An Updated Geographical Distribution of Leishmania Species Involved in Human Leishmaniasis in Colombia

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Abstract

Leishmaniasis in an endemic disease in Colombia. This country ranks second in number of cases of leishmaniasis in Latin America and is one of the four countries with the largest number of *Leishmania* species. Although leishmaniasis is a notifiable disease, the information about the geographic distribution of *Leishmania* species circulating in the country is fragmented. Since 1990, when the first distribution map was published in Colombia, very few new reports have described the *Leishmania* species present in Colombia and their geographic distribution. In this report we included 1,833 natural isolates obtained from human cases of leishmaniasis. Their geographical origin together with those reported by other authors let us confirm the presence of *Leishmania* species in 30 of 32 Colombian departments, being Quindío and San Andres/Providencia free of transmission. In this work, *L. braziliensis* is described for first time in the departments of Atlántico, Caldas and Vaupes and *L. panamensis* in the departments of Guajira and Vaupés. It is also confirmed that *L. panamensis* and *L. braziliensis* coexist in the same regions being the main causative agents of cutaneous leishmaniasis in Colombia; nonetheless, *L. panamensis* predominates in the north and western regions whereas *L. braziliensis* predominates in the southeast region.

Introduction

Leishmaniasis is a parasitic disease caused by species of the genus *Leishmania*. The parasites are maintained in reservoirs that may be wild or domestic animals, including humans, and are transmitted by the bite of numerous species belonging to the Psychodidae Family and Phlebotominae subfamily (*Lutzomyia*, *Phlebotomus*, *Warileya*, *Sergentomyia* and *Brumptomyia* genus). The disease is characterized by its large clinical polymorphisms and can be grouped into three major categories: cutaneous leishmaniasis (CL) with lesions circumscribed to the skin, mucosal leishmaniasis (ML) characterized by lesions affecting the mucous membranes of the nasooropharynx, and visceral leishmaniasis (VL), with lesions affecting organs and tissues, such as the spleen, liver, and bone marrow. Leishmaniasis in its different clinical forms is endemic in 98 countries located in tropical and subtropical regions worldwide [1, 2].

The World Health Organization (WHO) estimates that leishmaniasis continues to be a major health problem in 4 regions of the world: The Americas, East and North Africa and West and Southeast Asia. There are 92 countries or territories endemics for CL and 83 countries or territories endemic for VL. More than 1 billion people are at risk of infection because they live in endemic areas. It is estimated more than 1 million new cases of CL and 30,000 VL cases occur every year. In 2018, 253,435 new CL and 17,223 new VL cases were reported to WHO, 18% of them in the Americas region (AMR) [3]. In December 2019, the Pan American of Health Organization (PAHO) declared that 17 of the 18 endemic countries in the Americas reported a total of 46,041 new cases of leishmaniasis in the Region, of which, 84% were reported by Brazil (16,432), Colombia (6,362), Peru (6,321), Nicaragua (3,722), Bolivia (3,127) and Venezuela (2,612) [4].

In AMR, where current reporting rates are high, there was a slight decreasing trend in CL cases, from a peak of 71,866 cases in 2005 to 46,265 cases in 2018 [3]. Nevertheless, Colombia has still the second highest number of CL cases in the Americas, after Brazil [2, 3, 5]. Because many cases of CL and VL occur in remote regions, which makes it difficult to quantify the exact burden of the disease, the incidence for Latin America is estimated considering an underreporting of 2.8 - 4.6-fold [2]. To date, 22 species of *Leishmania* are described as human pathogens [1]. The identification has been based on the parasite´s isoenzymes content determined by a multilocus enzyme electrophoresis (MLEE) [6-8] or the detection of parasite membrane biomarkers with monoclonal antibodies (MoAb) [9-11].

Although the MLEE remains as the reference standard technique for the identification of *Leishmania* species, molecular techniques based on variations in homologous DNA sequences such as the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) targeting the hsp70 [12, 13], hsp20 [14], mpi and 6pgd [15], ITS [16, 17] kDNA [18], NAGT gene [19] and miniexon sequences [20] among others, have been used for this propose. More recently PCR coupled to High Resolution Melting (HRM) genotyping analysis [21-23] have also been implemented. Some additional reports of *Leishmania* identification have showed the use of direct Sanger sequence analysis of PCR-amplified genes, like the hsp70 [24, 25], or minicircle kDNA [26], among others.

Even though is well accepted that some species are correlated with some clinical forms, one species may cause different clinical forms. On the other hands, the clinical response to treatments also varies inside species, according to the susceptibility of *Leishmania* species strain to antileishmanial drugs [21]. Therefore, the WHO Expert Committee recommends treating cases with specific therapeutic strategies for the parasite species causing the disease. Hence, identification of the species that cause leishmaniasis is important not only from an epidemiological perspective to clarify the distribution of species in a certain region and the prognosis of the disease but also to make decisions regarding the therapeutic options to treat patients [1]. Thus, elucidating the species distribution in a given geographical area and determining the species of the parasite that is affecting the patient are necessary for the adjustment of treatment [1]. This issue is particularly important in countries such as Colombia, which is one of the four countries worldwide with the highest biodiversity of *Leishmania* species with at least 10 of them circulating in the territory [*L. braziliensis, L. panamensis, L. guyanensis, L. mexicana, L. amazonensis*, *L. infantum, L. colombiensis*, *L. equatorensis,L. lainsoni* and *L. naiffi*] [27-33], all of them isolated from patient samples. Other countries with a high number of *Leishmania* species include Brazil, Venezuela and Nicaragua [1].

