

## Short Report: Natural Infection of *Lutzomyia tortura* with *Leishmania (Viannia) naiffi* in an Amazonian Area of Ecuador

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**Abstract.** Natural infection of sand flies with *Leishmania* parasites was surveyed in an Amazonian area in Ecuador where leishmaniasis is endemic. Seventy-one female sand flies were dissected and one was positive for *Leishmania* protozoa. The species of this sand fly was identified as *Lutzomyia (Lu.) tortura* on the basis of morphologic characteristics. Analysis of the cytochrome *b* gene sequence identified the parasite as *L. (Viannia) naiffi*. We report the distribution of *L. (V.) naiffi* in Ecuador and detection of a naturally infected sand fly in the Ecuadorian Amazon and natural infection of *Lu. tortura* with *Leishmania* parasites in the New World.

Leishmaniasis is a protozoan disease caused by the genus *Leishmania*. It is distributed worldwide, especially in tropical and subtropical areas, and affects at least 12 million persons.<sup>1,2</sup> Disease occurs in three distinct manifestations (cutaneous, mucocutaneous, and visceral), and the clinical forms are largely associated with the *Leishmania* species responsible.<sup>1,2</sup> Parasites are transmitted by female sand flies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World.<sup>3,4</sup> At present, more than 800 sand fly species are known; however, only a portion of the species can transmit each particular *Leishmania* species.<sup>3,4</sup> Therefore, identification of the prevalent parasite and vector species is important for risk assessment and appropriate treatment.

Currently, little information is available on prevalent parasite and sand fly species in the Ecuadorian Amazon because of difficulty in gaining access to this region. In a previous study, cases of cutaneous and mucocutaneous leishmaniasis were recorded in these areas<sup>5</sup>; however, no parasitologic characterization of the causative agents has been made. Recently, we studied mucocutaneous leishmaniasis in these areas and found that *Leishmania (Viannia) braziliensis* is prevalent in Amazonian areas.<sup>6</sup> The sand fly species involved in the transmission cycle are not well characterized in these areas. In addition, no natural infection of sand flies with *Leishmania* species has been reported in the Ecuadorian Amazonia. In the present study, natural infection of sand flies with *Leishmania* species was surveyed in an area of the Ecuadorian rainforest where leishmaniasis is endemic.

Sand flies were captured with CDC light traps and protected human bait in tropical rainforests in Arajuno (1°23'S, 77°67'W, Department of Pastaza), Ecuador. In this area, 71 female sand flies were dissected for identification at the species level. On the basis of morphologic characteristics of spermathecae,<sup>7</sup> 11 species were recognized. The two most prevalent sand flies were *Lu. yuilli* (28 flies) and *Lu. tortura* (19 flies). Infection with *Leishmania* species was examined under a microscope and detected in one anthropophilic sand fly. The positive sand fly was identified as *Lu. tortura* or possibly *Lu.*

*hartmanni* on the basis of morphologic characteristics (Figure 1). To clarify this finding, we analyzed 18S ribosomal RNA gene sequences of the positive fly. The sequences did not match those of *Lu. hartmanni*. Therefore, the positive sand fly was identified as *Lu. tortura*. The presence of male *Lu. tortura* was also confirmed in the same study site, but no male *Lu. hartmanni* was found. *Lutzomyia tortura* has been reported in other Amazonian areas such as Taisha (2°38'S, 77°50'W, Department of Morona-Santiago) and Cotapino (0°70'S, 77°47'W, Department of Orellana),<sup>6,8,9</sup> but not on the Pacific coast subtropical and in Andean areas of Ecuador. This species was reported in the Colombian Amazon,<sup>7,10,11</sup> which suggests that *Lu. tortura* is distributed mainly in Amazonian regions. No natural infection of *Lu. tortura* with *Leishmania* parasites has been reported in the New World.

The dissected sample, including innumerable promastigotes, was fixed in absolute ethanol and genomic DNA was extracted. Using a portion of the DNA as a template, we amplified the leishmanial cytochrome *b* (*cyt b*) gene with a pair of primers, L.cyt-S (5'-GGGTAGGTTTTAGTYTAGG-3') and L.cyt-R (5'-CTACAATAAACAATCATAATATCAATT-3').<sup>12,13</sup> The sequence was determined and compared with sequences from 16 *Leishmania* and 2 *Endotrypanum* species. A phylogenetic analysis was also performed. The *cyt b* gene sequence had higher homology with that of *L. (V.) naiffi* (99.6%) than with others (88–97.9%) and phylogenetic tree analysis supported this result (Figure 2), which indicated that the parasite infecting the sand fly was *L. (V.) naiffi*. The parasite isolated was identified as ITOR/EC/07/Araj1.

