in a Hitachi FMBIO II with Internal Lane Standards (CXR 400).

Allele designations were made according to ystr.org with the aid of sequenced allelic ladders provided by Dr. Angel Carracedo, Instituto de Medicina Legal, Universidad de Santiago de Compostela, Galicia España and Dr. Daniel Corach, Servicio de Huellas Digitales Genéticas, Universidad de Buenos Aires, Buenos Aires Argentina and from reference samples provided by Dr. Lutz Roewer, Institute of Legal Medicine, Medical Faculty (Charite), Humboldt-University, Berlin, Germany.

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Table 1 AMOVA pairwise distance based on  $\Phi_{st}$  values between the Colombian Caucasian-Mestizo and Afro-Colombian populations groups with some African, African descent and Latin American populations

	$\phi_{\rm St}$	p-value
Afro-Colombian vs.		
Afro-USA	-0.00319	0.90042
Surinam	0.01405	0.07981
Equatorial Guinea	0.02345	0.00983
Angola (Cabinda)	0.04188	0.00266
Mozambique	0.04156	0.00049
Pygmies	0.09834	0.00029
Colombia Caucasian	0.26659	0.00000
Colombian Caucasian-Mestiz	o vs.	
Medellín	-0.00064	0.4738
Buenos Aires	0.00612	0.12354
El Salvador	0.02647	0.00105
Afro-Colombian	0.26659	0.00000

Quality control: Proficiency testing has been carried out for ystr.charite.de, GEP-ISFG WG, and Collaborative Testing Service (Sterling, VA). Laboratory accredited ISO 17025:2001 and certified ISO 9001:2000.

### 2. Data analysis

Haplotype frequencies, haplotype diversity, haplotype diversity S.E. and an analysis of molecular variance (AMOVA) in the form of  $\Phi_{st}$  values and the statistical significance (100.000 permutations) were determined with the aid of ARLEQUIN [6]. For population comparisons, the DYS385 locus was excluded from the analysis. The AMOVA analysis was carried out using the published data for Mozambique (n = 112) [7], Equatorial Guinea (n = 101) [8], El Salvador (n = 121) [9], Cabinda-Angola (n = 75) (unpublished data kindly provided by Sandra Beleza and