Although leishmaniasis is endemic in most of the Colombian territory and it is a mandatory notifiable disease since the end of the 1980's, information about the geographic distribution of the circulating *Leishmania* species in the country is very fragmented. To date, very few publications have described the *Leishmania* species present in Colombia and their geographical distributions. The present work describes and complement the current distribution of *Leishmania* species in Colombia, isolated from patients with different clinical forms of leishmaniasis attended in our laboratory and in endemic transmission foci studied in the last 35 years with the objective to update the information about the geographic distribution of *Leishmania* species in Colombia.

Results

During the work conducted between 1982 and 2020 (September), 8,604 patients with clinical and epidemiological lesions suspected of leishmaniasis were attended in site and during field work or active search. Of these, 4,539 (53%) had parasitological diagnoses of leishmaniasis and they came from 29 out of the 32 Colombia's departments and 398 localities (either municipalities, townships or districts). The majority (99%) of the patients were from rural areas. The largest number of patients diagnosed with leishmaniasis were from the departments of Antioquia and Caldas (Andean region), Chocó (Pacific coast region), Meta (Orinoquian region), Córdoba (Caribbean coast region), and Caquetá (Amazonian region), with 2,424, 447, 438, 182 and 169 patients, respectively (Figure 1).

The species were classified by extrinsic characteristics in 2 subgenera, *Viannia* and *Leishmania*. Of the 4,539 patients positive for leishmaniasis, the *Leishmania* species was identified in 1,833 (40%) patients. In 1,771 isolates the identified strains corresponded to 7 different species: *L. panamensis, L. braziliensis, L. amazonensis, L. guyanensis, L. mexicana, L. infantum* and *L. naiffi*. In the remaining 39 isolates the identification was only achieved for the genus *Leishmania* spp or subgenus *L.* (*Viannia*) spp. The number of the isolates by species and department/region is given in Table 1.

L. panamensis and *L. braziliensis* were the species identified in the highest percentages (78% and 20%, respectively). The remaining 2% of the species corresponded to *L. mexicana, L. infantum*, *L. guyanensis,* and *L. amazonesnsis* (0.1% for each) and mixed *L. braziliensis/L. panamensis* (0.2%). Although an attempt was made to identify all the species by the PCR-RFLP method, in the remained isolates (0.4%) it was not possible to find that they belonged to any known species.

Both *L. panamensis* and *L. braziliensis* were found in 17 of the 29 departments with leishmaniasis cases diagnosed in our laboratory (Figure 1). Mapping analysis by region did show that *L. panamensis* predominated in the northwest pacific coast and Andean regions, and was involved in 92% of cases diagnosed by us in these regions, whereas *L. braziliensis* predominated in the Southeast, Orinoquian and Amazonian regions towards the border with Brazil and being responsible for 74% of the leishmaniasis cases diagnosed by us in those regions.

Antioquia, Tolima, Meta and Cundinamarca departments have the highest diversity with six *Leishmania* species followed by Guaviare and Caquetá with five species of *Leishmania* affecting humans. Santander, Norte de Santander, Casanare and

Cauca have four *Leishmania* species, while the rest of departments have 3, 2 and 1 species.

In Arauca, Cauca, Sucre, Risaralda and Valle only *L. panamensis* was found, whereas in Atlántico and Magdalena *L. braziliensis* was only found (Figure 1). *L. mexicana* and *L. amazonensis* were found in Antioquia. *L. mexicana* and *L. guyanensis* were only found in the department of Meta, and *L. infantum* was found only in the department of Córdoba. In Antioquia and Meta departments, four different species were identified: 3 species were present in both departments (*L. braziliensis, L. panamensis, L. mexicana)* and *L. amazonensis* was found only in the Antioquia department, while *L. guyanensis was* in Meta department).

The comparison of the *Leishmania* species distribution found in the present study with the distribution previously reported by other authors, allowed the update of the information available for *Leishmania* species and their distribution by departments and regions. The table 2 summarizes the reported *Leishmania* species diversity for Colombia. It was observed that *L. panamensis* and *L. braziliensis* are the most frequently reported species. From seven authors that have reported the distribution of *Leishmania* species in Colombia, four of them have reported *L. panamensis* in the departments of Bolivar, Córdoba, Antioquia, Chocó, Caldas, Cundinamarca, Risaralda, Huila, Tolima, Boyacá, Santander and Meta. In turn, *L. braziliensis* have been reported in the departments of Cundinamarca, Santander, Meta and Vichada for four or more authors. Apparently, the departments of Quindío and San Andrés and Providencia archipelago remains free of autochthonous cases of leishmaniasis in Colombia, up to date.

Discussion

To date, there are about a dozen studies that have reported the presence of *Leishmania* species in different geographic regions in Colombia, most of them with a small number of isolates: 340 [27], 511 [28], 76 [29], 137 [30], 16 [31], 35 [21], 81 [33], 327 [32], 93 [12], 273 [34] and 96 [35]. Here we report the geographical distribution of 1,833 isolates of *Leishmania* from patients we have diagnosed from 29 departments in all geographic regions of the country. In this work no isolates from Vichada department were reported.

