Received: 2 May 2021 | Revised: 27 June 2021 | Accepted: 5 July 2021

DOI: 10.1111/tbed.14228

Transboundary and Emerging Diseases

WILEY

ORIGINAL ARTICLE

Tegumentary leishmaniasis by *Leishmania braziliensis* complex in Cochabamba, Bolivia including the presence of *L. braziliensis* outlier

Tegumentary leishmaniasis in Cochabamba, Bolivia

Mary Cruz Torrico[1,2,3] | Anna Fernández-Arévalo[3,4,**] | Cristina Ballart[3,5,**] | Marco Solano[1] | Ernesto Rojas[1] | Eva Ariza[3,4] | Silvia Tebar[3] | Daniel Lozano[1,2] | Alba Abras[3,4,6] | Joaquim Gascón[5] | Albert Picado[5,7] | Carmen Muñoz[4,8,9,#] | Faustino Torrico[1,2,#] | Montserrat Gállego[3,5,#]

[1] Facultad de Medicina, Universidad Mayor de San Simón, Cochabamba, Bolivia

[2] Fundación CEADES y Medio Ambiente, Cochabamba, Bolivia

[3] Secció de Parasitología, Departament de Biologia, Sanitat i Medi Ambient, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Barcelona, Spain

[4] Institut de Recerca Biomèdica Sant Pau, Barcelona, Spain

[5] Instituto de Salud Global de Barcelona (ISGlobal), Barcelona, Spain

[6] Laboratori d'Ictiologia Genètica, Departament de Biologia, Universitat de Girona, Girona, Spain

[7] Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland

[8] Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau Barcelona, Barcelona, Spain

[9] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain

Correspondence
Mary Cruz Torrico, Facultad de Medicina, UMSS. Av. Aniceto Arce N° 371, Cochabamba, Bolivia.
Email: mary.torrico@umss.edu
Montserrat Gállego, Secció de Parasitología, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona. Av. Joan XXIII, 27–31, 08028 Barcelona, Spain.
Email: mgallego@ub.edu

[**] Mary Cruz Torrico, Anna Fernández-Arévalo, and Cristina Ballart contributed equally to the work.
[#] Carmen Muñoz, Faustino Torrico, and Montserrat Gállego should be considered as joint senior author.

Abstract

Leishmaniasis is caused by protozoans of the *Leishmania* genus, which includes more than 20 species capable of infecting humans worldwide. In the Americas, the most widespread specie is *L. braziliensis*, present in 18 countries including Bolivia. The taxonomic position of the *L. braziliensis* complex has been a subject of controversy, complicated further by the recent identification of a particular subpopulation named *L. braziliensis* atypical or outlier. The aim of this study was to carry out a systematic analysis of the *L. braziliensis* complex in Bolivia and to describe the associated clinical characteristics. Forty-one strains were analyzed by sequencing an amplified 1245 bp fragment of the *hsp70* gene, which allowed its identification as: 24 (59%) *L. braziliensis*, 16 (39%) *L. braziliensis* outlier, and one (2%) *L. peruviana*. In a dendrogram constructed, *L. braziliensis* and *L. peruviana* are grouped in the same cluster, whilst *L. braziliensis* outlier appears in a separate branch. Sequence alignment allowed the identification of five non-polymorphic nucleotide positions (288, 297, 642, 993, and 1213) that discriminate

L. braziliensis and *L. peruviana* from *L. braziliensis* outlier. Moreover, nucleotide positions 51 and 561 enable *L. peruviana* to be discriminated from the other two taxa. A greater diversity was observed in *L. braziliensis* outlier than in *L. braziliensis*-*L. peruviana*. The 41 strains came from 32 patients with tegumentary leishmaniasis, among which 22 patients (69%) presented cutaneous lesions (11 caused by *L. braziliensis* and 11 by *L. braziliensis* outlier) and 10 patients (31%) mucocutaneous lesions (eight caused by *L. braziliensis*, one by *L. braziliensis* outlier, and one by *L. peruviana*). Nine patients (28%) simultaneously provided two isolates, each from a separate lesion, and in each case the same genotype was identified in both. Treatment failure was observed in six patients infected with *L. braziliensis* and one patient with *L. peruviana*.

KEYWORDS
Bolivia, clinical characteristics, *Leishmania braziliensis* complex, response to treatment, sequencing hsp70 gene, tegumentary human leishmaniasis

1 | INTRODUCTION

Leishmaniasis is caused by protozoans of the *Leishmania* genus, which includes more than 20 species capable of infecting humans worldwide. The parasites are transmitted to humans and vertebrate animals by phlebotomine sand flies (Organización Mundial de la Salud, 2021; Pan American Health Organization/World Health Organization, 2019a). The most extended species in the Americas is *L. braziliensis*, which is present in 18 countries (Pan American Health Organization/World Health Organization, 2019b). *Leishmania braziliensis* is widespread in areas of tropical forests, where it is transmitted in wild, peridomestic, and domestic cycles (Ballart et al., 2016; Campbell-Lendrum et al., 2001; Davies et al., 2000; Rojas et al., 2009). This specie is reported to cause tegumentary leishmaniasis (TL) in up to 90% of suspected cases (Garcia et al., 2007; Teles et al., 2015). Although patients with TL can suffer simultaneously from localized cutaneous (CL) and mucocutaneous leishmaniasis (MCL), more frequently the skin lesions evolve to a destructive mucosal inflammation years after their first appearance and when the CL has apparently healed (Burza et al., 2018; Kevric et al., 2015; Reithinger & Dujardin, 2007). After treatment, mucosal lesions can leave mutilating and disfiguring sequelae and even be fatal due to associated infections (Organización Panamericana de la Salud/Organización Mundial de la Salud, 2018; Pan American Health Organization/World Health Organization, 2019b). The other specie within the *L. braziliensis* complex, *L. peruviana*, is limited to regions of the inter-Andean valleys in Peru, and causes skin lesions, with rare affectation of the mucosa (Davies et al., 2000; Kato et al., 2019; Pérez et al., 2007).

The taxonomic position of the *L. braziliensis* complex has been a subject of controversy, *L. braziliensis* and *L. peruviana* being considered as variants of the same species or as two distinct species within the complex (Arana et al., 1990; Bañuls et al., 2000; Chouicha et al., 1997; Valdivia et al., 2015; Van der Auwera & Dujardin, 2015; Van den Broeck et al., 2020). Investigations carried out in Peru have reported the polymorphism of *L. braziliensis* complex, observing two genotypically different groups (groups 1 and 2), and *L. peruviana* has been included in group 1 (Van der Auwera et al., 2014). Other studies have identified hybrids of *L. braziliensis*/*L. peruviana* (Dujardin et al., 1995; Kato et al., 2019; Koarashi et al., 2016) that are capable of producing mucosal lesions (Nolder et al., 2007).

The application of different molecular tools, such as the amplified fragment length polymorphisms (AFLP) and the sequencing of a heat-shock protein *hsp70* gene fragment, has revealed high genetic diversity among isolates of the *L. braziliensis* complex and allowed the identification of a particular subpopulation named *L. braziliensis* atypical or outlier (Odiwuor et al., 2012; Van der Auwera et al., 2013). This subpopulation is apparently widespread in Latin America, being detected in Peru, Bolivia, and Panama (Fraga et al., 2013; Van der Auwera et al., 2016), although few clinical or epidemiological information has been described (Fraga et al., 2013). Previous studies have shown that *L. braziliensis* atypical or outlier strains exhibit a high degree of similarity (98.9-99.7%) for the *mpi*, *mdh*, *gpi*, and *6pgd* genes, with respect to the other isolates of *L. braziliensis*; however, it has several unique polymorphisms in the four genes (Tsukayama et al., 2009).

Considering the genetic variability of the *L. braziliensis* complex and the fact that atypical isolates of *L. braziliensis* have been reported in Bolivia, the aim of the present research was to carry out a systematic study of the complex in the Bolivian department of Cochabamba and identify atypical isolates of *L. braziliensis* by sequencing an *hsp70* gene fragment. Also, the clinical characteristics associated with the complex and the response to TL treatment were investigated. By updating the epidemiological situation of the disease, the results will help this pathology to be controlled in Bolivia.

1.1 | Study population

Thirty-two patients with cutaneous, mucosal, or mucocutaneous lesions, attending the Service of Dermatology of the Tropical Medicine

Center at the Faculty of Medicine, Universidad Mayor de San Simón (UMSS) (Cochabamba, Bolivia) from September 2014 to November 2015, were included. Those with suspected CL and MCL leishmaniasis were referred to the Parasitology laboratory service in the same center for the sample collection.

1.2 | Definitions

Clinical forms: Patients presenting only cutaneous lesions in any area of the body were classified as CL, and those with mucosal lesions in mouth and/or nostrils, or concomitant cutaneous and mucosal lesions, were classified as MCL.

Treatment failure: Patients who had a relapse due to treatment failure were identified from historical clinical data (Ballart et al., 2021). Treatment failures were considered as CL and MCL patients who had previously received a complete antileishmanial treatment in the past, regardless of its duration, and were not cured. In the case of MCL, patients presented an absent or incomplete scarring of lesion(s) and/or persistence of inflammation around the initial lesion, and/or clinical regression of a healed lesion and/or the presence of new mucosal lesion(s). In the case of CL, the previous treatment had to be directed to the same lesion(s) identified during our study.

