



Short communication

Natural infection of *cortelezzii* complex (Diptera: Psychodidae: Phlebotominae) with *Leishmania braziliensis* in Chaco, Argentina

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ABSTRACT

In Argentina, American Cutaneous Leishmaniasis (ACL) extends up to 29°S in the phytogeographic regions of the Yungas (west), Chaco (center) and Paranaense (east). Since the Phlebotominae vectors of this disease in the western Chaco (dry Chaco) are unknown, in the present work, we studied the natural infection in Phlebotominae by PCR-ERFLP and Dot blot in order to incriminate these organisms as potential vectors. Captures with CDC-type traps were performed monthly in the domicile, the peridomicile and the forest in the Municipio Misión Nueva Pompeya, Chaco, Argentina, in two sites with human cases of ACL: Los Pozos (24°54'S, 61°22'W) and Fortín Arenales (24°58'S, 61°21'W), from November 2006 to December 2007. A total of 1702 Phlebotominae were captured: *Mygonyemyia migonei* (83.8%), *cortelezzii* complex (11.1%), *Mycropogomyia peresi* (3.3%), *Mycropogomyia quinquefer* (1.2%), *Pintomyia torresi* (0.2%) and *Nyssomyia neivai* (0.2%). Although no significant differences were found in species diversity, there were significant differences in abundance between both sites studied. A total of 80 phlebotomine females were analyzed: 50 of the *cortelezzii* complex and 30 *My. migonei*. No intestinal flagellates were observed by light microscopy. Two pools of 10 individuals of the *cortelezzii* complex of the peridomicile and forest of Fortín Arenales were reactive by PCR and Dot blot for *Leishmania (Viannia) braziliensis*. In Argentina, *Evandromyia cortelezzii* has been incriminated as a likely vector of ACL because of its abundance in areas of sporadic outbreaks. In the present work, *Ev. cortelezzii* females were found naturally infected, thus reinforcing the hypothesis that the members of the *cortelezzii* complex act as vectors of the disease.

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In Argentina, leishmaniasis is distributed up to 29°S in nine provinces within the phytogeographic regions of the Yungas, Chaco and Paranaense (Salomón et al., 2001a). The parasite most frequently isolated from outbreaks of American Cutaneous Leishmaniasis (ACL), from both human patients and vectors, has been found to be *Leishmania (Viannia) braziliensis* (Segura et al., 2000; Córdoba Lanús et al., 2005). *Leishmania (Lesishmania) amazonensis* and *Leishmania (Viannia) guyanensis* have also been described by Frank et al. (2000) and Marco et al. (2005) as causative agents of

ACL cases and *Leishmania infantum* has been reported by Salomón et al. (2008a) as the causative agent of Visceral Leishmaniasis (VL) after the first autochthonous human case in 2006.

Of the 27 species of Phlebotominae described in Argentina, *Nyssomyia neivai* (Yungas region), *Nyssomyia whitmani* and *Mycropogomyia quinquefer* (Paranaense region) have been found naturally infected with *L. (V.) braziliensis*, whereas *Lutzomyia longipalpis* (Paranaense region) has been found naturally infected with *L. infantum* (Córdoba Lanús et al., 2006; Salomón et al., 2006). In the phytogeographic region of the humid Chaco, *Ny. neivai* is the most abundant, followed by *Mygonyemyia migonei*, similar to that observed in the regions of the Yungas and Paranaense. In the dry Chaco region, however, the dominant species are *My. migonei*, *Evandromyia cortelezzii* and *Evandromyia sallesi*, whereas *Ny. neivai* is less abundant or absent (Salomón et al., 2008b, 2011; Rosa et al., 2010). Given the lack of information on natural infection of sand flies and the continuous incidence of new cases of ACL in the west

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phytogeographic region of the province of Chaco and the low abundance of vectors incriminated in other regions, the purpose of this study was to demonstrate the natural infection of sand flies with *Leishmania* and typify the parasite species with the aim to better understand their involvement as vectors.

Study area and sampling site. The study was made from November 2006 to December 2007 in the Municipio Misión Nueva Pompeya (24°55'S, 61°35'W) in the dry phytogeographic region of Western Chaco, Chaco, Argentina. Two sites were selected: Fortín Arenales (24°58'S, 61°21'W), because it presented a human case of active ACL, and Los Pozos (24°54'S, 61°22'W), because it is a historic site of transmission (Fig. 1). Two dwellings were chosen at each site (Stations 1 and 2). The ecotones are quebracho (*Schinopsis* and *Aspidosperma*) and palo santo forests (*Bulnesia*), as well as cardonales and vinalares (*Prosopis ruscifolia*). The two sites showed evidence of deforestation and human impact on the forest.

Capture of *Phlebotominae*. Sand flies were caught with CDC-like light traps in the domicile (inside the house), in the peridomicile (hen-houses, lamb-pens or cattle-sheds, goats and horses) up to 10 m from the house, and in the forest, up to 110 m from the house. The traps were left for at least 12 h (19:00 to 07:00 h), monthly.