Our study confirms that leishmaniasis is present in almost the entire country with only two departments with apparently no reports of autochthonous cases of leishmaniasis: San Andres and Providencia and Quindío departments, despite some foci study conducted by us in those departments where no cases were not detected nor infected phlebotomine or reservoirs. The highest percentage of patients were mainly from the department of Antioquia followed by Meta, Chocó, Caquetá, and Caldas. This abundance of cases are because our laboratory is located in Medellín, the capital city of Antioquia department and close foci patients come to our consulting room and also because in these regions we have carried out the largest number of foci studies and clinical trials of the "leishvacin" vaccine between 1998 - 2008 [36] or some therapeutic alternatives such as miltefosine [37], thermotherapy [38] and Anfoleish® [39], or the implementation of the Leishmaniasis Control Program in military forces conducted by us from 2005-2008.

Results showed here agree with results previously reported by other authors, in which the department of Antioquia contributes the largest number of tegumentary leishmaniasis cases in Colombia but also with the highest number of *Leishmania* species (all identified species except *L. guyanensis* and *L. infantum*). Other departments with high numbers of cases in this work were Chocó and Meta followed by Córdoba, Caquetá and Caldas. Several factors explain why Antioquia is the department that reports the highest number of CL cases and they are related to human activities such as deforestation, mining, illicit crops and also as a consequence of the war against illicit crops and groups on the margins of the law.

We also confirm that *L. panamensis* and *L. braziliensis* coexist in the same regions in Colombia but in different proportions according to regions. Thus, *L. panamensis* predominates in the Pacific and Andean regions, whereas *L. braziliensis* predominates in the Orinoquian and Amazonian regions. The presence of *L. panamensis* in the Northwestern Colombia and, *L. braziliensis* in Southwestern Colombia, is understandable because it can be assumed that these species have gradually spread from Panama and Brazil to Colombia, respectively. It can be also assumed that the expansion of both species has found the barrier of the Andean and therefore the expansion has not been perhaps faster to the rest of Colombian territory.

However, there are zones with mixture presence of *L. panamensis* and *L. braziliensis* in the central area of Colombia. How the presence of both species of parasite was achieved in the same territory is something that needs to be determined; it could be likely due to migration in both routes of infected domestic reservoirs, through gallery forests that cross valleys and mountains. On the other hand, the higher prevalence of a species in a given area may be due to the presence and greater abundance of the main vector species in each geographic area or to the presence of new vectors of these *Leishmania* species in these areas. Nonetheless, these hypotheses remain to be validated.

The third most reported species is *L. infantum* which is present in 14 departments where cases of VL has been documented, with predominance in the Caribbean and Andean region but also in the departments of Meta (Orinoquian region) and Putumayo (Amazonian region) (Table 2). Recently, a first case of urban canine VL caused by *L. infantum* was described in Cali, Colombia (Valle department, Pacific region) [40].

The presence of *L. mexicana* and *L. amazonensis* are reported 14 departments of Colombia (Table 2). Both species co-circulating in almost the same departments, with the exception of Nariño, Caldas and Risaralda where only *L. mexicana* circulates and Chocó, Huila and Boyacá where only *L. amazonensis* has been reported [31, 32, 35]. There are no reports of *L. mexicana* nor *L. amazonensis* in the Caribbean region. This work reports *L. amazonensis* in Antioquia, with a strain identified in a patient from Anorí in 1991, maybe representing the first report for Antioquia. *L. mexicana* was reported in Antioquia in 2006 in a patient [41]. Notably, *L. mexicana* has been reported as causative agents of CL, but also as responsible of one case of diffuse CL [42] and other case of transfusion-transmitted VL in a immunocompromised patient [43].

L. guyanensis is reported in 12 departments in Colombia, especially those located in the Andean and Amazonian region but also in the departments of Cordoba and Sucre in the Caribbean region (Table 2). It is notorious that in the Amazon, only *L. guyanensis* has been reported [27] and there are no new reports since then. A recent publication that compiled the scientific literature available for *Leishmania* species identified in Colombia between 1985 and 2017 declares the presence of *L. guyanensis* in Valle, Guajira and Antioquia Departments [44]; nevertheless, it is not clear which authors are responsible for those reports. Few cases of *L. guyanensis* have been reported in Colombia; this could be due to the difficulties in taxonomic differentiation between *L. panamensis* and *L. guyanensis* [29].

Although *L. colombiensis* was identified early in the 80`s in samples from patients from the municipalities of Apartadó and San Carlos in the department of Antioquia [45], and in the sandfly *P. shannoni* from Santander in three individuals belonging to the sample bank of the Colombian National Institute of Health [32], in the present study this species of *Leishmania* was not found in any of the 138 isolates from San Carlos or the 181 isolates from Apartadó. Because no new reports of this species in Colombia have occur since long time ago, it is suggested that the taxonomic classification of this species is controversial.

It is not clear how other new species such as *L. lainsoni* or *L. equatorensis* arrived in some areas of the interior of the country. This does not seem to be due to geographic expansion of these species, as occurs with *L. panamensis* and *L. braziliensis*. Most probably the arrival could be occurred through a human or a reservoir. There is very few information about *L. lainsoni*. Since the description of this species has been recorded in three states of brazilian amazon basin [46], Perú [47], Bolivia [48, 49] and Ecuador [49].

