Title: ***Whole genome sequencing of Leishmania species causing Cutaneous Leishmaniasis in South America***

Leishmania protozoans cause significant disease burden in many regions of the world. Many *Leishmania* species endemic to South America cause Cutaneous Leishmaniasis (CL), with limited disease treatment options and efficacy. There are differences in the CL clinical outcomes, varying from a single to diffuse lesions and mucocutaneous manifestations, which might be a consequence of differences in the host and/or the parasite genome.

The delimitation of *Leishmania* species that causes CL species in Brazil is not completely understood, due to a lack of chromosomal level genome assemblies to represent the full gene repertoire of some species; and/or lack of substantial data from field isolates to estimate its intra-population variation. These parasites often have similar genomes, and natural hybridization was already detected in multiple populations, such as between *L. braziliensis* and *L. peruviana*, which hampers discerning between species limits and geographic variation. The occurrence of hybridization allows beneficial mutations to spread throughout populations, which is of great importance to study virulence factors and drug sensitivity .

Here we have generated chromosome-level assemblies of 12 strains/species of *Leishmania*: *L. braziliensis (M2903)*, *L. equatorensis*, two strains of *L. lainsoni*, *L. naiffi*, *L. panamensis*, *L. shawi*, *L. adleri*, *L. hertigi*, *L. pifanoi*, *L. colombiensis*, and an new *L. Viannia* hybrid isolate. Using a combination of Oxford Nanopore long reads and Illumina short reads, we have built a genome assembly pipeline using the Necat assembler, and extensive polishing steps before annotation. This resulted in a significant improvement in the quality and continuity of the genome assemblies. In the case of four of these species (L. lainsoni, L. panamensis, L. naiffi, and L. guyanensis) we have significantly improved the quality of genome assembly over the currently available data, while for the remaining species, we generated the first chromosomal level genome assembly reference.

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