1.3 | *Leishmania* isolation

Leishmania isolates were obtained by aspiration of the border of the lesions (Torrico-Rojas & Zubieta-Durán 2010) and cultured in TSTB media at 26-27°C (Bermúdez et al., 2005). Isolates were cryopreserved at −80°C in the laboratory of Parasitology at the UMSS in Bolivia and then sent to the Parasitology Laboratory at the University of Barcelona (UB), Spain. Promastigotes were recovered by thawing tubes containing isolates in a water bath at 37°C and cultured in parallel in NNN medium and Schneider's medium (Sigma-Aldrich) supplemented with 20% fetal bovine serum (Life Science Production) and 1% of sterile human urine. When the exponential growth stage was reached, cultures were washed with PBS, and the pellet resuspended in 1 mL of PBS to proceed with the DNA extraction.

1.4 | DNA extraction, amplification, purification and gene sequencing

DNA extraction was performed using the commercial QIAmp DNA Mini Kit (Qiagen) from 200 μL of culture in PBS that was treated with 20 μL of proteinase K following the manufacturer's instructions. The extracted DNA was eluted in 100 μL of AE buffer and stored at −20°C until use. Subsequently, the DNA was quantified in the EpochTM Multi-Volume Spectrophotometer System (BioTek) reader, and the extractions in which the DNA ratio 260/280 was equal to or less than 2 were processed.

The amplification of the 1245 bp *hsp70* gene was performed by using two PCRs that together cover this fragment: PCR-N (552 bp) and PCR-T (723 bp), according to a previously described protocol (Van der Auwera et al., 2013), with the following modifications: for each reaction were used 1 μM of buffer + $MgCl_2$, 200 μM dNTPs, 1 μM of primers F25 and R617 for PCR-N and 6F and R1310 for PCR-T, 1.5 U of DreamTaq DNA polymerase (Thermo Scientific), 5 μL of the extraction product and sterile distilled water to adjust the final volume to 50 μL. The PCR products, together with a negative control, were assessed by 1% agarose gel electrophoresis, 0.5% TBE buffer and ethidium bromide at 100V for 45 min. The fragments were identified in comparison with the DNA Molecular Weight Marker VIII (Roche).

Amplicons were enzymatically purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Scientific), in a ratio of 2:5 (4 μL of ExoSAP in 10 μL amplified product). Double-strand DNA was sequenced by the Sanger method. The sequencing was carried out at the Scientific and Technologic Centers of the UB (Spain).

1.5 | Sequence alignment, identification and polymorphism analysis

The sequences obtained were analyzed and edited with the MEGA 7.0.26 program and submitted to the GenBank databases under the accession numbers MW507486-MW507526 (http://www.ncbi.nlm.nih.gov). Minor errors, such as undefined or mismatched nucleotide positions, were manually corrected. Chromatogram positions with two overlapping nucleotide peaks were considered heterozygous and were corrected according to the IUPAC ambiguity codes (IUPAC, 2020). The alignment of the forward and reverse sequences of each fragment and the subsequent alignment of both fragments was performed with the SerialCloner 1.3.11 program to obtain the consensus sequence of the *hsp70* gene. To identify the strains sequenced, the BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) program was used, which compares the entered sequence with those published in the GenBank database, allowing its identification when regions of similarity are found and the statistical significance calculated.

Only the strains characterized within the *L. braziliensis* complex were taken into account for sequence alignment, population study and polymorphism analysis, in order to describe their genetic and phenotypic characteristics. The sequences obtained were analyzed together with additional sequences of 27 selected reference strains of the *L. braziliensis* complex (*L. braziliensis*, *L. peruviana*, *L. braziliensis* outlier) and other New World species (*L. lainsoni*, *L. naiffi*, *L. guyanensis*, *L. panamensis*) from GenBank (www.ncbi.nlm.nih.gov) (Supporting information Table S1) with the aim of evaluating the location of the studied *Leishmania* strains in the dendrogram. These reference strains were selected with the following criteria: they were published, covered at least 1245 bp of the *hsp70* gene, and were preferably isolated from humans in Bolivia or neighboring countries. Alignment was done with the ClutalW function of the MEGA 7.0.26 program. The ends of the sequences of the reference strains were trimmed to obtain a consensus length of 1245 bp of the *hsp70* gene in the studied Bolivian strains.

Dendrograms were built and the evolutionary relationship of the sequences was calculated using the Neighbor-joining statistical method. All nucleotides were duplicated in order not to lose the information of the heterozygous positions, and the clustering analysis was done using the MEGA 7.0.26 and BioNumerics 7.6.3 (Applied Maths) programs. As external group, a *Trypanosoma cruzi* sequence (KC960000.1) obtained from GenBank was added. The monophyletic groups were calculated with a bootstrap of 1000 replicates. Evolutionary distances were calculated using the p-distance method. In parallel, Neighbour-nets (NN) were constructed using Splitstree version 4.14.8 software (Huson & Bryant, 2006) from hsp70 datasets using the uncorrected p- method and an equal angles representation.

Polymorphism analysis was carried out manually, identifying the nucleotide positions with variability between isolates, which allowed us to discriminate them at the species level and to identify differences in the nucleotide positions between species and sub-species within the *L. braziliensis* complex.

The genetic diversity of sequences and the diversity of haplotypes for the *hsp70* gene were calculated using the program DnaSP version 5.10.01.

The clinical data collected were related to the typified species and sub-species.

1.6 | Ethical aspects

This research was carried out with isolates from patients with suspected TL attending the LABIMED, as part of a collaborative research project between ISGlobal (Barcelona, Spain) and CEADES Foundation (Cochabamba. Bolivia). The study protocol was approved by the Ethics Committees of the *Hospital Clínic de Barcelona* in Spain (HCB/2014/0582); *CEADES Salud y Medio Ambiente* and the *Facultad de Medicina* UMSS both in Bolivia. All suspected cases of leishmaniasis provided written informed consent (parents or guardians in case of patients under 18 years old) before participating to the study. All leishmaniasis suspects were diagnosed for free and CL and MCL confirmed cases were referred for treatment.

2 | RESULTS

2.1 | Identification of the *Leishmania braziliensis* complex

A total of 41 Bolivian strains of the *L. braziliensis* complex (obtained from 32 patients) were analyzed. On the basis of the BLAST results, 24 (59%) were identified as *L. braziliensis* and 16 (39%) as atypical forms of *L. braziliensis* (outlier). The percentage of similarity of these strains with respect to the GenBank reference strains was 99.76-100% (Table 1). One strain (MHOM/BO/2015/CUM-1372) presented the same percentage of similarity (99.92%) to both *L. peruviana* and *L. braziliensis* reference strains (LN907845.1, 1FR715987.1, respectively), but was characterized *as L. peruviana* because it is grouped with the reference strains of *L. peruviana* (Figure 1, bootstrap value 54), and nucleotide positions 51 (A) and 561 (A) enable to distinguish *L. peruviana* from the other two taxa (Table 2).

2.2 | Population study

The dendrogram obtained with the alignment of the 68 sequences (41 strains of the present study, plus the 26 reference strains of *Leishmania* obtained from GenBank and one sequence of *T. cruzi* obtained from the GenBank used as external group) is shown in Figure 1. Two clusters are observed, one corresponding to the subgenus *Viannia* and the other to the sub-genus *Leishmania* (*L. mexicana* and *L. amazonensis* reference strains). Within the *Viannia* subgenus, the *L. lainsoni* strains are separate from the others in their own branch. The *Leishmania guyanensis* and *L. braziliensis* complexes bifurcate from the other branch. *L. braziliensis* and *L. peruviana* appear grouped in a cluster, whilst *L. braziliensis* outlier is grouped in a separate branch together with *L. naiffi* (bootstrap values of 98 and 70, respectively). In the branch of *L. peruviana*, the Bolivian strain CUM-1372 is found in a sub-branch together with a reference strain of *L. braziliensis*.

The Neighbor-net analysis identifies three distinct genetic clusters showing that *L. brazilienis* outlier is clearly separated from *L. braziliensis* and the presence of possible hybrids of *L. braziliensis*/*L. peruviana* (CUM 1343, CUM 1352) located between *L. brazilienis* and *L. peruviana* clusters (Figure 2).

2.3 | Polymorphism analysis

The alignment of the *hsp*70 gene fragment of 1245 bp allowed the identification of three species of the *L. braziliensis* complex: *L. braziliensis* (24 strains), *L. peruviana* (1 strain), and *L. braziliensis* outlier (16 strains), which differ in seven nucleotide positions (Table 2). Five non-polymorphic nucleotide positions (288, 297, 642, 993, and 1213) allow *L. braziliensis* outlier to be discriminated from *L. peruviana* and *L. braziliensis* and two others (51 and 561) allow to discriminate *L. peruviana*. This study revealed 16 genotypes (1-16) among the Bolivian strains (six genotypes of *L. braziliensis*, nine of *L. braziliensis* outlier, and one of *L. peruviana*), plus six different genotypes found among the reference strains (17R-22R) (two genotypes of *L. braziliensis*, three of *L. braziliensis* outlier, and one of *L. peruviana*).

