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MOLECULAR KARYOTYPE CHARACTERIZATION OF *LEISHMANIA PANAMENSIS, LEISHMANIA MEXICANA,* AND *LEISHMANIA MAJOR*–LIKE PARASITES: AGENTS OF CUTANEOUS LEISHMANIASIS IN ECUADOR

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Abstract. Molecular karyotypes of *Leishmania* isolates from patients with cutaneous leishmaniasis in Ecuador were analyzed by pulsed-field gel electrophoresis (PFGE) and Southern blot hybridization. The DNA karyotypes of *L. major*–like parasites were similar between two human isolates from a lowland coastal and a highland Andean region, but were apparently different from those of eleven World Health Organization reference strains including *L. major.* The smallest chromosome of 240 kilobases in *L. major*–like parasites was found to belong to the 715-class of small linear chromosomal DNAs, which have been shown to appear in some lines of *Leishmania.* Chromosome banding patterns of *L. mexicana* isolates exhibited a novel, ordered, chromosomal ladder, and were identical among four human isolates and one canine isolate from a restricted geographic region in the Andes. On the other hand, minor chromosome size polymorphisms were observed among three *L. panamensis* isolates from different endemic regions near the Pacific Coast. Chromosomal locations of dihydrofolate reductase-thymidylate synthetase and P-glycoprotein genes revealed further differences in chromosomal organizations among these *Leishmania* species in Ecuador. These results indicate that karyotype analysis by PFGE is useful for epidemiologic studies of leishmaniasis in Ecuador.

Leishmaniasis is widespread and is a considerable public health problem in Ecuador. We have carried out epidemiologic studies of leishmaniasis in Ecuador since 1982 and have found a large number of cutaneous cases and a small number of mucocutaneous ones.[1] In tropical and subtropical lowland areas near the Pacific Coast, we have isolated *Leishmania panamensis* from patients with cutaneous lesions.[2] Recently, we discovered a new endemic area of cutaneous leishmaniasis (Paute) in the Andes (Figure 1).[3] By zymodeme, schizodeme, and serodeme analyses, the causative species were determined to be as *L. mexicana* and an unusual parasite closely related to *L. major.*[3]

The advent of pulsed-field gel electrophoresis (PFGE) has enabled us to study chromosomal organizations of protozoa including *Leishmania.* This technique has distinguished karyotypes among *Leishmania* species, subspecies, and strains.[4–8] Despite this variability, the molecular karyotype analysis can be used for grouping closely related species and for identification of new isolates in epidemiologic studies.[9–11] To further characterize *Leishmania* isolates from Ecuador, we have extended our preliminary study[12] by analyzing their molecular karyotypes using pulsed-field gradient gel electrophoresis in combination with Southern blot hybridization.

MATERIALS AND METHODS

Parasites

Ten *Leishmania* isolates from Ecuador and eleven World Health Organization reference strains of the same genus were used in the present study (Table 1). The Ecuadorian isolates have been classified as *L. panamensis, L. mexicana,* and *L. major*–like parasites by isoenzyme electrophoresis, kinetoplast DNA fingerprints, and reactivity against monoclonal antibodies.[2,3,13] The geographic distribution of these isolates is shown in Figure 1.

TABLE 1
Leishmania *isolates used in the present study*

Species	Designation*	Geographic origin	Abbreviation
Ecuadorian isolates			
L. panamensis (Lp)	MHOM/EC/87/G-05	Quininde, Esmeraldas	*L. panamensis* G5
	MHOM/EC/87/G-06	Z. Grande, Esmeraldas	*L. panamensis* G6
	MHOM/EC/87/G-07	S. Domingo, Pichincha	*L. panamensis* G7
L. mexicana (Lm)	MHOM/EC/88/Paute1	Paute, Azuay	*L. mexicana* P1
	MHOM/EC/88/Paute27	Paute, Azuay	*L. mexicana* P27
	MHOM/EC/88/Paute29	Paute, Azuay	*L. mexicana* P29
	MHOM/EC/88/Paute103	Paute, Azuay	*L. mexicana* P103
	MCAN/EC/88/PauteInu2	Paute, Azuay	*L. mexicana* PInu2
L. major–like (Lm1)	MHOM/EC/87/G-09	Quininde, Esmeraldas	*L. major*–like G9
	MHOM/EC/88/Paute115	Paute, Azuay	*L. major*–like P115
World Health Organization reference strains			
L. aethiopica	MHOM/ET/72/L100	Wollo, Ethiopia	*L. aethiopica* L100
L. tropica	MHOM/SU/58/StrainOD	Azerbaijan, USSR	*L. tropica* StrainOD
L. major	MHOM/SU/73/5-ASKH	Turkmen, USSR	*L. major* 5-ASKH
L. baziliensis	MHOM/BR/75/M2903	Para, Brazil	*L. braziliensis* M2903
L. pifanoi	MHOM/VE/57/LL1	Venezuela	*L. pifanoi* LL1
L. panamensis	MHOM/PA/71/LS94	Canal Zone, Panama	*L. panamensis* LS94
L. guyanensis	MHOM/BR/75/M4147	Para, Brazil	*L. guyanensis* M4147
L. mexicana	MHOM/BZ/82/BEL21	Belize	*L. mexicana* BEL21
L. amazonensis	MHOM/BR/73/M2269	Para, Brazil	*L. amazonensis* M2269
L. garnhami	MHOM/VE/76/JAP78	Venezuela	*L. garnhami* JAP78
L. aristidesi	MORY/PA/68/GML3	Darien, Panama	*L. aristidesi* GML3

* Designation code: host (M = Mammalia; CAN = *Canis familiaris*, ORY = *Oryzomys capito*).