It was expected that this species should be found in the Colombian Amazonian, as Araracuara, Caquetá where *Lu. ubiquitalis,* the insect vector of *L. lainsoni* [50] has been described [51]. Nevertheless, the presence of this species in Antioquia that is intriguing because no incrimination or occurrence of *L.ubiquitalis* has been recorded.

Leishmania and phlebotominae classification remain controversial and currently there is an increasing number of species described and phylogenetic analyses show difficulties with the current classification [52]. For example, the taxonomic classification of *L. archibaldi* as a species following its initial identification as a causative species of VL in Sudan remains controversial. Similarly, even though many authors have accepted that *L. chagasi* is synonymous with *L. infantum* based on its isoenzyme classification, other authors consider the parasite a different species or perhaps a subspecies of *L. infantum*.

In conclusion, this work reports the presence of *Leishmania* species in 30 departments and confirm that San Andres and Providencia and Quindio apparently remained free of transmission. Our finding also concludes that at least ten *Leishmania* species circulate in Colombia thus validating the great biodiversity of *Leishmania* species in this country. In addition, this work complements previous reports by other researchers and increase the area of distribution of *Leishmania* species in Colombia.

Methods

A retrospective study was performed with patients with a confirmed diagnosis of leishmaniasis (cutaneous, mucosal or visceral) treated during the active search for cases performed in multiple transmission foci of Colombia and during consultation at the medical facility of the Program for Study and Control of Tropical Diseases, Universidad de Antioquia, Medellín-Colombia. All experimental protocols were approved by the Human Bioethics Committee of the Sede de Investigación Universitaria, Universidad de Antioquia, Medellín-Colombia.

After sign the Inform Consent Form for use of samples and clinical data in research activities, patients were sampled for parasitological diagnosis of leishmaniasis and species identification. In the case of CL and ML samples were taken by scraping and/or aspirated material from the lesion (bottom and/or borders). After that, scraping material was stained with Giemsa for direct examination under the microscope and aspirated material was cultured in NNN medium (Novy, Nicolle and McNeal) and incubated at 26°C [1]. For patients with ML, samples were taken from aspirated material or scraped of mucosal lesions whiles in patients with VL samples were taken from spleen or bone marrow aspirates [53]. Conventional PCR was also used for diagnosis. All methods were carried out in accordance with WHO guidelines and regulations.

The clinical records of each patient were reviewed, and any information related to the clinical form was extracted, *Leishmania* species and, the most probable place of infection (municipality, county - “vereda” or both), according to the epidemiological information of the patient, displacements in weeks prior to the beginning of the injury, etc. All data analyzed were anonymized.

Over 30 years the identification of *Leishmania* species were carried out using the standardized methodologies recommended by the WHO, using MLEE and MoAb in the 80´s and 90´s and the PCR-RFLP in the last decade. For MLEE and MoAb, isolation of parasite and cultivation in laboratory conditions was mandatory. Thus, between 1984 and 1995, *Leishmania* species were identified according to the zymodeme patterns following the procedure described by Roiux (1990) [7]. From 1995 to 2008, strains were identified using MoAb [9, 11, 54] and since 2009, the identification has been conducted mainly using the PCR-RFLP technique and conducted with DNA isolated both, from parasites in culture or directly from DNA purified from the clinical samples with sets of primers targeting the cysteine B or the Hsp70 genes as described by others [12, 33, 55]. The restriction pattern generated allowed inferring the identity of the *Leishmania* species.

To address the geographical distribution of leishmaniasis cases and *Leishmania* species reported in the present work, a database was constructed with a list of patients with positive diagnosis of leishmaniasis and the and the most probable place of infection Although we have the location at the municipality and village level of most of the patients, the analysis was presented in a digital map at the department level for easier understanding.

Declarations

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Author contributions.

IDV conceptualized the work. SMR and CM wrote the main manuscript text and prepare figures and tables. All authors reviewed the manuscript.

Data availability.