Twenty-seven per cent of the strains (n = 11; 4/24 *L. braziliensis*, 1/1 *L. peruviana*, and 6/16 *L. braziliensis* outlier) showed heterozygosity in any of the nucleotide polymorphic positions analyzed. Traces of genetic exchange between *L. braziliensis* and *L. peruviana* were observed in some of the strains (genotypes 4, 5, 6).

The genetic diversity analysis of the *hsp70* gene fragment performed by the DnaSP version 5.10.01 program, including the *L. braziliensis*-*L. peruviana* group and the *L. braziliensis* outlier sequences, showed four polymorphic sites and four mutations in both groups analyzed (Table 3). A greater genetic diversity (Hd = 0.8) and nucleotide diversity (π = 0.00124) were observed in *L. braziliensis* outlier compared to *L. braziliensis*-*L. peruviana*.

TABLE 1 Identification of Bolivian strains of the Leishmania braziliensis complex by *hsp70* gene sequencing

WHO CODE	GenBank reference strain	Accession Number GenBank	Coverage (% of identity)	Species Identification
MHOM/BO/2014/CUM-1272	MCAN/PE/91/LEM2222	FR715991.1	1245/1245 (100%)	*L. braziliensis* outlier
MHOM/BO/2014/CUM-1275[1]	MHOM/BR/75/M2904	LS997627.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1276[1]	MHOM/BR/75/M2904	FR799003.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1279	MHOM/PE/02/LH2182	FN395040.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1281[2]	MHOM/BR/75/M2904	FR799003.1	1244/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2014/CUM-1282[2]	MHOM/BR/75/M2904	FR799003.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1284	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1286	MHOM/BO/–/CUM180	FN395039.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1288[3]	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1289[3]	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2014/CUM-1292	MHOM/BR/75/M2904	LS997627.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1293	MHOM/PE/–/LH3851	FR872763.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1294[4]	MHOM/PE/03/PER163/0	FR715990.1	1243/1245 (99.84%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1297	MHOM/PE/02/LH2182	FN395040.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1298	MHOM/BR/75/M2904	FR799003.1	1242/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2015/CUM-1307	MHOM/BO/–/CUM180	FN395039.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1309[4]	MHOM/PE/03/PER163/0	FR715990.1	1242/1245 (99.76%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1313[5]	MHOM/PE/03/PER163/0	FR715990.1	1242/1245 (99.76%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1314[5]	MHOM/PE/03/PER163/0	FR715990.1	1242/1245 (99.76%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1318	MHOM/BR/75/M2904	FR799003.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1319	MCAN/PE/91/LEM2222	FR715991.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1320[6]	MHOM/BR/75/M2904	FR799003.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1321[6]	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1330	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1339[7]	MHOM/BR/75/M2904	FR799003.1	1244/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2015/CUM-1343[7]	MHOM/BO/–/CUM68	FR872758.1	1244/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2015/CUM-1347	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1352	MHOM/BO/–/CUM68	FR872758.1	1244/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2015/CUM-1355	MHOM/BO/–/CUM555	FR872760.1	1243/1245 (99.84%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1361	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*
MHOM/BO/2015/CUM-1362[8]	MHOM/PE/–/LH3851	FR872763.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1364	MCAN/PE/91/LEM2222	FR715991.1	1245/1245 (100%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1365	MHOM/PE/03/PER163/0	FR715990.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1366	MHOM/PE/03/PER163/0	FR715990.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1367[9]	MHOM/PE/03/PER163/0	FR715990.1	1243/1245 (99.84%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1368[9]	MHOM/PE/03/PER163/0	FR715990.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1370[8]	MCAN/PE/91/LEM2222	FR715991.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1372	MHOM/PE/01/PER006/1 MHOM/PE/1990/HB86	FR715987.1 LN907845.1	1244/1245 (99.92%) 1244/1245 (99.92%)	*L. peruviana*
MHOM/BO/2015/CUM-1373	IWHI/BR/86/M10187	HF586369.1	1244/1245 (99.92%)	*L. braziliensis*
MHOM/BO/2015/CUM-1374	MHOM/PE/03/PER163/0	FR715990.1	1244/1245 (99.92%)	*L. braziliensis* outlier
MHOM/BO/2015/CUM-1376	IWHI/BR/86/M10187	HF586369.1	1245/1245 (100%)	*L. braziliensis*

The isolates from the same patient (nine patients with more than one isolate) are marked with a superscript 1,2,3,4, … 9 in the WHO code of the strain.

FIGURE 1 Neighbor joining tree based on the *hsp70* gene sequences. The bootstrap values are represented in the nodes. The bottom scale represents the proportional distance to the differences between the alignments.

◆*L. peruviana* strain identified in the study, with genetic profile of *L. peruviana* and *L. braziliensis*.

◇Strains identified in the study as possible *L. braziliensis*-*L. peruviana* hybrids

TABLE 2 Genotypes and nucleotide characteristics of the hsp70 gene (1245 pb) in *L. braziliensis* complex strains isolated in Bolivia and reference strains of GenBank

Species	Genotypes	N° of Strain Study/Ref	Nucleotide characters at the polymorphic sites													
			51	186	288	297	561	642	978	993	1119	1171	1200	1213	1223	1237
L. braziliensis Study 24/Ref.6	1	8/2	G	G	G	C	G	G	C	C	G	T	G	T	A	G
	2	2/1	G	G	G	C	G	G	C	C	G	T	G	T	A	A
	3	8/1	G	G	G	C	G	G	C	C	G	T	G	T	A	**R**
	4	3/0	G	G	G	C	**R**	G	C	C	G	T	G	T	A	G
	5	1/0	G	G	G	C	**R**	G	C	C	G	T	G	T	A	**R**
	6	2/0	**R**	G	G	C	**R**	G	**Y**	C	G	T	G	T	A	G
	17R	0/1	**R**	G	G	C	**R**	G	C	C	G	T	G	T	A	G
	18R	0/1*	A	G	G	C	A	G	T	C	G	T	G	T	A	G
L. peruviana	7	1/0	A	G	G	C	A	G	**Y**	C	G	T	G	T	A	G
Study 1/Ref. [5]	19R	0/5	A	G	G	C	A	G	C	C	G	T	G	T	A	G
L. b. outlier Study 16/Ref. [5]	8	2/0	G	G	A	T	G	A	C	A	G	T	G	A	A	G
	9	1/0	G	G	A	T	G	A	C	A	G	G	G	A	A	G
	10	3/0	G	G	A	T	G	A	C	A	G	G	T	A	C	G
	11	2/1	G	**R**	A	T	G	A	C	A	G	T	G	A	**M**	G
	12	2/0	G	G	A	T	G	A	C	A	G	T	G	A	**M**	G
	13	1/0	G	G	A	T	G	A	C	A	G	K	G	A	**M**	G
	14	1/0	G	G	A	T	G	A	C	A	G	K	G	A	C	G
	15	3/0	G	G	A	T	G	A	C	A	G	K	**K**	A	C	G
	16	1/0	G	G	A	T	G	A	C	A	G	G	**K**	A	C	G
	20R	0/2	G	**R**	A	T	G	A	C	A	**K**	G	G	A	A	G
	21R	0/1	G	**R**	A	T	G	A	C	A	G	T	G	A	A	G
	22R	0/1	G	G	A	T	G	A	C	A	G	**K**	**K**	A	**M**	G

Study: Strains analyzed in the present study (Bolivian strains), R/Ref.: reference strains (GenBank). Sequence variants are noted according to Den Dunnen et al. (2016) and the International Union of Pure and Applied Chemistry (IUPAC, 2020) nomenclature.

*Reference strain characterized as *L. braziliensis* in the GenBank (FR715987.1) but considered to be a sequence of *L. peruviana* by us and some other authors (Odiwuor et al., 2012; Van der Auwera et al., 2013).

Discriminative nucleotide positions are shaded and heterozygote positions are in bold.

TABLE 3 Genetic diversity parameters of *Leishmania braziliensis* complex hsp70 gene sequence

Specie	N	Region	S	Eta	HN	Hd	π	K
L. braziliensis-L. peruviana	25*	1245	4	4	5	0.598	0.00075	0.932
L. b. outlier	16	1245	4	4	6	0.8	0.00124	1.544

N, Number of sequences; S, Number of polymorphic sites; Eta, Total number of mutations; HN, Number of haplotypes; Hd, Haplotype diversity; π, Nucleotide diversity; K, Average number of nucleotide differences.

*In *L. braziliensis* are included the 24 isolates of *L. braziliensis* and 1 isolate of *L. peruviana* from Bolivia grouped in a cluster.