Pulsed-field gradient gel electrophoresis

Agarose blocks for PFGE were prepared as previously described.[12, 14] Promastigotes were grown in Schneider's *Drosophila* medium (Gibco, Grand Island, NY) with 20% heat-inactivated fetal calf serum at 25°C. The log-phase promastigotes were washed twice with Medium 199 (Gibco) and resuspended in the same medium at a concentration of 5×10^8/ml. The cell suspension was warmed to 37°C, and mixed with an equal volume of 1% agarose with a low-gelling temperature (Sea Plaque; FMC Bioproducts, Rockland, ME) in 75 mM phosphate buffer, pH 8.0 containing 65 mM NaCl and 1% glucose. Forty microliters of the mixture containing 1×10^7 organisms were solidified into 5 × 5 × 1.5-mm blocks. The agarose blocks were treated with 1% sarcosyl (Sigma, St. Louis, MO) and 2 mg/ml of proteinase K (Sigma) in 0.5 M EDTA, pH 8.0 at 50°C for two days. Samples were washed with 1× TBE (90 mM Tris, 90 mM boric acid, and 2 mM EDTA, pH 8.0) and stored in 0.5 M EDTA at 4°C until used. Electrophoresis was performed in 1.5% agarose at 180 V in 0.5× TBE at 10°C with a pulse interval of 60–100 sec for 35–38 hr using a turn-table type pulsed-field gel electrophoresis apparatus (Cross Field Gel Electrophoresis; ATTO Corp., Tokyo, Japan).[12] This apparatus was designed to make a 20-cm circular gel plate rotate at an angle of 110° alternately with a defined pulse time. After electrophoresis, the gel was stained with 0.1 μg/ml of ethidium bromide in 0.5× TBE for 45 min, destained with 0.5× TBE for 1 hr, and photographed. Chromosomal DNAs of *Saccharomyces cerevisiae* (FMC Bioproducts) were used as the molecular standard.

Southern blot hybridization

The gels used in PFGE were treated with 0.25 N HCl for 30 min and with 0.4 N NaOH plus 0.6 M NaCl for 30 min, followed by transfer to nylon membranes in the presence of 20× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.4). Transferred DNA on the membrane was fixed by ultraviolet cross-linking. The probes used in the present study were pRRv20, which contained the complete dihydrofolate reductase-thymidylate synthetase (DHFR-TS) coding region of *L. major*,[15] p7R50-P19, which contained a 1.9-kilobase (kb) *Pst* I fragment from the small linear chromosomal DNAs (slDNA)

present in the 7-R50 line of *L. major*,[16] pK25, which contained a 2.5-kb *Kpn* I fragment from the H region P-glycoprotein of *L. tarentolae* (ltpgpA),[17] and pCD-1-B8, which contained a 3.5-kb *Bam* HI fragment of LD1.[18] The DNA probes were labeled by random primer labeling method (Amersham, Arlington Heights, IL). Blots were hybridized with DNA probes overnight at 66°C in 6× SSC, 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's solution (1× Denhardt's solution = 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone), and 100 μg/ml of denatured salmon sperm DNA, and washed with 0.1× SSC and 0.1% SDS at 65°C for 1 hr before autoradiography at −70°C using intensifying screens. Some blots were rehybridized with different probes after removing the previous probe by washing the blot in 0.4 N NaOH at 42°C for 30 min followed by 0.2 M Tris-HCl, pH 7.4 containing 0.6 M NaCl at 42°C for 30 min.

RESULTS

Molecular karyotype of L. major*–like parasites*

Resolution of leishmanial chromosomal DNAs by PFGE was dependent on a pulse-time interval. Chromosomes less than 900 kb could be clearly separated with pulse intervals of 60–80 sec, and chromosomes from 900 kb to 1,100 kb were resolved at a 100-sec pulse time. Two clinical isolates of *L. major*–like parasites, *L. major*–like G9 from Quininde near the Pacific Coast and *L. major*–like P115 from Paute in the Andes, were analyzed (Table 1 and Figure 1). A minimum of 14 chromosomes in a range of 240–850 kb were resolved at a 60-sec pulse frequency (Figure 2A, lanes 6 and 7; Figure 2B, lanes 3 and 4; Figure 3, lanes 1 and 2). The chromosomal DNA banding patterns of these two isolates were very similar but not completely identical. A 240-kb chromosomal band with strong intensity was observed in strain P115, while two smaller chromosomes of 240 kb and 260 kb were seen in strain G9 (Figure 2B, lanes 3 and 4 and Figure 4).