Table 2
Eight loci Y-STR haplotype frequencies in African descent individuals from Colombia

Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
HNI	1	13	12	30	25	10	11	13	17, 18	0.0075
HN2	1	13	12	30	21	10	15	13	15, 16	0.0075
HN3	1	13	13	30	24	10	14	14	14, 17	0.0075
HN4	1	13	13	30	23	9	14	13	14, 16	0.0075
HN5	1	13	13	31	24	10	14	13	15, 15	0.0075
HN6	1	13	14	30	24	9	11	13	13, 14	0.0075
HN7	1	13	14	30	24	11	14	13	13, 18	0.0075
HN8	1	13	14	31	24	10	15	13	15, 17	0.0075
HN9	1	13	14	33	21	10	11	14	17, 18	0.0075
HN10	2	14	12	27	24	10	11	12	16, 17	0.0149
HN11	1	14	12	28	24	10	11	13	14, 14	0.0075
HN12	1	14	12	28	24	10	11	12	16, 17	0.0075
HN13	1	14	12	28	25	10	11	13	14, 14	0.0075
HN14	1	14	12	29	24	10	11	13	17, 18	0.0075
HN15	1	14	12	29	22	10	11	12	15, 15	0.0075
HN16	1	14	12	29	26	11	13	13	11, 14	0.0075
HN17	1	14	13	27	23	11	11	13	11, 14	0.0075
HN18	1	14	13	29	23	10	11	12	12, 17	0.0075
HN19	2	14	13	29	23	10	11	12	13, 19	0.0149
HN20	1	14	13	29	24	10	13	13	11, 14	0.0075
HN21	1	14	13	29	24	10	13	13	11, 15	0.0075
HN22	1	14	13	29	24	10	13	13	15, 16	0.0075
HN23	1	14	13	29	24	10	13	13	11, 16	0.0075
HN24	1	14	13	29	24	11	13	13	11, 15	0.0075
HN25	1	14	13	29	24	11	13	13	11, 14	0.0075
HN26	1	14	13	29	24	11	14	13	13, 17	0.0075
HN27	1	14	13	29	24	11	13	12	11, 13	0.0075
HN28	1	14	13	29	24	11	13	14	12, 14	0.0075
HN29	2	14	13	29	25	11	13	13	11, 14	0.0149
HN30	1	14	13	30	22	10	11	14	16, 17	0.0075
HN31	3	14	13	30	23	10	11	12	13, 15	0.0224
HN32	1	14	13	30	23	10	13	13	11, 14	0.0075
HN33	1	14	13	30	24	10	11	11	13, 14	0.0075
HN34	1	14	13	30	25	11	12	13	11, 14	0.0075
HN35	1	14	13	31	24	10	11	13	14, 18	0.0075
HN36	1	14	14	29	22	10	12	13	11, 12	0.0075