2.4 | Clinical characteristics of Bolivian *Leishmania braziliensis* complex strains

The 41 strains were obtained from 32 patients, 23 (69%) of which provided a single isolate. In the nine patients (31%) providing two isolates, each from a separate lesion and taken on the same day, the same genotype was identified for both isolates in each case. The clinical characteristics associated with the identified species are presented in Table 4. *Leishmania braziliensis* outlier was identified as a causal agent of mucosal involvement in one case (CUM-1307), and was not associated with treatment failure. One third of the patients (6 out of 19 patients, 32%) infected by *L. braziliensis* suffered treatment failure: four had skin lesions (one to three lesions) with an average evolution of 4 months (from 2 to 8 months); the single patient with a chronic skin lesion did

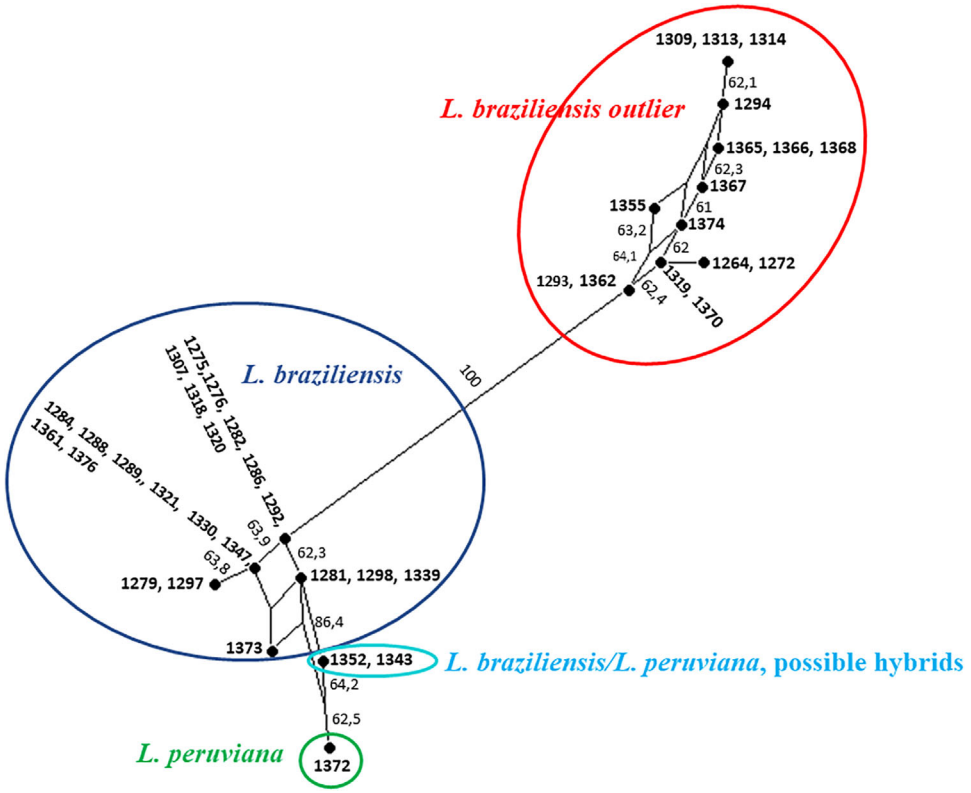

FIGURE 2 Genetic distance evaluation of *hsp70* by Neighbor-net (NN) in *Leishmania braziliensis* complex. Bootstrap values (1000 replicates) are shown on the edges (percentages)

TABLE 4 Leishmania braziliensis complex strains analyzed and their relationship with clinical characteristics

Species	N° of isolates	N° of patients	N° lesion per patient	Patients with CL	Patients with MCL	Patient with treatment failure
L. braziliensis	24	19	1 – 6	11	8	6*
L. peruviana	1	1	1	0	1	1
L. braziliensis outlier	16	12	1 – 3	11	1	0
Total	41	32	––	22	10	7

CL, cutaneous leishmaniosis; MCL, mucocutaneous leishmaniosis.
*Four patients with localized cutaneous lesions and two patients with mucocutaneous lesions.

not remember the time of onset, or did not the one patient with chronic mucosal lesions. The *L. peruviana* strain was isolated from a patient suffering from mucosal lesions and with treatment failure (CUM-1372).

The CUM-1372 strain, which was identified as *L. peruviana*, was isolated from a 71-year-old male patient living in Puerto Zudañes (Chapare province, Cochabamba), who had two scars, one on the face and one on an upper limb, and active lesions in the nostrils. The patient referred that the skin lesions were active approximately 5 years ago (around 2010) and were healed with one or two ampoules of meglumine antimoniate (Glucantime®) purchased from local pharmacies and applied repeatedly. In addition, the patient reported that in 2010 he already had mucosal lesions, and in 2015 he traveled to the city of Cochabamba to request medical attention being confirmed the diagnostic of MCL in our laboratory. He first received a complete treatment with Glucantime® but did not improve. After several months, he was hospitalized for 3 months to receive a complete treatment with amphotericin B, which led to an apparently complete recovery. In 2015, the mucosal lesions reactivated and he returned to Cochabamba to receive another full treatment with amphotericin B.

3 | DISCUSSION

3.1 | Identification of *the Leishmania braziliensis* complex and polymorphism analysis

Several techniques (biochemical, molecular, and proteomic) have been used to identify the *Leishmania* species, responsible for different clinical

forms of leishmaniasis around the world (Akhoundi et al., 2017; Arana et al., 1990; Lachaud et al., 2017; Van der Auwera et al., 2013, 2014). PCR and sequencing of conserved gene amplified products are currently widely employed. The genes encoding the *hsp*70 proteins were among the first to be used in kinetoplastid characterization, as they are highly conserved and present in various copies in tandem (Folgueira et al., 2007). They have been useful for phylogenetic and taxonomic studies of *Leishmania* in both the New and Old Worlds (Conter et al., 2019; Odiwuor et al., 2012; Van der Auwera & Dujardin, 2015; Van der Auwera et al., 2013) and have proved to be suitable and sensitive targets for the typing of neotropical *Leishmania* species from tissues (García et al., 2004) giving reproducible results.

In the present study, PCR and sequencing of a 1245 bp amplified fragment of the *hsp70* gene allowed the phylogenetic analysis of *L. braziliensis* complex strains isolated in Bolivia, as already carried out in other countries in Latin America (Fraga et al., 2010; Van der Auwera & Dujardin, 2015; Van der Auwera et al., 2014). The analysis showed the presence of *L. braziliensis*, *L. peruviana*, and *L. braziliensis* outlier. The identification of *L. braziliensis* outlier has epidemiological and clinical relevance, as no systematic studies of this type with TL-causing *Leishmania* strains have been carried out previously in Bolivia, or in other Latin American countries. This, despite the fact that several authors have reported atypical species in Peru, Panama, and Bolivia (Fraga et al., 2013; Odiwuor et al., 2012) named as "atypical *L. braziliensis*" (Fraga et al., 2013), *"L. braziliensis* type 2" (Van der Auwera et al., 2014), *"L. braziliensis* type 3" (Odiwuor et al., 2012) and "*L. braziliensis* outlier" (Van der Auwera et al., 2013). Indeed, there is not yet a consensus in their taxonomic name neither position, despite being more prevalent and easier to discriminate than *L. peruviana*. A more detailed phylogenetic study is needed to clarify its taxonomic status (Van der Auwera et al., 2015).

Based on the findings of this study regarding *L. peruviana*, the reference strain MHOM/PE/01/PER006/1 (= MHOM/PE/01/LH2140), characterized as *L. braziliensis* FR715987.1 (Adaui et al., 2011), may also belong to *L. peruviana* (genotype 19R of our study). In fact, this reference strain is reported to have mixed alleles from both species, clustering with *L. peruviana* in some studies and with *L. braziliensis* in others (Odiwuor et al., 2012; Van der Auwera et al., 2013).

This study as well demonstrated the genetic diversity of the *hsp70* gene among the *L. braziliensis* complex which is divided into two clearly differentiated clusters, the so-called *L. braziliensis* "outlier", and the second corresponds to *L. braziliensis*, where the Bolivian and reference strains of *L. peruviana* are also located. Similar results have been obtained in studies using AFLP of the *hsp70* gene or MLST analysis of four markers (*hsp70* gene, 7SLRNA gene, rDNA ITS1 and miniexon) (Odiwuor et al., 2012; Van der Auwera et al., 2014). However, in some cases, despite clustering separately, *L. braziliensis* outlier and *L. braziliensis* were considered to be sister clades, *L. peruviana* being grouped with the latter (Van der Auwera et al., 2013).

L. braziliensis is known to be the predecessor of *L. peruviana*, which evolved in adaptation to the different ecosystem in Peru (Dujardin et al., 1995), with hybrids of *L. braziliensis*/*L. peruviana* being reported (Kato et al., 2016; 2019; Nolder et al., 2007; Odiwuor et al., 2012). However, nothing is known about the evolution of *L. braziliensis* outlier. The genetic diversity index showed that *L. braziliensis* outlier has greater diversity than *L. braziliensis*, these data supporting that *L. braziliensis* outlier diverges from *L. braziliensis*, which can also be observed in both the Neighbor-joining dendrogram, and in the Neighbor-Net analysis.

A geographical origin could be ruled out, as *L. braziliensis* and *L. braziliensis* outlier are both widely distributed in Latin America, namely in Peru, Panama and Bolivia (Fraga et al., 2013), and it seems that the two groups of parasites are sympatric. Due to parasites included in this study were isolated during two consecutive years, the identified genotypes existed simultaneously and the possibility of a time bias can be excluded (Odiwuor et al., 2012).