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. World Health Organization. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. World Health Organ *Tech Rep Ser.* **949**, 1–186 (2010).
2. Alvar, J. *et al*. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. **7**, e35671; 10.1371/journal.pone.0035671 (2012).
3. World Health Organization. 2020. Weekly Epidemiological Record, **95**, 265–280 (2020).
4. Organización Panamericana de la Salud. Leishmaniasis: Informe Epidemiológico de las Américas. Washington, D.C.: OPS. Preprint at http://iris.paho.org/xmlui/handle/123456789/50505 (2019).
5. Bern, C., Maguire, J. H. & Alvar, J. Complexities of assessing the disease burden attributable to leishmaniasis. *PLoS Negl Trop Dis*. **2**, e313; 10.1371/journal.pntd.0000313 (2008). Lannote 1986
6. Rioux, J. A. *et al*. Taxonomy of *Leishmania*: Use of isoenzymes. Sugestions for a new classification. *Ann Parasitol Hum Comp*. **65**, 111–125 (1990).
7. Thomaz-Soccol, V. *et al*. Phylogenetic taxonomy of New World *Leishmania*. *Ann Parasitol Hum Comp*. **68**, 104-106 (1993).
8. McMahon-Pratt, D. & David, J. R. Monoclonal antibodies that distinguish between New World species of *Leishmania*. *Nature***291**, 581-593 (1981).
9. Grimaldi, G. Jr, David, J. R. & McMahon-Pratt, D. Identification and distribution of New World *Leishmania* species characterized by serodeme analysis using monoclonal antibodies. *Am J Trop Med Hyg*. **36**, 270-287 (1987).
10. Grimaldi, G. & McMahon-Pratt, D. Monoclonal antibodies for the identification of New World Leishmania species. *Mem Inst Oswaldo Cruz*. **91**, 37-42 (1996).
11. Montalvo, A. M. *et al*. Detection and identification of *Leishmania* spp.: application of two hsp70-based PCR-RFLP protocols to clinical samples from the New World. *Parasitol Res*. **116**, 1843-1848 (2017).
12. Espada, C. R, *et al*. Identification of *Leishmania (Viannia)* species and clinical isolates of *Leishmania (Leishmania) amazonensis* from Brazil using PCR-RFLP of the heat-shock protein 70 gene reveals some unexpected observations. *Diagn Microbiol Infect Dis*. **91**, 312-318 (2018).
13. Montalvo, A. M. *et al*. Improving the sensitivity of an hsp20-based PCR for genus detection of Leishmania parasites in cutaneous clinical samples: a proof of concept. *Parasitol Res*. **119**, 345-349 (2020).
14. Kato, H. *et al*. Further insight into the geographic distribution of *Leishmania* species in Peru by cytochrome b and mannose phosphate isomerase gene analyses. *PLoS Negl Trop Dis.* **13**, e0007496; 10.1371/journal.pntd.0007496 (2019).
15. Ajaoud, M. *et al*. Detection and molecular typing of *Leishmania tropica* from *Phlebotomus sergenti* and lesions of cutaneous leishmaniasis in an emerging focus of Morocco. *Parasit Vectors***6**, 217;10.1186/1756-3305-6-217 (2013).
16. Mohammadi, M. A. *et al*. Comparison of Three PCR-based Methods for Simplicity and Cost Effectiveness Identification of Cutaneous Leishmaniasis Due to *Leishmania tropica*. *Iran J Parasitol*. **12**, 215–223 (2017).
17. Abdolmajid, F. *et al*. Identification of Leishmania species by kinetoplast DNA-polymerase chain reaction for the first time in Khaf district, Khorasan-e-Razavi province, *Iran. Trop Parasitol*. **5**, 50-4 (2015)

18. Saberi, R. *et al*. Identification of *Leishmania*species using *N-acetylglucosamine-1-phosphate transferase*gene in a zoonotic cutaneous leishmaniasis focus of Iran. *J Vector Borne Dis*. **55**, 14-19 (2018)
19. Marfurt, J. *et al*. Identification and differentiation of *Leishmania* species in clinical samples by PCR amplification of the miniexon sequence and subsequent restriction fragment length polymorphism analysis. *J Clin Microbiol*. **41**, 3147-3153 (2003).
20. Hernández, C. *et al*. Identification of six New World *Leishmania* species through the implementation of a High-Resolution Melting (HRM) genotyping assay. *Parasit Vectors*. **7**, 501; 10.1186/s13071-014-0501-y (2014).
21. Kuang, Z., Zhang, C., Pang, H. & Ma, Y. A rapid high-resolution melting method for differentiation of Leishmania species targeting lack gene. *Acta Trop*. **178**, 103-106 (2018).
22. Ghafari, S. M., Fotouhi-Ardakani, R. & Parvizi, P. Designing and developing a high-resolution melting technique for accurate identification of *Leishmania* species by targeting amino acid permease 3 and cytochrome oxidase II genes using real-time PCR and *in silico* genetic evaluation. *Acta Trop*. **211**, 105626; 10.1016/j.actatropica.2020.105626 (2020).
23. Requena, J. M. *et al*. Sequence analysis of the 3'-untranslated region of HSP70 (type I) genes in the genus *Leishmani*a: its usefulness as a molecular marker for species identification. *Parasites Vectors***5**, 87; 10.1186/1756-3305-5-87 (2012).
24. Herrera, G., Higuera, A., Patiño, L. H., Ayala, M. S. & Ramírez, J. D. Description of *Leishmania* species among dogs and humans in Colombian Visceral Leishmaniasis outbreaks. *Infection, Genetics and Evolution*. **64**, 135-138 (2018).
25. Kocher, A., Valière, S., Bañuls, A. & Murienne, J. High-throughput sequencing of kDNA amplicons for the analysis of *Leishmania* minicircles and identification of Neotropical species. *Parasitol.***145**: 585-594 (2018).
26. Corredor, A. *et al*. Distribution and etiology of leishmaniasis in Colombia. *Am J Trop Med Hyg*. **42**, 206-214 (1990).
27. Saravia, N. G. *et al*. Epidemiologic, genetic, and clinical associations among phenotypically distinct populations of *Leishmania (Viannia)* in Colombia. *Am J Trop Med Hyg*. **59**, 86-94 (1998).
28. Thomaz-Soccol, V. *et al*. Enzymatic polymorphism and phylogenetic relationships in Leishmania Ross, 1903 (Sarcomastigophora: Kinetoplastida): A case study in Colombia. *Syst Parasitol*. **46**, 59-68 (2000).
29. Ovalle, C. E., Porras, L., Rey, M,. Ríos , M. & Camargo, Y. C. Distribución geográfica de especies de Leishmania aisladas de pacientes consultantes al Instituto Nacional de Dermatología Federico Lleras Acosta, E.S.E., 1995-2005.*Biomédica* **26**, 145-151 (2006).
30. Urbano, J. *et al*. Characterization of cutaneous isolates of *Leishmania* in Colombia by isoenzyme typing and kDNA restriction analysis. *Rev Ibero-Latinoam Parasitol*. **70**, 16-24 (2011).
31. Ramírez, J. D. *et al*. Taxonomy, diversity, temporal and geographical distribution of Cutaneous Leishmaniasis in Colombia: A retrospective study. *Sci Rep*. **6**, 28266; 10.1038/srep28266 (2016).
32. Montalvo, A. M. *et al*. Molecular identification of *Leishmania* spp. clinical isolates from Colombia based on hsp70 gene. *Biomedica***36**, 37-44 (2016).
33. Patino, L. H. *et al*. Spatial distribution, *Leishmania*species and clinical traits of Cutaneous Leishmaniasis cases in the Colombian army. *PLoS Negl Trop Dis*. **11**, e0005876; 10.1371/journal.pntd.0005876 (2017).
34. Ovalle-Bracho, C., Camargo, C., Díaz-Toro, Y. & Parra-Muñoz, M. Tipificación molecular de *Leishmania (Leishmania) amazonensis* y especies del subgénero *Viannia* asociadas a leishmaniasis cutánea y mucosa en Colombia: un estudio de concordancia. *Biomédica***38**, 1-32 (2018).
35. Vélez, I. D. *et al*. Safety and immunogenicity of a killed *Leishmania (L.) amazonensis* vaccine against cutaneous leishmaniasis in Colombia: a randomized controlled trial. *Trans R Soc Trop Med Hyg*. **94**, 698-703 (2000).
36. Vélez, I. *et al*. Efficacy of miltefosine for the treatment of American cutaneous leishmaniasis. *Am J Trop Med Hyg*. **83**, 351-356 (2010).
37. López, L. *et al*. Thermotherapy. An alternative for the treatment of American cutaneous leishmaniasis. *Trials***13**, 58; 10.1186/1745-6215-13-58 (2012).