The karyotype of *L. major*–like parasites was apparently different from those of three reference strains for Old World cutaneous leishmaniasis: *L. aethiopica* L100, *L. tropica* strain OD, and *L. major* 5-ASKH (Figure 2A, lanes 1, 4 and 5, respectively), and from eight reference strains for American leishmaniasis: *L. garnhami* JAP78, *L. aristidesi* GML3 (Figure 2A, lanes 2 and 3), *L. braziliensis* M2903, *L. pifanoi* LL1 (Figure 2B, lanes 1 and 2), *L. guyanensis* M4147, *L. panamensis* LS94, *L. amazonensis* M2269, and *L. mexicana* BEL21 (Figure 3A, lanes 3, 4, 9 and 10, respectively).

FIGURE 1. Geographic location of *Leishmania* isolates from Ecuador used in this study. Lp = *L. panamensis;* Lm = *L. mexicana;* Lml = *L. major*–like. The dotted area on map indicates the Andean highland region more than 1,000 meters above sea level.

The DHFR-TS gene was located on a single chromosome of approximately 450 kb in both the *L. major*–like parasites G9 and P115 (Figures 3B and 4), as well as in the *L. major* 5-ASKH reference strain. The p7R50-P19 probe from the slDNAs of the 715-class in *L. major* hybridized with a chromosome of 240 kb in both *L. major*–like strains (Figures 3C and 4). The slDNA probe was shown to hybridize with a 200-kb chromosomal DNA in *L. braziliensis* M2903, which has been previously shown to contain amplified 715-class DNA sequences[16] related to LD1 sequences.[18] The pCD-1-B8 probe from LD1 also hybridized with the corresponding chromosomes of the *L. major*–like isolates and *L. braziliensis* M2903. The pk25 probe from P-glycoprotein gene of *L. tarentolae* hybridized with a single chromosome of approximately 690 kb in both isolates of *L. major*-like parasites (Figures 3D and 4).

Molecular karyotype of L. mexicana *isolates*

A minimum of 19 DNA bands in a range of 220–1,090 kb were recognized in *L. mexicana*