Table 2 (Continued)

Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
A CONTRACTOR	1	2012	Tanga T	2000 Pro 2000 Pro 2000 Pro	200	1100	100000		100 7000	TO THE REST OF THE PARTY OF THE
HN37	1	14	14	30 30	24	11	13 13	13 13	11, 13 12, 14	0.0075 0.0075
HN38	1	14	14	30	24 24	11	13	13	11, 15	0.0075
HN39	1	14	14	30	24	11	13	13	11, 13	0.0075
HN40	1	14	14		23		13	12		0.0075
HN41	1	14	14	30		11	11	14	16, 16	0.0075
HN42	1	14	14	31	20	12		13	17, 18	0.0075
HN43	1	15	12	28	24	10	11		13, 17	
HN44	1	15	12	28	22	10	11	12	15, 15	0.0075
HN45	1	15	12	29	22	10	11	13	14, 15	0.0075
HN46	1	15	13	27	24	10	11	14	12, 13	0.0075
HN47	1	15	13	29	21	9	11	14	17, 17	0.0075
HN48	1	15	13	30	21	9	11	14	17, 17	0.0075
HN49	1	15	13	29	22	11	11	13	11, 12	0.0075
HN50	1	15	13	30	21	10	11	15	16, 17	0.0075
HN51	1	15	13	30	21	10	11	13	15, 15	0.0075
HN52	1	15	13	30	21	10	11	14	16, 16	0.0075
HN53	3	15	13	30	21	10	11	13	16, 17	0.0224
HN54	3	15	13	30	21	10	11	14	13, 17	0.0224
HN55	1	15	13	30	21	10	11	14	16, 18	0.0075
HN56	2	15	13	30	21	11	11	15	16, 18	0.0149
HN57	1	15	13	30	21	11	11	13	17, 17	0.0075
HN58	1	15	13	30	22	10	11	14	16, 17	0.0075
HN59	3	15	13	30	24	10	11	14	12, 13	0.0224
HN60	1	15	13	31	19	10	10	13	16, 18	0.0075
HN61	1	15	13	31	21	10	11	13	15, 17	0.0075
HN62	1	15	13	31	21	10	11	13	17, 17	0.0075
HN63	1	15	13	31	21	10	11	13	16, 16	0.0075
HN64	1	15	13	31	21	10	11	13	16, 17	0.0075
HN65	1	15	13	31	21	10	11	13	15, 18	0.0075
HN66	1	15	13	31	21	10	11	14	15, 19	0.0075
HN67	1	15	13	31	21	11	11	13	16, 16	0.0075
HN68	1	15	13	31	21	11	11	13	17, 17	0.0075
HN69	1	15	13	32	21	11	11	13	16, 17	0.0075
HN70	1	15	14	29	21	10	11	14	14, 14	0.0075
HN71	1	15	14	30	22	10	11	14	16, 17	0.0075
HN72	1	15	14	30	22	11	11	12	14, 15	0.0075
HN73	3	15	14	31	21	11	11	13	16, 18	0.0224
HN74	1	15	14	31	23	11	13	13	11, 16	0.0075
HN75	1	15	14	31	24	10	13	13	14, 14	0.0075
HN76	1	15	14	32	21	10	11	13	17, 17	0.0075
HN77	1	15	14	32	21	10	11	14	16, 16	0.0075
HN78	1	15	14	32	24	11	13	13	14, 14	0.0075
HN79	1	15	14	32	21	10	11	14	17, 19	0.0075
HN80	1	16	12	30	22	10	12	12	13, 13	0.0075
HN81	1	16	13	30	21	9	11	15	17, 17	0.0075
HN82	1	16	13	30	21	10	11	13	16, 16	0.0075
HN83	1	16	13	30	21	10	12	15	18, 20	0.0075
HN84	1	16	13	30	21	10	11	14	13, 15	0.0075
HN85	2	16	13	30	22	11	11	14	15, 17	0.0149
HN86	1	16	13	30	24	10	14	14	11, 15	0.0075
HN87	1	16	13	31	24	10	11	13	11, 11	0.0075
HN88	i	16	13	31	21	10	11	13	16, 17	0.0075
HN89	1	16	13	31	21	10	11	14	14, 14	0.0075
HN90	i	16	13	31	21	10	11	15	16, 17	0.0075
HN91	1	16	13	31	21	10	11	13	15, 18	0.0075
HN92	1	16	14	31	21	10	11	14	17, 18	0.0075
	3	16	14	31	21	10	11	15	18, 18	0.0224
HN93	1		14	31	22	10	11	13	15, 17	0.0075
HN94	1	16	14	31						
HN95		16	14	32	21	10	11	14	17, 19	0.0075

