Whole genome sequencing of *Leishmania braziliensis* in clinical samples demonstrates long-term recurrent recombination in a population of parasites from Southern Peru

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Peru is one of the countries with the highest burden of tegumentary leishmaniasis (TL) in the world. In the Amazonian Forest, *L. braziliensis* is the most prevalent species of *Leishmania*, which is known to be genetically very heterogeneous. Using whole genome sequencing (WGS) of cultivated parasites isolated from 1994 to 2002, our group discovered ancestral populations isolated in patches of tropical rainforest [Heeren et al., 2023]. In addition, a large number of parasites from Southern Peru showed mixed ancestry, resulting from multiple intra-species hybridization events, probably favored by environmental destruction in that zone, together with migration of human and hemerophile reservoir like dogs and rats. Interestingly, these hybrids were often associated with treatment failure and 80% of them were shown to harbour the endosymbiotic LRV1 virus. The presence of these hybrids in that region could be of relevance for public health, and their fate since the early sampling was studied in the present follow-up study.

More specifically, we aimed to assess if these hybrid parasites are still dominant today in southern Peru and if they continue to recombine or predominantly reproduce clonally. We sampled patients from Southern Peru in 2019-2020 and used a culture-independent WGS protocol to avoid selection biases associated with *in vitro* maintenance. Our study consist of two parts. **1)** the validation of a robust direct sequencing protocol on clinical samples with extremely low parasitaemia levels (median 0.01% of parasite DNA): we compared the performance of two direct sequencing methods (Selective Whole Genome Amplification [SWGA] and SureSelect-based target genome capture [SuSL]) on 17 samples collected from TL patients in the departments of Cusco and Madre de Dios. SuSL outperformed SWGA by providing more even and higher genomic coverage which allowed reliable analysis of population genomic structure, somy levels, and maxicircle sequence, identification of major copy number variations, and better coverage of genes associated with drug resistance and virulence. **2)** population genomic analyses of these clinical samples was integrated with our previously obtained genomic data of cultured isolates collected in this region. SuSL allowed identification and ancestry analysis of hybrid parasites: 13 out of the 17 clinical samples from 2019-2020 exhibited admixed ancestry patterns as the cultured/cryopreserved isolates from 1991-2003: close inspection of local ancestry assignment in individual chromosomes revealed that all hybrid parasites had unique mosaic ancestry patterns between their ancestral components, suggesting that *L. braziliensis* has recurrently recombined in this region for several decades. Besides the technological innovation of SuSL, our study confirms the importance of sexual recombination in *L. braziliensis*, the dominance of hybrids in Southern Peru and the need for genomic surveillance.

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