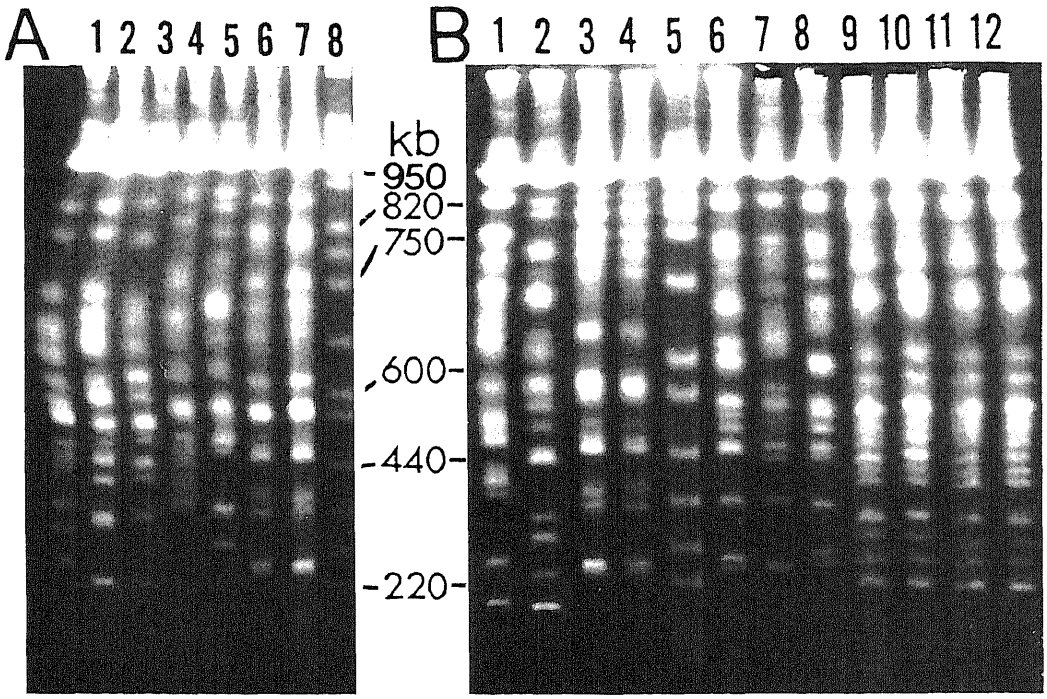

FIGURE 2. Separation of chromosomal DNAs of *Leishmania* promastigotes by pulsed-field gel electrophoresis. This technique was performed using 1.5% agarose gels run at 180 V with a 60-sec pulse time for 36 hr (**A**) or 35 hr (**B**). **A**, lane 1, *L. aethiopica* L100; lane 2, *L. garnhami* JAP78; lane 3, *L. aristidesi* GML3; lane 4, *L. tropica* strain OD; lane 5, *L. major* 5-ASKH; lane 6, *L. major*–like G9; lane 7, *L. major*–like P115; lane 8, *Saccharomyces cerevisiae.* **B**, lane 1, *L. braziliensis* M2903; lane 2, *L. pifanoi* LL1; lane 3, *L. major*–like P115; lane 4, *L. major*–like G9; lane 5, *S. cerevisiae;* lane 6, *L. panamensis* G5; lane 7, *L. panamensis* G6; lane 8, *L. panamensis* G7; lane 9, *L. mexicana* P27; lane 10, *L. mexicana* P29; lane 11, *L. mexicana* PInu2; lane 12, *L. mexicana* P103. kb = kilobases.

isolates. The karyotype was highly conserved among four human isolates (*L. mexicana* P1, P27, P29, and P103) and one canine isolate (*L. mexicana* PInu2) from Paute. It is notable that four smaller chromosomal bands of 220, 250, 280, and 325 kb appear to form a chromosome ladder (Figure 2B, lanes 9–12; Figure 3A, lanes 11–15; Figure 4).

Southern blot hybridization analysis revealed that the DHFR-TS gene was located on a chromosome of approximately 450 kb in all *L. mexicana* strains (Figure 3B). The p7R50-P19 probe hybridized with none of chromosomes < 1,100 kb in the isolates (Figure 3C). However, the pk25 probe from the P-glycoprotein gene recognized broad DNA bands ranging from 700 kb to 750 kb in these strains (Figures 3D and 4).

Molecular karyotype of L. panamensis *isolates*

Three human isolates of *L. panamensis*, G5, G6, and G7 from three different geographic regions near the Pacific Coast, were analyzed (Figure 1). Chromosome size polymorphisms were seen among these isolates, especially with respect to chromosome 1 and chromosomes between 500 kb and 750 kb in size, although chromosomes > 800 kb were similar to each other. For example, two chromosomes of approximately 240 kb and 260 kb were seen in strains G5 and G7, while a single DNA band of 240 kb was observed in strains G6, *L. panamensis* LS94, and *L. guyanensis* M4147 (Figure 2B, lanes 6–8 and Figure 4).

The DHFR-TS gene was located on a single chromosome of approximately 480 kb in three Ecuadorian *L. panamensis* isolates, as well as in the *L. guyanensis* M4147 and *L. panamensis* LS94 reference strains (Figure 3B, lanes 3–7). The probe from slDNAs did not hybridize with chromosomes < 1,100 kb (Figure 3C). The probe from the P-glycoprotein gene hybridized with a single chromosomal DNA, which was approxi-