Table 2 (Continued)

Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
HN96	1	16	14	33	21	10	11	13	16, 16	0.0075
HN97	1	17	12	28	21	11	11	14	17, 19	0.0075
HN98	1	17	12	30	22	10	12	12	13, 13	0.0075
HN99	1	17	12	30	21	10	11	13	16, 17	0.0075
HN100	1	17	12	31	22	10	12	12	14, 17	0.0075
HN101	2	17	13	28	23	10	11	13	12, 12	0.0149
HN102	1	17	13	30	21	10	11	14	11, 19	0.0075
HN103	1	17	13	30	21	10	12	14	16, 19	0.0075
HN104	1	17	13	29	21	10	11	15	16, 19	0.0075
HN105	1	17	13	30	21	10	11	15	17, 19	0.0075
HN106	1	17	13	30	21	11	11	14	18, 18	0.0075
HN107	6	17	13	30	21	11	11	14	17, 19	0.0448
HN108	1	17	14	30	26	10	11	13	10, 14	0.0075
HN109	2	17	14	31	21	10	11	15	16, 18	0.0149
HN110	1	17	14	32	21	10	11	15	16, 18	0.0075

Table 3
Eight loci Y-STR haplotype frequencies in Caucasian-Mestizo individuals from Colombia

Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
HC1	1	13	11	29	24	10	14	14	12, 19	0.0073
HC2	1	13	12	29	23	10	11	12	16, 17	0.0073
HC3	1	13	12	29	24	10	11	13	14, 18	0.0073
HC4	1	13	13	29	22	9	15	13	14, 16	0.0073
HC5	1	13	13	29	23	10	12	13	16, 16	0.0073
HC6	1	13	13	29	23	10	13	13	14, 14	0.0073
HC7	1	13	13	29	24	10	15	13	14, 16	0.0073
HC8	1	13	13	29	24	10	14	13	11, 14	0.0073
HC9	1	13	13	29	24	10	11	13	13, 14	0.0073
HC10	1	13	13	29	24	10	14	14	15, 18	0.0073
HC11	1	13	12	29	24	11	13	14	11, 14	0.0073
HC12	1	13	13	29	24	11	11	13	13, 14	0.0073
HC13	1	13	13	29	24	11	14	13	11, 14	0.0073
HC14	1	13	13	29	25	10	15	13	14, 16	0.0073
HC15	1	13	13	30	23	10	11	13	16, 20	0.0073
HC16	1	13	13	30	24	11	11	13	16, 18	0.0073
HC17	1	13	13	30	24	10	11	13	18, 18	0.0073
HC18	1	13	13	30	24	10	11	13	17, 17	0.0073
HC19	1	13	13	30	24	10	11	13	16, 16	0.0073
HC20	1	13	13	30	25	10	11	14	14, 18	0.0073
HC21	1	13	13	31	23	10	11	12	16, 17	0.0073
HC22	1	13	13	31	24	10	13	15	13, 13	0.0073
HC23	1	13	13	31	25	9	11	13	16, 16	0.0073
HC24	1	13	13	32	23	10	14	12	14, 19	0.0073
HC25	1	13	13	32	23	10	14	13	12, 18	0.0073
HC26	1	13	14	30	24	9	11	13	13, 14	0.0073
HC27	1	13	14	30	24	9	11	13	14, 14	0.0073
HC28	1	13	15	31	25	10	15	13	15, 17	0.0073
HC29	1	14	12	28	22	10	11	13	13, 14	0.0073
HC30	1	14	12	28	23	11	13	13	12, 14	0.0073
HC31	1	14	12	28	24	11	13	13	11, 14	0.0073
HC32	1	14	12	28	24	11	14	13	12, 14	0.0073
HC33	1	14	12	28	25	12	13	13	11, 14	0.0073
HC34	1	14	12	29	22	10	11	13	14, 14	0.0073
HC35	1	14	12	29	24	10	13	13	11, 14	0.0073
HC36	1	14	12	29	24	11	13	13	11, 14	0.0073
HC37	1	14	12	30	24	10	11	12	13, 16	0.0073
HC38	1	14	12	30	23	10	15	13	15, 17	0.0073

Table 3 (Continued)