Information on infections with *L. (V.) naiffi* is limited and most cases have been reported in Brazil. *Leishmania (V.) naiffi* has been isolated from armadillos, the sand fly species *Lu. squamiventris*, *Lu. paraensis*, *Lu. davisii*, and *Lu. hirsuta*, and humans with cutaneous leishmaniasis in Brazil.<sup>14–19</sup> Outside Brazil, infection with *L. (V.) naiffi* was reported in a patient with cutaneous leishmaniasis who was infected in French Guiana, Martinique, or Guadeloupe.<sup>20</sup> Four human cases of cutaneous leishmaniasis caused by *L. (V.) naiffi* have been reported; three of the patients were infected in French Guiana or Martinique and one was suspected to be infected in Ecuador or Peru because the patient had been on a seven-week trip to Ecuador and Peru that included four days in the Ecuadorian Amazon.<sup>21</sup> This report suggested that *L. (V.)*

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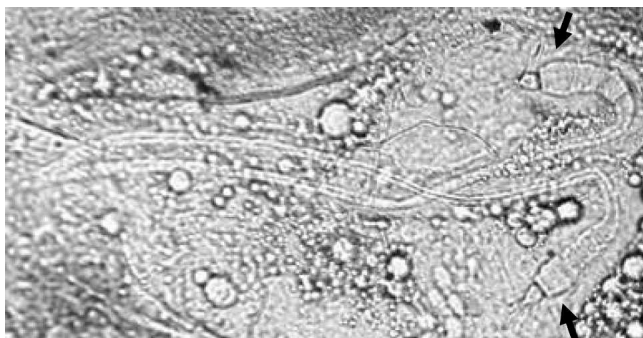


FIGURE 1. Spermathecae (arrows) and ducts of a female *Lutzomyia tortura* sand fly.

*naiffi* may have spread in Ecuador. Other than this one suspected case, no case of cutaneous leishmaniasis caused by *L. (V.) naiffi* has been reported in Ecuador, probably because of limited research in the Amazonian regions.

During our field research, one suspected case of cutaneous leishmaniasis was found and tissue material from this person (a four-year-old girl) from an active cutaneous lesion was placed on an FTA Classic Card (Whatman BioScience, Newton, MA). DNA was extracted from the specimen and the leishmanial *cyt b* gene was amplified. The *cyt b* gene sequence had the highest degree of homology with that of *L. (V.) guyanensis* (99.9%), and phylogenetic tree analysis supported the result (Figure 2), indicating that the patient was infected with *L. (V.) guyanensis*. The presence of *L. (V.) braziliensis* and hybrid genotypes between *L. (V.) panamensis/guyanensis* and *L. (V.) braziliensis* in the Ecuadorian Amazon has been re-

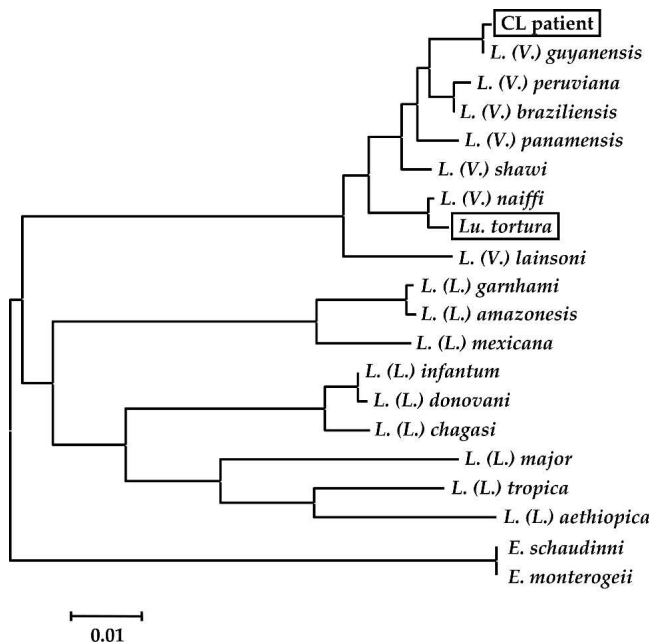


FIGURE 2. Phylogenetic tree of cytochrome *b* gene sequences among species. The leishmanial *cyt b* genes were amplified from a patient with cutaneous leishmaniasis (CL patient) and a positive sand fly (*Lu. tortura*), and the sequences were determined. Phylogenetic tree analysis was performed with sequences obtained and those from 16 *Leishmania* species and 2 *Endotrypanum* species as described in the text. Scale bar indicates 0.01% divergence.

ported.<sup>6,9,22</sup> We have shown that *L. (V.) guyanensis* is present in the Amazonian areas of Ecuador, but no human cases infected with *L. (V.) naiffi* were found in the present study. However, this species may be circulating in this area because it was detected in a highly anthropophilic species of sand fly. Further research in Amazonian regions will provide more information on human cases of infection with *L. (V.) naiffi*.

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