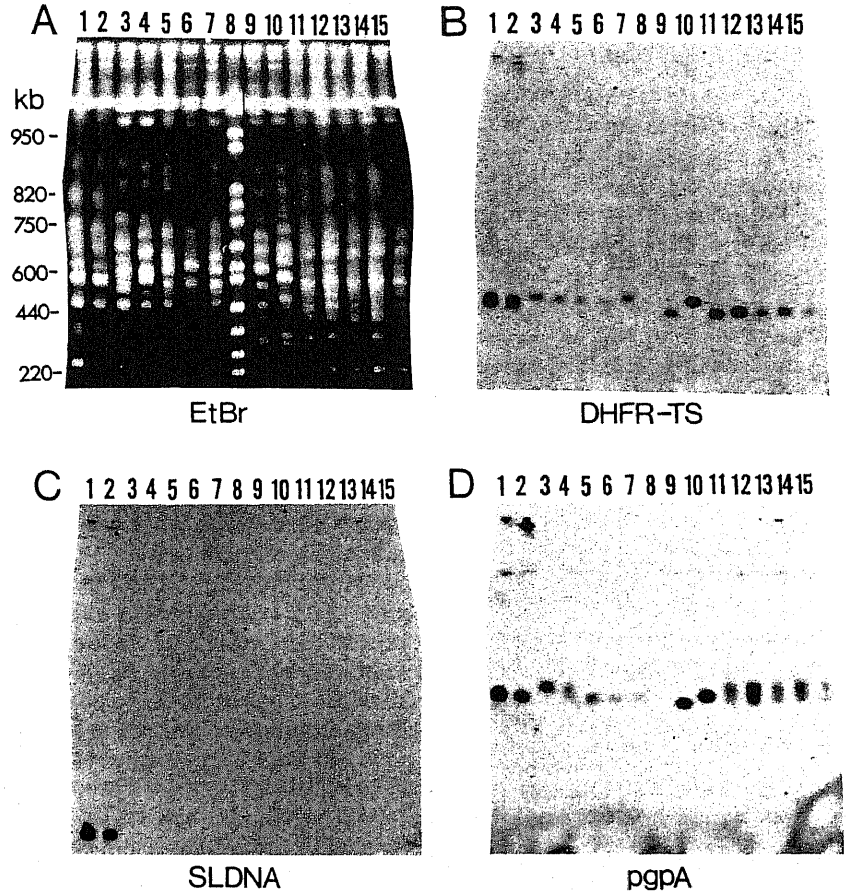

FIGURE 3. Southern blot hybridization analysis of *Leishmania* isolates from Ecuador. Pulsed-field gel electrophoresis was performed using 1.5% agarose gels run at 180 V with a 70-sec pulse time for 38 hr and stained with ethidium bromide (EtBr) (**A**). The blot was hybridized with probe pRRv20 from the dihydrofolate reductase-thymidylate synthetase (DHFR-TS) gene (**B**), probe p7R50-P19 from the small linear chromosomal DNA (SLDNA) (**C**), or probe pK25 from the P-glycoprotein A gene (pgpA) (**D**). Lane 1, *L. major*–like P115; lane 2, *L. major*-like G9; lane 3, *L. guyanensis* M4147; lane 4, *L. panamensis* LS94; lane 5, *L. panamensis* G5; lane 6, *L. panamensis* G6; lane 7, *L. panamensis* G7; lane 8, *Saccharomyces cerevisiae;* lane 9, *L. amazonensis* M2269; lane 10, *L. mexicana* BEL21; lane 11, *L. mexicana* P27; lane 12, *L. mexicana* P29; lane 13, *L. mexicana* PInu2; lane 14, *L. mexicana* P103; lane 15, *L. mexicana* P1. kb = kilobases.

mately 690 kb in the Ecuadorian isolates, but was approximately 750 kb in the reference strains M4147 and LS94 (Figure 3D, lanes 3–7 and Figure 4).

DISCUSSION

In the present study, we report the molecular karyotype characteristics of three different *Leishmania* species, *L. panamensis, L. mexicana,* and *L. major*–like parasites, that are known to be the causative agents of cutaneous leishmaniasis in Ecuador. The karyotype analysis indicates the complexity and diversity of *L. panamensis* in the Pacific coastal regions but relatively simple epidemiologic feature of *L. mexicana* and *L. major*–like infections in the Andes.

Leishmania major–like parasites were initially designated as Brazilian *Leishmania* because they were indistinguishable from *L. major* reference strains by zymodeme and serodeme analyses.[19] We have isolated *L. major*–like parasites from patients at two geographically different regions in Ecuador, one from Quininde in the Pacific lowland region and the other from Paute, which is 2,300–2,500 meters above sea level in the An-

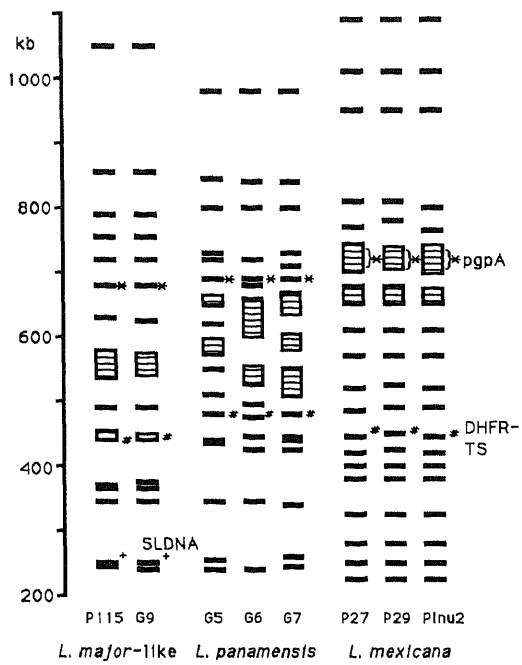

FIGURE 4. Summary of DNA karyotypes and chromosomal locations of specific DNA sequences in Ecuadorian *Leishmania* isolates. Areas with transverse lines indicate broadly stained zones that may contain more than two chromosomal DNAs. Hybridization with the dihydrofolate reductase-thymidylate synthetase probe of *L. major*, the 715-class small linear chromosomal DNAs of *L. major*, and the H region P-glycoprotein A gene of *L. tarentolae* is indicated by DHFR-TS (#), SLDNA (+), and pgpA (*), respectively. kb = kilobases.

des Mountains (Figure 1).[3] Karyotype similarity of the two isolates (Figures 2 and 4) suggests that this strain of *L. major*–like parasite might be widely distributed in Ecuador. Determination of DNA karyotypes of *L. major*–like strains in Brazil is required for further defining the epidemiologic features of this novel parasite.