The live specimens trapped were separated into containers suitable for transport to the laboratory for studies of natural infection with *Leishmania* and taxonomic identification highlighting both *Ev. sallesi* and *Ev. cortezezzii* females were identified as belonging to the *cortezezzii* complex, because these sand flies are indistinguishable by morphological characteristics (Galati, 2003).

Natural infection. Dissection. Female sand flies were dissected individually to observe intestinal flagellates by light microscopy, and then identified and pooled in groups of 10 specimens of the same species for DNA extraction and PCR technique.

DNA extraction and multiplex PCR-Dot blot hybridization. The insects (pools of unfed males and females) were subjected to molecular analysis for *Leishmania* infection. The pools were stored at -20°C until DNA extraction. DNA was extracted as previously described by Pita Pereira et al. (2005) and rigorous procedures were assumed to control potential contamination, e.g. we included negative control groups (male sand flies) in the DNA extraction step and decontaminated instruments and working areas with diluted chloride solution and ultraviolet light. The multiplex PCR was designed to simultaneously amplify the cacophony gene IVS6 region in sand flies of the neotropical genus *Lutzomyia* (as an internal control for

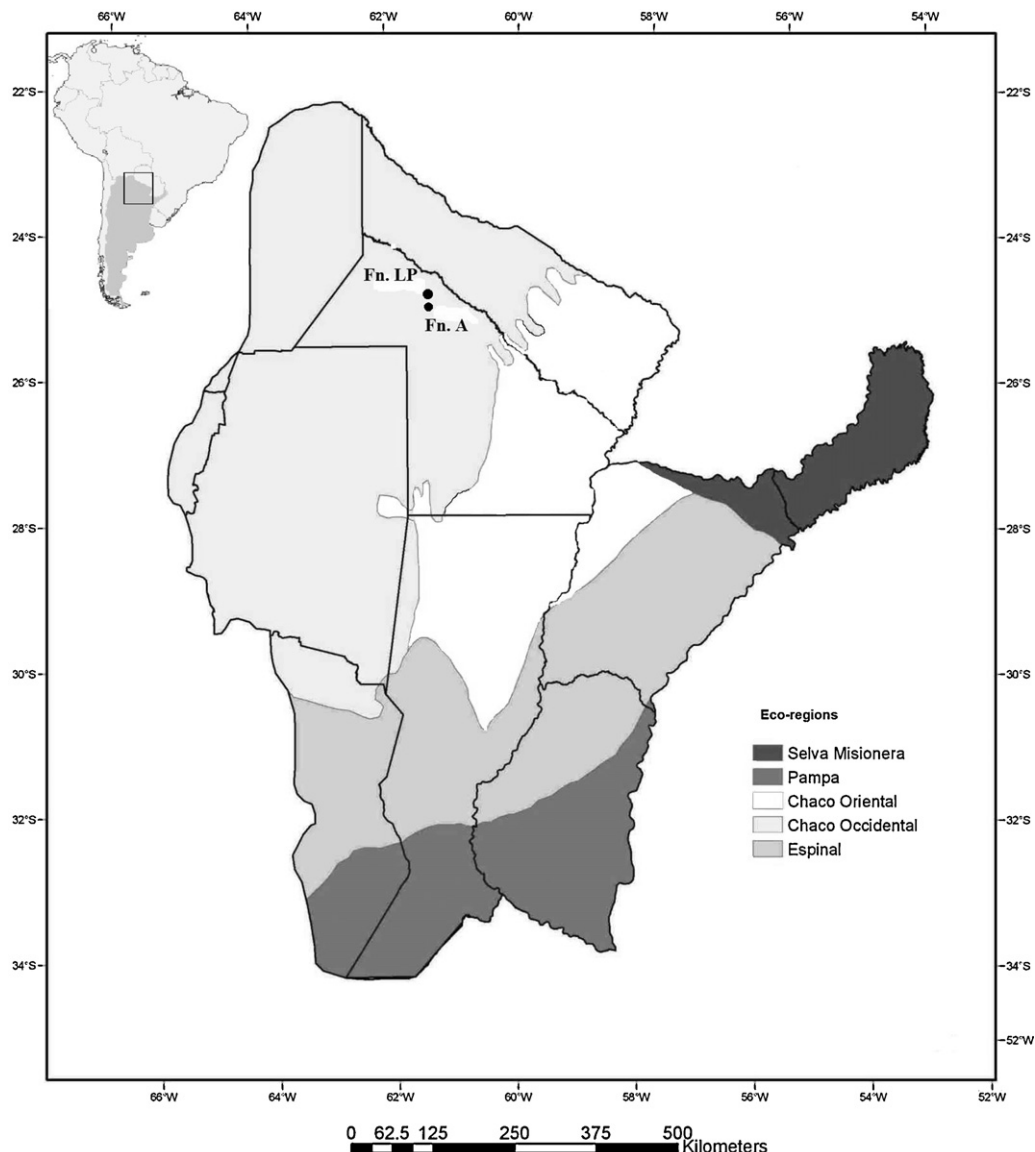


Fig. 1. Study area and its location in South America.

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