Table 3 (Continued)										
Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
HC39	1	14	13	28	23	10	11	12	14, 15	0.0073
HC40	1	14	13	28	23	10	13	13	11, 14	0.0073
HC41	1	14	13	29	23	10	13	13	11, 14	0.0073
HC42	2	14	13	29	23	10	13	13	12, 13	0.0146
HC43	1	14	13	29	23	11	13	13	11, 15	0.0073
HC44	1	14	13	29	24	9	13	13	11, 14	0.0073
HC45	4	14	13	29	24	10	13	13	11, 14	0.0292
HC46	1	14	13	29	24	10	13	13	12, 14	0.0073
HC47	1	14	13	29	24	10	13	13	11, 15	0.0073
HC48	1	14	13	29	24	10	14	13	11, 15	0.0073
HC49	1	14	13	29	24	10	14	12	11, 15	0.0073
HC50	1	14	13	29	24	11	12	13	11, 14	0.0073
HC51	5	14	13	29	24	11	13	13	11, 14	0.0365
HC52	1	14	13	29	24	11	14	13	11, 15	0.0073
HC53	1	14	13	29	24	11	13	13	12, 13	0.0073
HC54	2	14	13	29	24	11	13	13	11, 13	0.0146
HC55	2	14	13	29	24	11	13	13	10, 14	0.0146
HC56	1	14	13	29	24	11	13	13	11, 17	0.0073
HC57	1	14	13	29	24	11	13	13	12, 14	0.0073
HC58	1	14	13	29	25	11	14	13	11, 12	0.0073
HC59	1	14	13	29	25	10	13	13	11, 15	0.0073
HC60	1	14	13	29	25	11	13	12	11, 14	0.0073
HC61	1	14	13	29	25	11	13	13	11, 13	0.0073
HC62	1	14	13	30	23	9	13	12	18, 19	0.0073
HC63	1	14	13	30	23	10	13	13	11, 14	0.0073
HC64	1	14	13	30	23	11	11	13	11, 18	0.0073
HC65	1	14	13	30	23	10	13	12	11, 14	0.0073 0.0073
HC66	1	14	13	30	24	10	13	13	11, 15 11, 14	0.0073
HC67	1	14	13	30	24	10	14 15	14 12	14, 16	0.0073
HC68	1	14	13	30	24	10 11	13	13	11, 14	0.0073
HC69	1	14	13	30	24 24	11	13	13	14, 15	0.0073
HC70	1	14	13	30 31	23	10	11	12	13, 18	0.0073
HC71	1	14	13 13	31	25	12	11	12	13, 17	0.0073
HC72	1	14 14	13	31	25	11	13	13	11, 14	0.0073
HC73 HC74	1	14	14	29	23	9	13	13	13, 13	0.0073
HC75	1	14	14	29	24	11	13	13	11, 14	0.0073
HC76	1	14	14	30	24	10	13	13	11, 14	0.0073
HC77	1	14	14	30	24	10	11	12	13, 15	0.0073
HC78	1	14	14	30	24	10	14	13	11, 14	0.0073
HC79	1	14	14	30	24	11	13	13	11, 15	0.0073
HC80	4	14	14	30	24	11	13	13	11, 14	0.0292
HC81	1	14	14	30	24	11	13	13	11, 11	0.0073
HC82	1	14	14	30	24	11	13	13	11, 18	0.0073
HC83	1	14	14	31	24	10	13	13	14, 14	0.0073
HC84	1	14	14	31	24	10	13	13	11, 14	0.0073
HC85	1	14	14	31	24	11	13	13	11, 14	0.0073
HC86	1	14	15	32	24	10	13	13	11, 15	0.0073
HC87	1	14	15	32	24	11	11	13	11, 15	0.0073
HC88	1	15	11	28	23	9	11	14	13, 14	0.0073
HC89	1	15	12	28	21	10	11	14	13, 13	0.0073
HC90	1	15	12	28	22	10	15	13	14, 16	0.0073
HC91	1	15	12	28	22	10	11	14	12, 14	0.0073
HC92	1	15	12	28	24	11	11	12	16, 18	0.0073
HC93	1	15	12	28	24	10	11	12	16, 18	0.0073
HC94	1	15	12	29	23	10	13	13	12, 14	0.0073
HC95	1	15	13	29	21	10	11	14	12, 15	0.0073
HC96	1	15	13	29	24	11	13	12	11, 15	0.0073
HC97	1	15	13	29	24	11	11	13	11, 14	0.0073

Table 3 (Continued)