The present study using PFGE revealed that the DNA karyotype of Ecuadorian *L. major*–like parasites was totally different from that of the *L. major* 5-ASKH reference strain (Figure 2A, lanes 5–7) and from those reported for five other *L. major* stocks (MRHO/SU/59/P, MHOM/IL/67/Jericho II, strain D-1, strain WR300, and strain Bokkara).[20] For example, the size of the smallest chromosome in these reference strains was approximately 350 kb, whereas it was 240 kb in the Ecuadorian *L. major*–like parasite. It has been also reported that schizodeme profiles of *L. major*–like from Brazil and Ecuador were different from those of *L. major* reference stains including MHOM/SU/73/5-ASKH, MHOM/IL/79/LCR-L251, MHCM/SN/IL/00/LRC-223, and MHOM/SN/00/DK99.[3,19] Taken together, it is suggested that the *L. major*–like parasites in the New World differ from the *L. major* in the Old World in terms of both kinetoplast DNA (kDNA) and genomic DNA organization.

Furthermore, it was demonstrated that the *L. major*–like isolates contained the 715-class or LD1 DNA sequences in the smallest chromosome of 240 kb (Figure 3C), while the *L. major* reference strain 5-ASKH did not contain the 715-class DNA sequences in the low molecular weight chromosomes. The 715-class slDNAs were designated to new chromosomal DNAs that appeared de novo in several lines derived from the LT252 isolate of *L. major*.[16] The slDNA was present in multiple copies, stably maintained, linear, related to LD1 DNA sequences, and had a size range of 180–220 kb. The LD1 was found to be a 27.5-kb DNA molecule and was present within large chromosomes of 1.5–2 megabases in all *Leishmania* stocks examined.[18] The LD1 is also present in smaller (< 550 kb) chromosomes in some stocks including *L. donovani*, *L. chagasi*, *L. braziliensis*, *L. amazonensis*, and *L. mexicana*, or it forms circular molecules designated CD1.[18,21,22] It is also suggested that the slDNA and LD1/CD1 may arise from an evolutionarily conserved large chromosome by chromosomal rearrangement,[16,18,23] although the functions of these DNA sequences are unknown. Thus, the present study provides new evidence that the *L. major*–like stocks in Ecuador contained LD1 sequences in the 240-kb chromosome.

In our previous paper, we reported that schizodeme profiles of *L. mexicana* from Paute were identical with eight human isolates (MHOM/EC/88/Paute1, 6, 7, 23, 25, 27, 29, and 103), the canine strain (MCAN/EC/88/PauteInu2), and the sand fly isolate (IAYA/EC/89/PAI1).[3] This study revealed identical karyotypes among four human isolates (MHOM/EC/88/Paute1, 27, 29, and 103) and the canine isolate (MCAN/EC/88/PauteInu2) (Figures 2B and 3A). These similarities in kDNA fingerprints and genomic DNA karyotypes further suggest that the *L. mexicana* strains from Paute may belong to a homologous population and that dogs may play a role as reservoir hosts in the endemic region.

In addition to Paute, we found patients with cutaneous leishmaniasis in Alausi (elevation 2,300 meters), which is located approximately 80 km north of Paute. Since one of the human isolates from Alausi (MHOM/EC/89/AL1) was found to be very similar to the strains of *L. mexicana* from Paute in terms of schizodeme, serodeme, and zymodeme, this strain was determined to be *L. mexicana*.[3] Thus, the karyotype of the isolate from Alausi is probably identical to that from Paute. In addition, we recently found cutaneous leishmaniasis patients in Huigra (elevation 1,300 meters) located approximately 20 km southwest of Alausi, and two human isolates showed DNA karyotypes similar to the *L. mexicana* stains from Paute (unpublished data). Isolation of *L. mexicana* was also reported from patients with cutaneous leishmaniasis in Alausi by other investigators[24] and in the western Andean cordillera of Colombia at an elevation of 1,500 meters.[25]

Interestingly, the clinical aspects of leishmaniasis in Paute, Alausi, and Huigra resemble uta caused by *L. peruviana* infections in the Peruvian Andes.[3, 24, 26, 27] The DNA karyotype of the Ecuadorian *L. mexicana* is characterized by the presence of four low molecular weight chromosomes of 220, 250, 280 and 325 kb, in which chromosomes 1 and 4 showed strong ethidium bromide staining intensities (Figure 2B, lanes 9–12). A similar chromosomal ladder was seen in the rodent isolate in Belize (MNYC/BZ/62/WR592),[10] but not in other strains of the *L. mexicana* complex.[5, 10, 11] Thus, the DNA karyotyping of leishmanial isolates is definitely useful for identification of new isolates in epidemiologic studies and in the clinical diagnosis of the Andean cutaneous leishmaniasis.