38. López, L. *et al*. A phase II study to evaluate the safety and efficacy of topical 3% amphotericin B cream (Anfoleish) for the treatment of uncomplicated cutaneous leishmaniasis in Colombia. *PLoS Negl Trop Dis*. **12**, e0006653; 10.1371/journal.pntd.0006653 (2018).
39. Arbeláez N. *et al*. First Report of an Urban Case of Canine Visceral Leishmaniasis in the Municipality of Cali, Colombia. *Am J Trop Med Hyg*. **102**, 289-293 (2020).
40. Sierra, D. *et al*. Leishmania (Leishmania) mexicana en el corregimiento de San Matías, municipio de Gómez Plata, Antioquia, Colombia. *Biomédica***26**, 232-235 (2006).
41. Pérez, C., Solías, Y. & Rodríguez ,G. [Diffuse cutaneous leishmaniasis in a patient with AIDS]. *Biomedica***26**, 485-97 (2006). Erratum in: *Biomedica***27**, 149 (2007).
42. Mestra, L. *et al*. Transfusion-transmitted visceral leishmaniasis caused by *Leishmania (Leishmania) mexicana* in an immunocompromised patient: a case report. *Transfusion***51**, 1919-1923 (2011).
43. Salgado-Almario, J., Hernández, C. A., Ovalle, C. E. Geographical distribution of *Leishmania* species in Colombia, 1985-2017. *Biomedica*. **39**, 278-290 (2019).
44. Kreutzer, R. D. *et al*. Characterization of *Leishmania colombiensis* sp. n (Kinetoplastida: Trypanosomatidae), a new parasite infecting human, animals, and phlebotomine sand flies in Colombia and Panama. *Am J Trop Med Hyg*. **44**, 662-675 (1991).
45. 4 Silveira, F. T., Shaw, J. J., Braga, R. R. & Ishikawa, E. Dermal leishmaniasis in the Amazon region of Brazil: *Leishmania (Viannia) lainsoni* sp.n., a new parasite from the state of Pará. *Mem Inst Oswaldo Cruz***82**, 289-292 (1987).
46. Lucas, C.M. *et al*. *Leishmania (Viannia) lainsoni*: first isolation in Peru. *Am J Trop Med Hyg*.**51**, 533-537 (1994).
47. Martinez, E., Le Pont, F., Mollinedo, S. & Cupolillo, E. A first case of cutaneous leishmaniasis due to *Leishmania (Viannia) lainsoni* in Bolivia. *Trans R Soc Trop Med Hyg*. **95**, 375-377 (2001).
48. Bastrenta, B. *et al*. First evidence of transmission of *Leishmania (Viannia) lainsoni* in a sub-Andean region of Bolivia. *Acta Trop*.**83**, 249-253 (2002).
49. Kato, H. *et al*. First Human Cases of *Leishmania (Viannia) lainsoni* Infection and a Search for the Vector Sand Flies in Ecuador. *PLoS Negl Trop Dis*. 10, e0004728. doi: 10.1371/journal.pntd.0004728 (2016).
50. Silveira, F. T. *et al*. Cutaneous leishmaniasis in the Amazon region: natural infection of the sandfly *Lutzomyia ubiquitalis* (Psychodidae: Phlebotominae) by *Leishmania (Viannia) lainsoni* in Pará state, Brazil. *Mem Inst Oswaldo Cruz* **86**, 127-130 (1991).
51. Morales, A. & Minter, D. M. Estudio sobre Flebotomineos en Araracuara Caquetá, Colombia S.A Incluyendo la descripción de *Lutzomyia araracuarensis* (Diptera, Psychodidae). *Biomedica* **1**, 94-116 (1981).
52. Akhoundi, M. *et al*. A Historical Overview of the Classification, Evolution, and Dispersion of *Leishmania* Parasites and Sandflies. *PLoS Negl Trop Dis*. **10**, e0004349; 10.1371/journal.pntd.0004349 (2016).
53. Ramírez, J. R. *et al*. Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. *J Clin Microbiol*. **38**, 3768-3773 (2000).
54. Saravia, N. G. *et al*. Heterogeneity, geographic distribution, and pathogenicity of serodemes of *Leishmania viannia* in Colombia. *Am J Trop Med Hyg*. **66**, 738-744 (2002).
55. Montalvo, A. M. *et al*. Differentiation of *Leishmania (Viannia) panamensis* and *Leishmania (V.) guyanensis* using BccI for hsp70 PCR-RFLP. *Trans R Soc Trop Med Hyg*. **104**, 364-367 (2010).
56. Velez, I. D. *et al*. Evaluación ecoepidemiológica de un foco de leishmaniasis visceral en la comunidad indígena Zenú de san Andrés de Sotavento, Córdoba: Primer paso para su control. *Rev Col Entomol*. **21**, 111-122 (1995).
57. Arroyo, C. & Garzón, J. Investigación de un foco de leishmaniasis cutánea en la zona andina del departamento de Nariño. *Biomédica*. **16**, 25-31 (1995).
58. Rodríguez-Barraquer, I. *et al*. Etiologic agent of an epidemic of cutaneous leishmaniasis in Tolima, Colombia. *Am J Trop Med Hyg*. **78**, 276-282 (2008).