The finding of 16 genotypes within the *L. braziliensis* complex, indicates a high degree of polymorphism in the *hsp*70 gene, especially among *L. braziliensis* outlier strains. Four types of ambiguities were identified (R, Y, K, M), with up to three ambiguities in the strains from genotype 6 (CUM-1343 and CUM-1352) corresponding to possible hybrids of *L. braziliensis*/*L. peruviana*. This finding is clinically relevant because hybrids can potentially cause mucosal leishmaniasis (Nolder et al., 2007). In our study, only the CUM-1343 strain was associated with a chronic mucocutaneous lesion with evolution time unknown (> 12 months), whereas the CUM-1352 strain was associated with skin lesion with three months of evolution. None of these two strains responded to the treatment with meglumine antimoniate (Glucantime®).

A previous investigation carried out with isolates from Peru, Panama and Bolivia, based on the analysis of a 1380 bp sequence of the *hsp70* gene, also showed ambiguities: up to four in group 1 (*L. braziliensis*) and group 3 (*L. braziliensis* atypical), and as many as seven in intermediate isolates between group 1 and 3 (Odiwuor et al., 2012), which may be due to *L. braziliensis*/*L braziliensis* outlier hybrids (Van der Auwera et al., 2013). The presence of numerous ambiguities could be related to genetic recombination events among parasite populations, as shown in a study of the *L. braziliensis* complex, or to genetic exchange, as demonstrated in *L. donovani* (Boité et al., 2012;; Fernández-Arévalo et al., 2020; Lukeš et al., 2007). The presence of *L. braziliensis* outlier with three nucleotide ambiguities suggest low level of genetic recombination in the strains circulating in Bolivia or possibly clonal and occasional sexual reproduction (Cupolillo et al., 1998). The sequencing analysis of four genes (*mpi*, *mdh*, *gpi*, and *6pgd*) with atypical patterns in MLEE, has allowed the characterization of New World *Leishmania* species. Moreover, as a high number of single nucleotide polymorphisms in these genes are found on different chromosomes, it is thought that the variation is distributed throughout the genome, indicating that the divergence of this group of atypical parasites did not occur recently (Tsukayama et al., 2009).

On the other hand, the CUM-1372 strain (identified as *L. peruviana*) presented a single ambiguity (Y) in position 978, which is the only difference with the sequences of the *L. peruviana* reference strains used in this study. Thus, this could represent the first report of *L. peruviana* in Bolivia, or a possible hybrid of *L. braziliensis*/*L. peruviana*, as it has the genetic profile of both species.

A previous study with complete genome sequencing including 67 strains of the *L. braziliensis* complex from Peru suggested that deforestation in the last 150,000 years has influenced the speciation and diversity of parasites, and whole genome analysis demonstrated a meiotic-like recombination between Andean and Amazonian *Leishmania* species, resulting in a full-genome hybrid (Van den Broeck et al., 2020). The identification of possible hybrids in Bolivia is therefore another reason to suspect that *L. peruviana* could also be circulating in this country.

3.2 | Clinical characteristics of Bolivian *Leishmania braziliensis* complex strains

The *L. braziliensis* complex was identified as responsible for TL in 85.4% of the isolates obtained in the region of Cochabamba in Bolivia. Our results agree with those described in other endemic areas of TL in Bolivia, such as 93% in Chapare, as well department of Cochabamba, and 65.5% in the department of La Paz (Bilbao-Ramos et al., 2017). Similar results have been obtained in other Latin American countries (Davies et. al., 2000; Montalvo et al., 2016; Teles et al., 2015).

In our study28% of patients presented chronic mucosal lesions with more than 12 months of evolution and involvement of the oral and nasal mucosa: one patient by *L. braziliensis* outlier, one by *L. peruviana* and seven by *L. braziliensis*. According to previous reports, from 1 to 10% of skin lesions caused by *L. braziliensis* tend to develop serious and disfiguring mucocutaneous lesions, and can even lead to pneumonia (Burza et al., 2018; Pan American Health Organization/World Health Organization, 2019b).

In the present study, *L. braziliensis* outlier was isolated from skin lesions more frequently (87.5%). Two strains of *L. braziliensis* outlier were isolated simultaneously from chronic mucosal lesions (oral and nasal > 12 months of evolution) corresponding to the same patient. So far, few publications have described the clinical and epidemiological characteristics of infections with *L. braziliensis* outlier in other countries; it has been isolated from patients with skin lesions (Odiwuor et al., 2012), whereas in a study that typified five strains as *L. braziliensis* outlier, three were obtained from mucocutaneous lesions and two from skin lesions (Tsukayama et al., 2009). In addition, hybrids of *L. braziliensis/L. braziliensis* outlier have also been reported, but without a description of the clinical lesions (Van der Auwera et al., 2013).

We identified *L. peruviana* in a patient with mucosal lesions in Bolivia without a history of traveling to Peru, and who works in agriculture in the Chapare region (Cochabamba). The distribution of *L peruviana* was thought to be restricted to endemic areas in the rural Andean and inter-Andean valleys of Peru (between 1000 and 3000 m above sea level) (Arevalo et al., 2007; Koarashi et al., 2016; Kato et al., 2019; Lucas et al., 1998), but it has also been reported from a lowland area and other ecoregions of Peru including the Amazonian jungle (Arévalo et al., 2007). *L. peruviana* usually causes a benign form of cutaneous leishmaniasis known as uta (Laison & Shaw, 1987; Van den Broeck et al., 2020), but cases of mucosal and disseminated leishmaniasis associated with this species have been reported (Espinoza-Morales et al., 2017; Lucas et al., 1998; Organización Mundial de la Salud., 2010).

In a study using *hsp70* PCR RFLP, a Bolivian *L. braziliensis* strain isolated from a mucocutaneous lesion (CUM-29, FN395041) also showed an *L. peruviana* profile (Montalvo et al., 2010). Therefore, the authors considered that more intra-species studies with a higher number of isolates of the *L. braziliensis* complex need to be carried out to explain this behavior. In addition, we identified possible hybrids of *L. braziliensis/L. peruviana* (CUM-1343 and CUM-1352), which are also suspected in Colombia (Montalvo et al., 2016). Hybrids of *L. braziliensis/L. peruviana* have been identified in humans, dogs and sand flies in Huánuco, Peru, where both species are endemic, and are potentially causative of mucosal lesions (Dujardin et al., 1995; Kato et al., 2016; Nolder et al., 2007). Likewise, the hybridization of strains could have clinical implications related to the behavior of the parasite, adaptation to the vector and response to treatment (Hamad et al., 2011).

In Bolivia, leishmaniasis is generally treated with meglumine antimoniate for skin lesions and amphotericin B for mucosal lesions; miltefosine, ketoconazole and itraconazole, among others, are also used with variable results (Ministerio de Salud de Bolivia, 2015). In our study, 22% of the patients infected with the *L. braziliensis* complex presented treatment failure; six patients infected with *L. braziliensis* failed to respond to meglumine antimoniate, whereas only the single patient infected with *L. peruviana* did not improve with amphotericin B. Previous studies in Bolivia reported treatment failure with pentavalent antimonials, miltefosine and even amphotericin B deoxycholate, resulting in the use of combined treatment strategies (Rojas Cabrera et al., 2017) or perilesional treatment (Rojas Cabrera et al., 2019).

Another study in Brazil found an effectiveness of 53.8% in patients infected with *L. braziliensis* treated with meglumine antimoniate whereas a study in Peru reported therapeutic failure in 30.4% of patients infected with *L. braziliensis* and 24.5% for *L. peruviana* (Arevalo et al., 2007). Likewise, it was reported that lesions <5 weeks of evolution, multiple lesions and infection by *L. braziliensis* have the highest risk of therapy failure with sodium stibogluconate (Pentostan®) (Llanos-Cuentas et al., 2008). Comparing these results with ours with meglumine antimoniate, similarities can be observed in the group: multiple lesions (in our study, two and three), chronic evolution (>8 weeks) and the species, as all were typified as *L. braziliensis sensu stricto*.

In our sample, none of the patients infected with *L. braziliensis* outlier presented treatment failure during the time of study including the patient with mucosal lesions. There are no scientific reports referring to the clinical and epidemiological characteristics of *L. braziliensis* outlier infection, possibly because the typing techniques routinely used cannot discriminate the species within the *L. braziliensis* complex (Odiwuor et al., 2012). Thus, more molecular studies on species discrimination and their clinical and epidemiological relevance are required (Tsukayama et al., 2009).

An important observation in our study, not previously reported in Latin America, is that a patient with chronic mucosal lesions (isolate CUM-1372, *L. peruviana*) failed to respond to treatment with both meglumine antimoniate and amphotericin B deoxycholate. Amphotericin B is not usually recommended due to its toxicity (renal, hepatic, etc.), and

is only used as an alternative in cases of treatment failure with first-line drugs or in special situations (Organización Panamericana de la Salud, 2013; Rodríguez Galvis et al., 2020). Treatment failure is known to be more likely in mucosal lesions (Ministerio de Salud de Bolivia, 2015), possibly because this clinical manifestation is related to the immunocompromised status of the patient (perhaps due to difficulty in feeding), a factor that could contribute to the severity of the infection as well as the treatment failure of amphotericin B (Nweze et al., 2020; Van Griensven et al., 2014).