Another unique karyotype profile of the *L. mexicana* was the chromosomal loci of genes homologous to the P-glycoprotein. The probe from ltpgpA hybridized with several DNA bands of all Ecuadorian *L. mexicana* isolates examined. The same probe recognized only the single chromosome of approximately 700 kb in other strains including *L. amazonensis* M2269 and *L. mexicana* BEL21 (Figure 3D). It is possible that P-glycoprotein genes are dispersed in different chromosomes in the Ecuadorian *L. mexicana*. At least three P-glycoprotein genes have been reported in *L. tarentolae*, but they appear to be located on a single chromosome.[28]

The DNA karyotypes of the *L. braziliensis* complex have been analyzed in more detail.[5, 9, 11] The chromosomal DNA patterns of *L. guyanensis*, *L. panamensis*, and *L. braziliensis* were essentially identical above 900 kb, but were distinct for each isolate below 900 kb.[5] Karyotypes of three Ecuadorian *L. panamensis* isolates (G5, G6, and G7) were closely related to each other and indistinguishable above 800 kb, but showed size polymorphisms below 730 kb (Figure 2B). Since consensus chromosomes have been reported in *L. panamensis*,[9, 11] at least the chromosomes of 240, 340, 480, and 690 kb appear to be conserved in the Ecuadorian isolates. Furthermore, the DNA karyotype of strain G6 showed stable characteristics after in vitro passages for eight months (unpublished data).

Leishmania panamensis is the most commonly isolated *Leishmania* species in Colombia, representing 54% of the total isolates.[25] Some Colombian strains of *L. panamensis* with identical isoenzyme phenotypes differed genotypically in schizodeme profiles/or molecular karyotypes.[9] Isoenzyme electrophoresis profiles of three Ecuadorian *L. panamensis* isolates were similar to those of the *L. panamensis* LS94 strain except for phosphogluconate dehydrogenase.[2] On the other hand, schizodeme profiles and molecular karyotypes of these three isolates were different, although they are closely related to each isolate.[13] This genotypic variation among *L. panamensis* isolates from Ecuador and Colombia may reflect a great diversity of mammalian reservoir hosts and sand fly vectors in the Pacific coastal lowlands of Central and South America. Indeed, leishmanial parasites have been isolated from four mammalian species that serve as reservoir hosts from the coastal regions in Ecuador.[1] In addition, more than 50 *Lutzomyia* species have been identified in Ecuador and we found that at least three of them (*Lu. trapidoi*, *Lu. hartmanni* and *Lu. gomezi*) were probable vectors for leishmaniasis in the lowlands.[1] This is in sharp contrast to the Andean highlands in Ecuador, in which only one sand fly species (*Lu. ayacuchensis*) was identified as the vector for *L. mexicana*.[1, 3] Since a relatively small, restricted, geographic area can be endemic with a great diversity of *Leishmania* infections,[9] careful epidemiologic studies are required to elucidate the transmission mechanisms of cutaneous leishmaniasis in Ecuador.

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REFERENCES

1. Hashiguchi Y, Gomez EAL, 1991. A review of leishmanisis in Ecuador. *Bull Pan Am Health Organ 25:* 64–76.
2. Mimori T, Grimaldi G Jr, Kreutzer RD, Gomez EAL, McMahon-Pratt D, Tesh RB, Hashiguchi Y, 1989. Identification, using isoenzyme electrophoresis and monoclonal antibodies, of Leishmania isolated from humans and wild animals of Ecuador. *Am J Trop Med Hyg 40:* 154–158.
3. Hashiguchi Y, Gomez EAL, De Coronell VV, Mimori T, Kawabata M, Furuya M, Nonaka S, Takaoka H, Alexander JB, Quizhpe AM, Grimaldi G Jr, Kreutzer RD, Tesh RB, 1991. Andean leishmaniasis in Ecuador caused by infection with Leishmania mexicana and L. major-like parasites. *Am J Trop Med Hyg 44:* 205–217.
4. Giannini SH, Schittini M, Keithly JS, Warburton PW, Cantor CR, Van der Ploeg LHT, 1986. Karyotype analysis of Leishmania species and its use in classification and clinical diagnosis. *Science 232:* 762–765.
5. Scholler JK, Reed SG, Stuart K, 1986. Molecular karyotype of species and subspecies of Leishmania. *Mol Biochem Parasitol 20:* 279–293.
6. Samaras N, Spithill TW, 1987. Molecular karyotype of five species of Leishmania and analysis of gene locations and chromosomal rearrangement. *Mol Biochem Parasitol 25:* 279–291.
7. Bishop RP, Miles MA, 1987. Chromosome size polymorphisms of Leishmania donovani. *Mol Biochem Parasitol 24:* 263–272.
8. Pages M, Bastien P, Veas F, Rossi V, Bellis M, Wincker P, Rioux J-A, Roizes G, 1989. Chromosome size and number polymorphisms in Leishmania infantum suggest amplification/deletion and possible genetic exchange. *Mol Biochem Parasitol 36:* 161–168.
9. Saravia NG, Weigle K, Segura I, Giannini SH, Pacheco R, Labrada LA, Goncalves A, 1990. Recurrent lesions in human Leishmania braziliensis infection-reactivation or reinfection?. *Lancet 336:* 398–402.
10. Grogl M, Kreutzer RD, McHugh CP, Martin RK, 1991. Characterzation of a Leishmania isolate from the rodent host Neotoma micropus collected in Texas and comparison with human isolates. *Am J Trop Med Hyg 45:* 714–722.
11. Lighthall GK, Giannini SH, 1992. The chromosomes of Leishmania. *Parasitol Today 8:* 192–199.
12. Katakura K, Matsumoto Y, Furuya M, Gomez EAL, Hashiguchi Y, 1992. Karyotype analysis of Leishmania isolates from Ecuador by pulsed field gel electrophoresis. Hashiguchi Y, ed. *Studies on New World Leishmaniasis and Its Transmission, with Particular Reference to Ecuador.* Res Rep Ser No. 3. Kochi, Japan: Kyowa Printing Co., 11–15.
13. Mimori T, Gomez EA, Hashiguchi Y, 1990. Further characterization of Leishmania isolated from humans and animals of Ecuador, using restriction enzyme analysis of kinetoplast DNA. Hashiguchi Y, ed. *Studies on New World Leishmaniasis and Its Transmission, with Particular Reference to Ecuador.* Res Rep Ser No. 2. Kochi, Japan: Kyowa Printing Co., 31–39.
14. Katakura K, Peng Y, Pithawalla R, Detke S, Chang K-P, 1991. Tunicamycin-resistant variants from five species of Leishmania contain amplified DNA in extrachromosomal circles of different sizes with a transcriptionally active homologous region. *Mol Biochem Parasitol 44:* 233–244.
15. Beverley SM, Ellenberger TE, Cordingley JS, 1986. Primary structure of the gene encoding the bifunctional dihydrofolate reductase-thymidylate synthase of Leishmania major. *Proc Natl Acad Sci USA 83:* 2585–2588.
16. Beverley SM, Coburn CM, 1990. Recurrent de novo appearance of small linear DNAs in Leishmania major and relationship to extrachromosomal DNAs in other species. *Mol Biochem Parasitol 42:* 133–142.
17. Petrillo-Peixoto ML, Beverley SM, 1988. Amplified DNAs in laboratory stocks of Leishmania tarentolae: extrachromosomal circles structurally and functionally similar to the inverted H region amplification of methotrexate-resistant L. major. *Mol Cell Biol 8:* 5188–5199.
18. Tripp CA, Myler PJ, Stuart K, 1991. A DNA