59. Martínez, L. P., Rebollo, J. A., Luna, A. L., Cochero, S. & Bejarano, E. E. Molecular identification of the parasites causing cutaneous leishmaniasis on the Caribbean coast of Colombia. *Parasitol Res.***106**, 647–652 (2010).

60. Sánchez, J. P. *et al*. Ecoepidemiología de la leishmaniasis visceral en Colombia (1943-2019): revisión sistemática. *Hechos Microbiol.***11**, 22-60 (2020).

Tables

Table 1. Origin and number of the *Leishmania* isolates

Region	Department	*Leishmania* species[a]									
		L. b	*L. p*	*L. g*	*L. m*	*L. a*	*L. i*	*L. n*	*L. viannia*	*L.* spp	*L. b/L. p*
Caribbean	Córdoba	9	41				1			3	1
	Sucre		4								
	Bolivar	6	5							1	
	Atlántico	1									
	Magdalena	2									
	Cesar									1	
	Guajira	1									2
	San Andrés and Providence										
Pacific	Chocó	4	183							7	
	Valle		3								
	Cauca		1								
	Nariño	2	11								
Andean	Antioquia	50	933		1	1		1		18	
	Caldas	16	57							3	
	Risaralda		3								
	Quindío										
	Cundinamarca	16	22								
	Huila	1	3								
	Tolima	1	30								
	Santander	3	25								
	Norte Santander	5	1								
	Boyacá	7	9								
Oronoquian	Casanare									1	
	Arauca		1								
	Meta	181	56	1	1				2		1
	Vichada										
Amazonian	Caquetá	44	17						1		
	Guainía	1	1								
	Guaviare	21	6								
	Putumayo	2	2								
	Vaupés	6	1								
	Amazonas	1	1								
Not specified location		2	3				1				
TOTAL	1,833	375	1,420	1	2	1	2	1	3	27	2

[a] *L. b: Leishmania braziliensis; L. p: L. panamensis; L. g: L. guyanensis; L. m: L. mexicana; L. a: L. amazonensis; L. i: L. infantum; L. n: L. naiffi; L.* spp*: Leishmania* species*; L. b/L. p: L. braziliensis/L. panamensis* (mixed infection)

Table 2. Geographic distribution of *Leishmania* species identified and reported since 1990 to 2020

egion	Department	*Leishmania* species									
		L. b[a]	*L. p*	*L. g*	*L. m*	*L. a*	*L. i*	*L. c*	*L. l*	*l. e*	*L.n*
ribbean	Córdoba	32,í[b]	27,30,32,34,í	34			56,60,í				
	Sucre	59	27,32,59, í	59			60				
	Bolívar	í	27,30,32,34,í				60				
	Atlántico	í									
	Magdalena	33,34,í	32								
	Cesar	27					60				
	Guajira	32,í	í				27,32,60				
	San Andrés/Providence										
acific	Chocó	27,32,í	27,30,32-34,í			32					
	Valle		27,í		30	35	40				
	Cauca	27,	27,í		30	31					
	Nariño	34,58,59	34,35,58,í		27, 57						
lean	Antioquia	21,27,32,34,í	21,27,30,32-35,í		21,32,í	21,32,í		32,45[c]	32	32	33
	Caldas	í	27, 30,32,35,í		30						
	Risaralda		27,30,32,35,í		27						
	Quindío										
	Cundinamarca	27, 30, 32, 35,í	27, 0,32,35,í	27, 35	30,32	31	27,32,60				
	Huila	27, 32, í	27,30,32,í				27,32,60				
	Tolima	32, 33, 35,í	27,30,32-35,58,í	34,35,58	32	32	27,32,60				
	Santander	27, 32, 33, 35,í	21,27,30,31-34,í	21	21	35	21,60	45			
	Norte Santander	27, 32, 34,í	27, 32,34,í		32	27		16			
	Boyacá	31, 32, 34,í	21,27,30-32,35,í	21		21	60				
noquian	Casanare	27, 32	27,31, 32		30	35					
	Arauca		27, í								
	Meta	27, 30, 21 32, 12,35, í	27, 30,21, 32 33-34,í	21,32,34,í	32,34,í	27,32	21,32		34		
	Vichada	27, 30, 32, 34	32								
azonian	Caquetá	27, 33,	30,32,í	27,32,33	30	35					