In conclusion, this first systematic genetic analysis of *L. braziliensis* complex isolates from Bolivia has revealed the presence of all the species, *L. braziliensis*, *L. braziliensis* outlier *L. peruviana*, at least in the department of Cochabamba. Regarding their associated clinical characteristics, *L. braziliensis* outlier was frequently isolated in skin lesions, in one case with mucosal involvement. Unlike *L. braziliensis*, no phenotype of treatment failure was observed for infection with this atypical species. The study of populations and the analysis of polymorphisms showed that both groups presented nucleotide ambiguities indicative of genetic recombination processes. In addition, the detection of nucleotide positions allowed groups of the complex to be differentiated. This study demonstrates the feasibility of performing similar interventions that are required in other endemic areas in Bolivia. Also, emphasize the relevance of determining the genetic characteristics, geographical distribution and clinical impact of the *L. braziliensis* complex in order to obtain more knowledge about the epidemiology of TL and its control.

ACKNOWLEDGMENTS

The work carried out in Bolivia was supported by AECID (14-CO1-558). The participating UB and ISGbobal investigators are part of the GREPIMER group (Grup de Recerca en Patología Importada i Malaties Emergents i Re-emergents), which received support from the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR, 2014 SGR 26 and 2017 SGR 924). ISGlobal receives support from the Tropical Disease Cooperative Research Network (RICET) (RD12/0018/0010) and the Spanish Ministry of Science, Innovation and Universities through the "Centro de Excelencia Severo Ochoa 2019-2023" Program (CEX2018-000806-S). ISGlobal is a member of the Centres de Recerca de Catalunya (CERCA) Programme, Government of Catalonia (Spain).

The authors would like to thank all the staff of LABIMED and IIBISMED for their support in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

DATA AVAILABILITY STATEMENT

Sequence data are available in GenBank under the accession numbers MW507486 - MW507526

ORCID

Mary Cruz Torrico https://orcid.org/0000-0001-5478-4881
Anna Fernández-Arévalo https://orcid.org/0000-0003-4925-7184

REFERENCES

Adaui, V., Castillo, D., Zimic, M., Gutierrez, A., Decuypere, S., Vanaerschot, M., De Doncker, S., Schnorbusch, K., Maes, I., Van Der Auwera, G., Maes, L., Llanos-Cuentas, A., Arevalo, J., & Dujardin, J. C. (2011). Comparative gene expression analysis throughout the life cycle of *Leishmania braziliensis*: Diversity of expression profiles among clinical isolates. *PLoS Neglected Tropical Diseases*, *5*(5), e1021. https://doi.org/10.1371/journal.pntd.0001021

Akhoundi, M., Downing, T., Votýpka, J., Kuhls, K., Lukeš, J., Cannet, A., Ravel, C., Marty, P., Delaunay, P., Kasbari, M., Granouillac, B., Gradoni, L., & Sereno, D. (2017). *Leishmania* infections: Molecular targets and diagnosis. *Molecular Aspects of Medicine*, *57*(3), 1–29. https://doi.org/10.1016/j.mam.2016.11.012

Arana, M., Evans, D. A., Zolessi, A. L., Llanos Cuentas, A., & Arévalo, J. (1990). Biochemical characterization of *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Viannia*) *peruviana* by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *84*(4), 526–529. https://doi.org/10.1016/0035-9203(90)90025-A

Arévalo, J., Ramírez, L., Adaui, V., Zimic, M., Tulliano, G., Miranda-Verástegui, C., Lazo, M., Loayza-Muro, R., De Doncker, S., Maurer, A., Chappuis, F., Dujardin, J. C., & Llanos-Cuentas, A. (2007). Influence of Leishmania (Viannia) Species on the Response to Antimonial Treatment in Patients with American Tegumentary Leishmaniasis. *Journal of Infectious Diseases*, *195*(12), 1846–1851. https://doi.org/10.1086/518041

Ballart, C., Torrico, M. C., Vidal, G., Torrico, F., Lozano, D., Gállego, M., Pinto, L., Rojas, E., Aguilar, R., Dobaño, C., Ares-Gomez, S. S., & Picado, A. (2021). Clinical and immunological characteristics of tegumentary leishmaniasis cases in Bolivia. *PLoS Neglected Tropical Diseases*, *15*(3), 1–15. https://doi.org/10.1371/journal.pntd.0009223

Ballart, C., Vidal, G., Picado, A., Cortez, M. R., Torrico, F., Torrico, M. C., Godoy, R., Lozano, D., & Gállego, M. (2016). Intradomiciliary and peridomiciliary captures of sand flies (Diptera: Psychodidae) in the leishmaniasis endemic area of Chapare province, tropic of Cochabamba, Bolivia. *Acta Tropica*, *154*, 121–124. https://doi.org/10.1016/j.actatropica.2015.11.007

Bañuls, A. L., Dujardin, J. C., Guerrini, F., De Doncker, S., Jacquet, D., Arévalo, J., Noel, S., Le Ray, D., & Tibayrenc, M. (2000). Is *Leishmania* (*Viannia*) *peruviana* a Distinct species? A MLEE/RAPD Evolutionary Genetics Answer. *Journal of Eukaryotic Microbiology*, *47*(3), 197–207. https://doi.org/10.1111/j.1550-7408.2000.tb00039.x

Bermúdez, H., Solano, M., Torrico-Rojas, M. C., Carballo-Montero, M., Lafuente-Covarrubias, O., Lara-Arias, M. P., & Paredes-Torrico, P. (2005). Diagnóstico de Leishmaniasis Utilizando Medio de Cultivo TSTB en Pacientes del Trópico de Cochabamba. *Gaceta Médica Boliviana*, *28*(2), 31–35. Retrieved on January 18, 2020, from: http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S1012-29662005000200006&lng=es&tlng=es

Bilbao-Ramos, P., Dea-Ayuela, M. A., Cardenas-Alegría, O., Salamanca, E., Santalla-Vargas, J., Benito, C., Flores, N., & Bolás-Fernández, F. (2017). Leishmaniasis in the major endemic region of plurinational State of Bolivia: Species identification, phylogeography and drug susceptibility implications. *Acta Tropica*, *176*, 150–161. https://doi.org/10.1016/j.actatropica.2017.07.026

Boité, M., Mauricio, I., Miles, M., & Cupolillo, E. (2012). New insights on taxonomy, phylogeny and population genetics of *Leishmania* (*Viannia*) parasites based on multilocus sequence analysis. *PLoS Neglected Tropical Diseases*, *6*(11), e1888. https://doi.org/10.1371/journal.pntd.0001888

Burza, S., Croft, S., & Boelaert, M. (2018). Leishmaniasis. *Lancet*, *392*(10151), 951–970. https://doi.org/10.1016/S0140-6736(18)31204-2

Campbell-Lendrum, D., Dujardin, J. P., Martinez, E., Feliciangeli, M. D., Perez, J. E., Passerat De Silans, L. N. M., & Desjeux, P. (2001). Domestic and peridomestic transmission of American cutaneous leishmaniasis: Changing epidemiological patterns present new control opportunities. *Memórias do Instituto Oswaldo Cruz*, *96*(2), 159–162. https://doi.org/10.1590/S0074027620010002000004

Chouicha, N., Lanotte, G., Pratlong, F., Cuba Cuba, C. A., Velez, I. D., & Dedet, J. P. (1997). Phylogenetic taxonomy of *Leishmania* (*Viannia*) *braziliensis* based on isoenzymatic study of 137 isolates. *Parasitology*, *115*(4), 343–348. https://doi.org/10.1017/S0031182097001376

Conter, C., Mota, C., Andreo dos Santos, B., de Souza Braga, L., De Souza Terron, M., Rocha Navasconi, T., Bekner Silva Fernandes, A., Galhardo Demarchi, I., Reinhold de Castro, K., Alessi Aristides, S., Campana Lonardoni, M., Vieira Teixeira, J., & Verzignassi Silveira, T. (2019). PCR primers designed for new world *Leishmania*: A systematic review. *Expimental Parasitology*, *207*, 107773. https://doi.org/10.1016/j.exppara.2019.107773

Cupolillo, E., Momen, H., & Grimaldi, G., Jr. (1998). Genetic diversity in natural populations of new world *leishmania*. *Memórias do Instituto Oswaldo Cruz*, *93*(5), 663–668. https://doi.org/10.1590/S0074-02761998000500018

Davies, C. R., Reithinger, R., Campbell-Lendrum, D., Feliciangeli, D., Borges, R., & Rodriguez, N. (2000). The epidemiology and control of leishmaniasis in Andean countries. *Cadernos de Saúde Pública*, *16*(4), 925–950. https://doi.org/10.1590/S0102-311X2000000400013

Den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., McGowan-Jordan, J., Roux, A. F., Smith, T., Antonarakis, S. E., & Taschner, P. E. (2016). HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human mutation*, *37*(6), 564–569. https://doi.org/10.1002/humu.22981

Dujardin, J. C., Bañuls, A. L., Llanos-Cuentas, A., Alvarez, E., De Doncker, S., Jacquet, D., Le Ray, D., Arevalo, J., & Tibayrenc, M. (1995). Putative *Leishmania* hybrids in the Eastern Andean valley of Huanuco, Peru. *Acta Tropica*, *59*(4), 293–307. https://doi.org/10.1016/0001-706X(95)00094-U

