

Lutzomyia Sand Fly Diversity and Rates of Infection by *Wolbachia* and an Exotic *Leishmania* Species on Barro Colorado Island, Panama

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Abstract

Background: Sand flies (Diptera, Psychodidae, Phlebotominae) in the genus *Lutzomyia* are the predominant vectors of the protozoan disease leishmaniasis in the New World. Within the watershed of the Panama Canal, the cutaneous form of leishmaniasis is a continuous health threat for residents, tourists and members of an international research community. Here we report the results of screening a tropical forest assemblage of sand fly species for infection by both *Leishmania* and a microbe that can potentially serve in vector population control, the cytoplasmically transmitted rickettsia, *Wolbachia pipientis*. Knowing accurately which *Lutzomyia* species are present, what their evolutionary relationships are, and how they are infected by strains of both *Leishmania* and *Wolbachia* is of critical value for building strategies to mitigate the impact of this disease in humans.

Methodology and Findings: We collected, sorted and then used DNA sequences to determine the diversity and probable phylogenetic relationships of the Phlebotominae occurring in the understory of Barro Colorado Island in the Republic of Panama. Sequence from CO1, the DNA barcoding gene, supported 18 morphology-based species determinations while revealing the presence of two possible "cryptic" species, one (*Lu. sp. nr vespertilionis*) within the *Vespertilionis* group, the other (*Lu. gomezi*) within the *Lutzomyia-cruciata* series. Using ITS-1 and "minicircle" primers we detected *Leishmania* DNA in 43.3% of *Lu. trapidoi*, 26.3% of *Lu. gomezi* individuals and in 0% of the other 18 sand fly species. Identical ITS-1 sequence was obtained from the *Leishmania* infecting *Lu. trapidoi* and *Lu. gomezi*, sequence which was 93% similar to *Leishmania (viannia) naiffi* in GenBank, a species previously unknown in Panama, but recognized as a type of cutaneous leishmaniasis vectored broadly across northern and central South America. Distinct strains of the intracellular bacterium *Wolbachia* were detected in three of 20 sand fly species, including *Lu. trapidoi*, in which it frequently co-occurred with *Leishmania*.

Conclusions: Both morphological and molecular methods were used to examine an assemblage of 20 sand fly species occurring in the forests of the Panama Canal area. Two of these species, members of separate clades, were found to carry *Leishmania* at high frequency and hence are likely vectors of leishmaniasis to humans or other mammal species. A single *Leishmania* species, identified with high confidence as *Le. naiffi*, was carried by both species. That *Le. naiffi* is known to cause cutaneous lesions in South America but has hitherto not been reported or implicated in Panama opens the possibility that its range has recently expanded to include the Isthmus or that it occurs as a recent introduction. The occurrence of *Leishmania* and *Wolbachia* in *Lu. trapidoi* identifies one important vector of the disease as a potential target for gene introductions using *Wolbachia* population sweeps.

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