Haplotype	n	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
HC98	1	15	13	29	25	11	14	13	11, 14	0.0073
HC99	1	15	13	29	25	11	13	13	11, 14	0.0073
HC100	1	15	13	29	25	10	14	11	12, 18	0.0073
HC101	1	15	13	29	25	10	11	13	11, 14	0.0073
HC102	1	15	13	30	22	11	11	13	14, 15	0.0073
HC103	1	15	13	30	23	11	11	12	12, 17	0.0073
HC104	1	15	13	30	23	10	12	13	14, 16	0.0073
HC105	1	15	13	30	23	10	13	12	14, 16	0.0073
HC106	1	15	13	30	24	9	12	12	15, 15	0.0073
HC107	1	15	13	30	24	11	13	13	11, 14	0.0073
HC108	1	15	13	31	22	11	11	13	17, 18	0.0073
HC109	2	15	14	29	25	10	14	11	12, 18	0.0146
HC110	1	15	14	30	23	10	13	13	11, 11	0.0073
HC111	1	15	14	30	25	10	11	13	11, 14	0.0073
HC112	1	15	14	31	24	10	11	12	11, 14	0.0073
HC113	1	16	12	27	25	10	11	12	13, 16	0.0073
HC114	1	16	13	27	21	10	11	14	17, 18	0.0073
HC115	1	16	13	28	23	10	11	13	12, 12	0.0073
HC116	1	16	13	30	24	9	14	14	14, 14	0.0073
HC117	1	16	13	30	25	10	12	15	12, 15	0.0073
HC118	2	16	13	31	25	11	11	13	11, 14	0.0146
HC119	1	16	13	32	25	11	11	13	11, 15	0.0073
HC120	1	16	14	30	24	11	13	13	12, 14	0.0073
HC121	1	17	13	29	22	10	11	13	14, 14	0.0073
HC122	1	17	14	29	23	10	11	13	12, 12	0.0073

Leonor Gusmao, IPATIMUP, Portugal), Afro-Americans (n = 698) obtained previously from YSTR.org/USA, Surinam [3], Central African Pygmies (n = 32) [3], Buenos Aires (n = 100) [3] as well as a sample of 225 individuals from Medellín (Colombia) obtained from YSTR.org reported by Builes J.J. (Genes Ltda., Medellín, Colombia). The discrimination power was determined based on the number of individual haplotypes/total number of individuals analyzed.

# 3. Other remarks

The haplotype diversity for the Colombian Caucasian-Mestizo population was  $0.9971 \pm 0.0016$  and discrimination power of 0.8905. For the Afro-Colombian population the haplotype diversity was  $0.9955 \pm 0.0019$  and discrimination power of 0.8208. The AMOVA analysis for the Afro-Colombian and Colombian Caucasian-Mestizo populations based on  $\Phi_{st}$  values are shown in Table 1. As expected, the lowest pairwise difference between the African descent populations tested was the Afro-Colombian and Afro-American population pair, followed by the Afro-Colombian and the population from Surinam. These results are likely due to the same geographic origin and genetic pool in Africa of these population groups as we postulated earlier [10,11]. The lowest pairwise difference for the Colombian Caucasian-Mestizo population was obtained with the population sample from Medellín-Colombia (as expected) followed by the population from Buenos Aires and El Salvador.

The most frequent haplotype for both the Afro-Colombian (HN107 in Table 2) and Caucasian-Mestizo population (HC51 in Table 3) samples tested were searched against the YHDB.org. The Afro-Colombian haplotype (HN107) was found only once (1:28650 haplotypes) in YHDB.org in an Afro-Caribbean population residing in London UK. On the other hand, the most frequent haplotype in the Colombian Caucasian-Mestizo populations was found a total of 1116

Table 4
Y-STR haplotype sharing statistics between Caucasian-Mestizo and
African descent populations of Colombia

	Caucasian Andean region	African descent Chocó		
No. of individuals	137	134		
No. of haplotypes	122	110		
Discrimination (%)	89.05	82.08		
Haplotype class				
Single				
No.	114	96		
Proportion	0.934	0.8727		
Multiple				
No.	8	14		
Proportion	0.066	0.1272		
Haplotype diversity	0.9971	0.9955		
Haplotype diversity S.E.	0.0016	0.0019		

instances with similar frequencies in several European countries such as Spain (Madrid, Barcelona, Asturias, Santiago de Compostela), England, Germany, Portugal and Italy (see Table 4).

This paper follows the guidelines for publication of population data requested by the journal [12].

#### 3.1. Access to the data

Available upon requested at jyunis@hotmail.com.

# Acknowledgements

This work was supported by Servicios Medicos Yunis Turbay y Cia. and by a grant from DINAIN, Universidad Nacional de Colombia, DI00C377 to J.J. Yunis and Luis E. Acevedo.

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