Espinoza-Morales, D., Lucchetti Rodríguez, A., Silva-Caso, W., Suarez-Ognio, L., Pons, M. J., & Del Valle Mendoza, J. (2017). An atypical case of disseminated cutaneous leishmaniasis due to *Leishmania peruviana* in the valleys of Ancash-Peru. *Asian Pacific Journal of Tropical Medicine*, *10*(11), 1101–1103. https://doi.org/10.1016/j.apjtm.2017.10.001

Fernández-Arévalo, A., El Baidouri, F., Ravel, C., Ballart, C., Abras, A., Lachaud, L., Tebar, S., Lami, P., Pratlong, F., Gállego, M., & Muñoz, C. (2020). The *Leishmania donovani* species complex: A new insight into taxonomy. *International Journal for Parasitology*, *50*(13), 1079–1088. https://doi.org/10.1016/j.ijpara.2020.06.013

Folgueira, C., Cañavate, C., Chicharro, C., & Requena, J. M. (2007). Genomic organization and expression of the HSP70 locus in New and Old World *Leishmania* species. *Parasitology*, *134*(3), 369–377. https://doi.org/10.1017/S0031182006001570

Fraga, J., Montalvo, A. M., De Doncker, S., Dujardin, J. C., & Van der Auwera, G. (2010). Phylogeny of *Leishmania* species based on the heat-shock protein 70 gene. Infection. *Genetics and Evolution*, *10*(2), 238–245. https://doi.org/10.1016/j.meegid.2009.11.007

Fraga, J., Montalvo, A. M., Maes, L., Dujardin, J. C., & Van der Auwera, G., (2013). HindII and SduI digests of heat-shock protein 70 PCR for *Leishmania* typing. *Diagnostic Microbiology and Infectious Disease*, *77*(3), 245–247. https://doi.org/10.1016/j.diagmicrobio.2013.07.023

Garcia, A. L., Parrado, R., De Doncker, S., Bermudez, H., & Dujardin, J. C. (2007). American tegumentary leishmaniasis: Direct species identification of *Leishmania* in non-invasive clinical samples. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *101*(4), 368–371. https://doi.org/10.1016/j.trstmh.2006.06.009

García, L., Kindt, A., Bermudez, H., Llanos-Cuentas, A., De Doncker, S., Arévalo, J., Wilder Quispe Tintaya, K., & Dujardin, J. C. (2004). Culture-Independent Species Typing of Neotropical Leishmania for Clinical Validation of a PCR-Based Assay Targeting Heat Shock Protein 70 Genes. *Journal of Clinical Microbiology*, *42*(5), 2294–2297. https://doi.org/10.1128/JCM.42.5.2294-2297.2004

Garcia, L., Kindt, A., Bermúdez, H., Llanos-Cuentas, A., De Doncker, S., Arévalo, J., Quispe, K., & Dujardin, J. C. (2004). Culture-independent species typing of neotropical *leishmania* for clinical validation of a PCR-based assay targeting heat shock protein 70 genes. *Journal of Clinical Microbiology*, *42*(5), 2294–2297. https://doi.org/10.1128/JCM.42.5.2294-2297.2004

Hamad, S. H., Musa, A. M., Khalil, E. A. G., Abebe, T., Younis, B. M., Elthair, M. E. E., El-Hassan, A. M., Hailu, A., & Bart, A. (2011). *Leishmania*: Probable genetic hybrids between species in Sudanese isolates. *Journal of Microbiology and Antimicrobial*, *3*(6), 142–145.

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution*, *23*, 254–267, https://doi.org.10.1093/molbev/msj030

IUPAC. (2020). IUPAC ambiguity codes. Nucleotide ambiguity code. Nomenclature for Incompletely Specified Bases in Nucleic Acid Sequences. Retrieved from: https://www.dnabaser.com/articles/IUPAC%2Fambiguity%2Fcodes.html

Kato, H., Cáceres, A. G., & Hashiguchi, Y. (2016). First Evidence of a Hybrid of *Leishmania* (*Viannia*) *braziliensis*/*L.* (*V.*) *peruviana* DNA detected from the Phlebotomine sand fly *Lutzomyia tejadai* in Peru. *PLoS Neglected Tropical Diseases*, *10*(1), 1–9. https://doi.org/10.1371/journal.pntd.0004336

Kato, H., Cáceres, A. G., Seki, C., Silupu García, C. R., Holguín Mauricci, C., Castro Martínez, S. C., Moreno Paico, D., Castro Muniz, J. L., Troyes Rivera, L. D., Villegas Briones, Z. I., Guerrero Quincho, S., Sulca Jayo, G. L., Tineo Villafuerte, E., Manrique de Lara Estrada, C., Arias, F. R., Passara, F. S., Ruelas Llerena, N., Kubo, M., Tabbabi, A., … Hashiguchi, Y. (2019). Further insight into the geographic distribution of *Leishmania* species in Peru by cytochrome b and mannose phosphate isomerase gene analyses. *PLoS Neglected Tropical Diseases*, *13*(6), e0007496. https://doi.org/10.1371/journal.pntd.0007496

Kevric, I., Cappel, M. A., & Keeling, J. (2015). New world and old world *leishmania* infections: A practical review. *Dermatologic Clinics*, *33*(3), 579–593. https://doi.org/10.1016/j.det.2015.03.018

Koarashi, Y., Cáceres, A. G., Saca, F. M. Z., Flores, E. E. P., Trujillo, A. C., Alvares, J. L. A., Yoshimatsu, K., Arikawa, J., Katakura, K., Hashiguchi, Y., & Kato, H. (2016). Identification of causative *Leishmania* species in Giemsa-stained smears prepared from patients with cutaneous leishmaniasis in Peru using PCR-RFLP. *Acta Tropica*, *158*, 83–87. https://doi.org/10.1016/j.actatropica.2016.02.024

Lachaud, L., Fernández-Arévalo, A., Normand, A. C., Lami, P., Nabet, C., Donnadieu, J. L., Piarroux, M., Djenad, F., Cassagne, C., Ravel, C., Tebar, S., Llovet, T., Blanchet, D., Demar, M., Harrat, Z., Aoun, K., Bastien, P., Muñoz, C., Gállego, M., & Piarroux, R. (2017). Identification of *Leishmania* by matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry using a free web-based application and a dedicated mass-spectral library. *Journal of Clinical Microbiology*, *55*(10), 2924–2933. https://doi.org/10.1128/JCM.00845-17

Lainson, R., & Shaw, J. (1987). Evolution, classification and geographical distribution. In W. Peters & R. Killick-Kendrick (Eds.), *The Leishmaniases in Biology and Medicine* (Vol., 1). London: Academic Press Inc., pp. 1–120. Retrieved from: https://patua.iec.gov.br/handle/iec/2715?locale-attribute=es

Llanos-Cuentas, A., Tulliano, G., Araujo-Castillo, R., Miranda-Verastegui, C., Santamaria-Castrellon, G., Ramirez, L., Lazo, M., De Doncker, S., Boelaert, M., Robays, J., Dujardin, J. C., Arévalo, J., & Chappuis, F. (2008). Clinical and parasite species risk factors for pentavalent antimonial treatment failure in cutaneous leishmaniasis in Peru. *Clinical Infectious Diseases*, *46*(2), 223–231. https://doi.org/10.1086/524042

Lucas, C. M., Franke, E. D., Cachay, M. I., Tejada, A., Cruz, M. E., Kreutzer, R. D., Barker, D. C., McCann, S. H. E., & Watts, D. M. (1998). Geographic distribution and clinical description of leishmaniasis cases in Peru. *American Journal of Tropical Medicine and Hygiene*, *59*(2), 312–317. https://doi.org/10.4269/ajtmh.1998.59.312

Lukeš, J., Mauricio, I. L., Schönian, G., Dujardin, J. C., Soteriadou, K., Dedet, J. P., Kuhls, K., Tintaya, Q. K. W., Jirků, M., Chocholová, E., Haralambous, C., Pratlong, F., Oborník, M., Horák, A., Ayala, F. J., & Miles, M. A. (2007). Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(22), 9375–9380. https://doi.org/10.1073/pnas.0703678104

Ministerio de Salud de Bolivia. (2015). Norma Nacional y Manual de Procedimientos Técnicos de Leishmaniasis, 365. Retrieved from: https://www.minsalud.gob.bo/images/Documentacion/dgss/Epidemiologia/Leishmaniasis/365-Norma_Nacional_y_Manual_de_Procedimientos_Tcnicos_de_Leishmaniasis-2015.pdf

Montalvo, A. M., Fraga, J., Montano, I., Monzote, L., Van der Auwera, G., Marín, M.., & Muskus, C. (2016). Identificación molecular con base en el gen hsp70 de aislamientos clínicos de *Leishmania* spp. en Colombia. *Biomedica*, *36*(Supl.1), 37–44. https://doi.org/10.7705/biomedica.v36i2.2688

Montalvo, A. M., Fraga, J., Monzote, L., Montano, I., De Doncker, S., Dujardin, J. C., & Van Der Auwera, G. (2010). Heat-shock protein 70 PCR-RFLP: A universal simple tool for *Leishmania* species discrimination in the New and Old World. *Parasitology*, *137*(8), 1159–1168. https://doi.org/10.1017/S0031182010000089