sequence (LD1) which occurs in several genomic organizations in Leishmania. *Mol Biochem Parasitol 47:* 151–160.

19. Momen H, Grimaldi G Jr, Pacheco RS, Jaffe CL, McMahon-Pratt D, Marzochi MCA, 1985. Brazilian Leishmania stocks phenotypically similar to Leishmania major. *Am J Trop Med Hyg 34:* 1076–1084.
20. Giannini SH, Curry SS, Tesh RB, Van der Ploeg LHT, 1990. Size-conserved chromosomes and stability of molecular karyotype in cloned stocks of Leishmania major. *Mol Biochem Parasitol 39:* 9–22.
21. Gajendran N, Dujardin JC, Le Ray D, Matthyssens G, Muyldermans S, Hamers R, 1989. Abnormally migrating chromosome identifies Leishmania donovani populations. Hart DT, ed. *Leishmaniasis: The Current Status and New Strategies for Control.* New York: Plenum Press, 539–547.
22. Stuart K, Tarr P, Aline R, Smiley B, Scholler J, Keithly J, 1989. Small nucleic acids in Leishmania. Hart DT, ed. *Leishmaniasis: The Current Status and New Strategies for Control.* New York: Plenum Press, 555–562.
23. Liu J, Gajendran N, Muthi D, Muyldermans S, Dujardin J-C, De Doncker S, Jacquet D, Le Ray D, Mathieu-Daude F, Hamers R, 1991. Chromosome rearrangement in Leishmania mexicana M379. *Mol Biochem Parasitol 46:* 53–60.
24. Armijos RX, Chico ME, Cruz ME, Guderan RH, Kreutzer RD, Berman JD, Rogers MD, Grogl M, 1990. Human cutaneous leishmaniasis in Ecuador: identification of parasites by enzyme electrophoresis. *Am J Trop Med Hyg 42:* 424–428.
25. Corredor A, Kreutzer RD, Tesh RB, Boshell J, Palau MT, Caceres E, Duque S, Pelaez D, Rodriguez G, Nichols S, Hernandez CA, Morales A, Young DG, Ferro de Carrasquilla C, 1990. Distribution and etiology of leishmaniasis in Colombia. *Am J Trop Med Hyg 42:* 206–214.
26. Lumbreras H, Guerra H, 1985. Leishmaniasis in Peru. Chang K-P, Bray RS, eds. *Leishmaniasis.* Amsterdam: Elsevier, 297–311.
27. Lainson R, Shaw JJ, 1987. Evolution, classification and geographical distribution. Peters W, Killick-Kendrick R, eds. *The Leishmaniases in Biology and Medicine.* Volume 1. London: Academic Press, 1–120.
28. Quellette M, Hettema E, Wust D, Fase-Fowler F, Borst P, 1991. Direct and inverted DNA repeats associated with P-glycoprotein gene amplification in drug resistant Leishmania. *EMBO J 10:* 1009–1016.