Guainía	34,35, í 34,35,í	32, í					
Guaviare	27,30,33,34,í	30,31,32,34,í	27,30,33	32,34	35		
Putumayo	32,34,í	34,í	32,34				32
Vaupés	34,í	í					
Amazonas			27				

[a] *L. b: Leishmania braziliensis; L. p: L. panamensis; L. g: L. guyanensis; L. m: L. mexicana; L. a: L. amazonensis; L. i: L. infantum; L. l: L. laissoni; L. e: L. equatoriensis; L. c: L. colombiensis; L. n: L. naiffi.* [b] í: Current report. [c] detected in the *Phlebotomus shannoni* sandfly

Figures

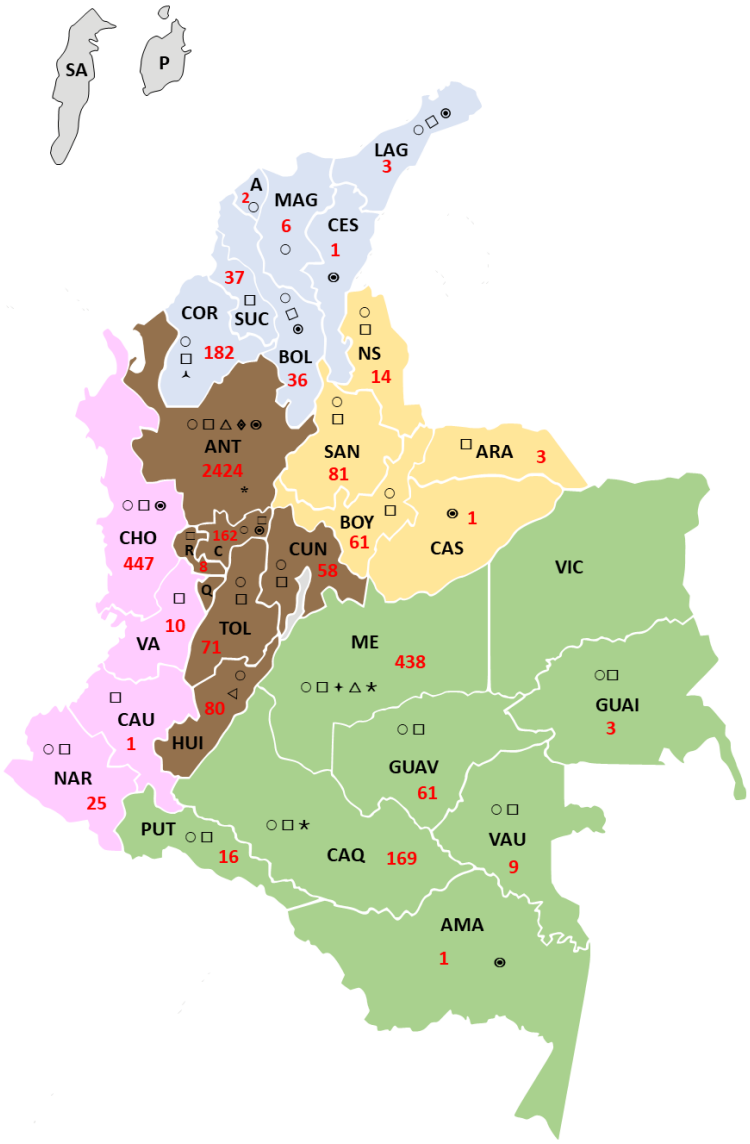

Figure 1

Geographic distribution of cases of leishmaniasis and Leishmania species diagnosed at PECET, Medellin-Colombia. Distribution according regions. Caribbean coast region (blue): departments of Atlántico (A), Bolivar (BOL), Cesar (CES), Córdoba (COR), La Guajira (LAG), Magdalena (MAG), San Andrés and Providence (SAP) and Sucre (SUC). Pacific coast region (pink): departments of Chocó (CHO), Valle del Cauca (VA), Cauca (CAU) and Nariño (NAR). Andean region (brown): departments of Antioquia (ANT), Caldas (CAL), Risaralda (R), Quindío (Q), Cundinamarca (CUN); Huila (HUI) and Tolima (TOL). Orinoquian region (yellow): departments of Arauca (ARA), Boyacá (BOY), Casanare (CAS), Norte de Santander (NS) and Santander (SAN). Amazonian region (green): departments of Amazonas (AMA), Caquetá (CAQ), Guainía (GUAI), Guaviare (GUAV), Meta (MET), Putumayo (PUT), Vaupés (VAU), Vichada (VIC). L. braziliensis (), L. panamensis (), L. guyanensis (), L. mexicana (), L. amazonensis (), L. infantum (), L. viannia (), Leishmania spp ().