Nolder, D., Roncal, N., Davies, C., Llanos-Cuentas, A., & Miles, M. A., (2007). Multiple hybrid genotypes of *Leishmania* (*Viannia*) in a focus of mucocutaneous leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, *76*(3), 573–578. https://doi.org/10.4269/ajtmh.2007.76.573

Nweze, J. A., Nweze, E. I., & Onoja, U. S. (2020). Nutrition, malnutrition, and leishmaniasis. *Nutrition (Burbank, Los Angeles County, Calif.)*, *73*, 110712. https://doi.org/10.1016/j.nut.2019.110712

Odiwuor, S., Veland, N., Maes, I., Arévalo, J., Dujardin, J. C., & Van der Auwera, G. (2012). Evolution of the *Leishmania braziliensis* species complex from amplified fragment length polymorphisms, and clinical implications. *Infection, Genetics and Evolution*, *12*(8), 1994–2002. https://doi.org/10.1016/j.meegid.2012.03.028

Organización Mundial de la Salud. (2010). Control de la leishmaniasis. Informe de una reunión del Comité de Expertos de la OMS sobre el Control de las Leishmaniasis, Ginebra, 22 a 26 de marzo de 2010. Serie de Informes Técnicos *949*. Retrieved from: https://apps.who.int/iris/bitstream/handle/10665/82766/WHO_TRS_949_spa.pdf?sequence=1&isAllowed=y

Organización Mundial de la Salud. (2021). Leishmaniasis. Retrieved from https://www.who.int/es/news-room/fact-sheets/detail/leishmaniasis

Organización Panamericana de la Salud. (2013). Leishmaniasis en las Américas: Recomendaciones para el tratamiento. Washington, DC. Retrieved from: https://iris.paho.org/handle/10665.2/7704

Organización Panamericana de la Salud/Organización Mundial de la Salud. (2018). Leishmaniasis: Informe Epidemiológico de las Américas, *6*. Retrieved from: http://iris.paho.org/xmlui/bitstream/handle/123456789/34858/LeishReport6_spa.pdf?sequence=5&isAllowed=y

Pan American Health Organization/World Health Organization. (2019a). Leishmaniasis: Epidemiological Report in the Americas, *8*. Retrieved from: https://iris.paho.org/bitstream/handle/10665.2/51734/leishreport8_eng.pdf?sequence=1&isAllowed=y

Pan American Health Organization/World Health Organization. (2019b). Manual of procedures for surveillance and control of leishmaniasis in the Americas. Retrieved from https://iris.paho.org/handle/10665.2/51838

Pérez, J. E., Veland, N., Espinosa, D., Torres, K., Ogusuku, E., Llanos-Cuentas, A., Gamboa, D., & Arévalo, J. (2007). Isolation and molecular identification of *Leishmania* (*Viannia*) *peruviana* from naturally infected *Lutzomyia peruensis* (Diptera: Psychodidae) in the Peruvian Andes. *Memórias do Instituto Oswaldo Cruz*, *102*(5), 655–658. https://doi.org/10.1590/S0074-02762007005000077

Reithinger, R., & Dujardin, J. C. (2007). Molecular diagnosis of leishmaniasis: Current status and future applications. *Journal of Clinical Microbiology*, *45*(1), 21–25. https://doi.org/10.1128/JCM.02029-06

Rodríguez Galvis, M. C., Pérez Franco, J. E., Casas Vargas, M. Y., & Ordoñez Rubiano, M. F. (2020). Effectiveness and safety of amphotericin B deoxycholate, amphotericin B colloidal dispersion, and liposomal amphotericin B as third-line treatments for cutaneous and mucocutaneous leishmaniasis: A retrospective study. *The American Journal of Tropical Medicine and Hygiene*, *102*(2), 274–279. https://doi.org/10.4269/ajtmh.18-0514

Rojas, E., Parrado, R., Delgado, R., Reithinger, R., & García, A. L. (2009). Leishmaniasis in Chaparé, Bolivia. *Emerging Infectious Diseases*, *15*(4), 678–680. https://doi.org/10.1002/hep.22184

Rojas Cabrera, E., Paz, D., Verduguez-Orellana, A., Córdova Rojas, M., & Guzmán Rivero, J. M. (2017). Tratamiento combinado de Leishmaniasis mucosa posterior a falla terapéutica con tratamiento convencional: Reporte de caso clínico. *Gaceta Médica Boliviana*, *40*(1), 46–48.

Rojas Cabrera, E., Verduguez-Orellana, A., Córdova Rojas, M., & Guzmán-Rivero, J. M. (2019). Antimoniato de meglumine perilesional en leishmaniasis cutánea con falla terapéutica sistémica: Serie de casos. *Gaceta Médica Boliviana*, *42*(1), 74–78. Retrieved from: http://www.scielo.org.bo/scielo.php?script=sci_arttext&pid=S1012-29662019000100013&lng=es&tlng=es https://doi.org/10.47993/gmb.v42i1.62

Teles, C. B., Medeiros, J. F., Santos, A. P., de Freitas, L. A., Katsuragawa, T. H., Cantanhede, L. M., Ferreira, R., & Camargo, L. M. (2015). Molecular characterization of American cutaneous leishmaniasis in the triborder area of Assis Brazil, Acre State, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*, *57*(4), 343–347. https://doi.org/10.1590/S0036-46652015000400012

Torrico-Rojas, M. C., & Zubieta-Durán, M. F. (2010). Manual de normas y procedimientos técnicos de laboratorio (para Leishmaniasis); *Ministerio de Salud y Deportes Bolivia*, *20*(4), 2010-06-07, ISBN: 978-9995450-13-7

Tsukayama, P., Lucas, C., & Bacon, D. J. (2009). Typing of four genetic loci discriminates among closely related species of New World *Leishmania*. *International Journal for Parasitology*, *39*(3), 355–362. https://doi.org/10.1016/j.ijpara.2008.08.004

Valdivia, H. O., Reis-Cunha, J. L., Rodrigues-Luiz, G. F., Baptista, R. P., Baldeviano, G. C., Gerbasi, R. V., Dobson, D. E., Pratlong, F., Bastien, P., Lescano, A. G., Beverley, S. M., & Bartholomeu, D. C. (2015). Comparative genomic analysis of *Leishmania* (*Viannia*) *peruviana* and *Leishmania* (*Viannia*) *braziliensis*. *Bmc Genomics [Electronic Resource]*, *16*(1), 1–10. https://doi.org/10.1186/s12864-015-1928-z

Van den Broeck, F., Savill, N. J., Imamura, H., Sanders, M., Maes, I., Cooper, S., Mateus, D., Jara, M., Adaui, V., Arevalo, J., Llanos-Cuentas, A., Garcia, L., Cupolillo, E., Miles, M., Berriman, M., Schnaufer, A., Cotton, J. A., & Dujardin, J. C. (2020). Ecological divergence and hybridization of Neotropical *Leishmania* parasites. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(40), 25159–25168. https://doi.org/10.1073/pnas.1920136117

Van der Auwera, G., Bart, A., Chicharro, C., Cortes, S., Davidsson, L., Di Muccio, T., Dujardin, J., Felger, I., Paglia, M. G., Grimm, F., Harms, G., Jaffe, C., Manser, M., Ravel, C., Robert-Gangneux, F., Roelfsema, J., Töz, S., Verweij, J. J., & Chiodini, P. L. (2016). Comparison of *Leishmania* typing results obtained from 16 European clinical laboratories in 2014. *Eurosurveillance*, *21*(49), 30418. https://doi.org/10.2807/1560-7917.ES.2016.21.49.30418

Van der Auwera, G., & Dujardin, J. C. (2015). Species typing in dermal leishmaniasis. *Clinical Microbiology Review*, *28*(2), 265–294. https://doi.org/10.1128/CMR.00104-14

Van der Auwera, G., Maes, I., De Doncker, S., Ravel, C., Cnops, L., Van Esbroeck, M., Van Gompel, A., Clerinx, J., & Dujardin, J. C. (2013). Heat-shock protein 70 gene sequencing for *Leishmania* species typing in European tropical infectious disease clinics. *Eurosurveillance*, *18*(30), 20543. https://doi.org/10.2807/1560-7917.ES2013.18.30.20543

Van der Auwera, G., Ravel, C., Verweij, J. J., Bart, A., Schonïan, G., & Felger, I. (2014). Evaluation of four single-locus markers for *Leishmania*

species discrimination by sequencing. *Journal of Clinical Microbiology*, *52*(4), 1098–1104. https://doi.org/10.1128/JCM.02936-13

Van Griensven, J., Carrillo, E., López-Vélez, R., Lynen, L., & Moreno, J. (2014). Leishmaniasis in immunosuppressed individuals. *Clinical Microbiology and Infection*, *20*(4), 286–299. https://doi.org/10.1111/1469-0691.12556

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Torrico, M. C., Fernández-Arévalo, A., Ballart, C., Solano, M., Rojas, E., Ariza, E., Tebar, S., Lozano, D., Abras, A., Gascón, J., Picado, A., Muñoz, C., Torrico, F., & Gállego, M. (2021). Tegumentary leishmaniasis by *Leishmania braziliensis* complex in Cochabamba, Bolivia, including the presence of *L. braziliensis* outlier. *Transboundary and Emerging Diseases*, 1–14. https://doi.org/10.1111/tbed.14228