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Application of Polymerase Chain Reaction to Screen Rodents from New Mexico, Texas, Bolivia, and Nicaragua for Leishmania and an Assessment of the Specificity of Primers

Robert Alan Miranda
University of the Incarnate Word

APPLICATION OF POLYMERASE CHAIN REACTION TO SCREEN RODENTS FROM NEW MEXICO, TEXAS, BOLIVIA, AND NICARAGUA FOR *LEISHMANIA* AND AN ASSESSMENT OF THE SPECIFICITY OF PRIMERS

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ROBERT ALAN MIRANDA

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ROBERT ALAN MIRANDA

APPROVED:

Sara F. Kerr, Chairman of Committee

Richard S. Peigler, Member

Russell W. Raymond, Member

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ABSTRACT

Application of polymerase chain reaction to screen rodents from New Mexico, Texas, Bolivia, and Nicaragua for *Leishmania* and an assessment of the specificity of primers

Robert Alan Miranda, B. S. University of the Incarnate Word

Polymerase chain reaction was used to screen rodents from four regions for *Leishmania* and to screen isolates of *L. enriettii* and *L. hertigi* using *L. mexicana* complex primers and *L.* (*Viannia*) primers to examine the specificity of these primers. Twenty-five rodent specimens from Bolivia, 42 from Nicaragua, 84 from New Mexico, and 74 from Texas were tested for *Leishmania.* Two *Oryzomys acritus* and two *Oryzomys nitidus* from Bolivia and one *Neotoma micropus* from Texas tested positive for *Leishmania* and additionally tested positive with *L. mexicana* complex-specific primers. Isolates of *L. enriettii* and *L. hertigi* reacted with *L. mexicana* complex primers and did not react with primers specific to the subgenus *Viannia. Leishmania hertigi* and *L. enriettii* were positive and negative, respectively, using primers specific to the genus *Leishmania.*

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INTRODUCTION

The members of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) form an important group of protozoan parasites. They are transmitted by phlebotomine sand flies (Diptera: Psychodidae) and are infective to six orders of mammals (Noyes et al., 1997). According to Lainson (1997), there are currently 30 named species of *Leishmania*, with 20 in the New World and ten in the Old World. The parasites affect 88 countries (66 in the Old World and 22 in the New World), of which 72 are classified as developing countries (Desjeux, 2004). Infection can lead to a variety of diseases collectively known as the leishmaniases. Human leishmaniasis affects about 12 million people worldwide from a population of approximately 350 million at risk (Magill, 1995). Disease symptoms range from self-healing skin lesions to mucocutaneous lesions to a sometimes fatal visceral disease. There are about 1.0-1.5 million new cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis each year, and disability-adjusted life years lost due to leishmaniasis are close to 2.4 million (Desjeux, 2004).

Leishmaniasis is a zoonotic disease with transmission between sand fly vectors and animal reservoirs. Of the six genera of sand flies only *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. of the New World are responsible for transmitting leishmaniasis (World Health Organization, 1990). The promastigote form of the parasite, found in the infected female sand fly's alimentary tract, is transmitted to an animal host while taking a blood meal. The promastigotes then enter cells of monocyte or macrophage lineage via specific receptors and transform

into amistigotes where they persist and replicate by binary fission. Humans are incidental hosts that become infected when in close contact with the animal/sand fly cycles (Magill, 1995).

Some *Leishmania* species have been identified in both the Old World and New World, including the neotropical species *L. enriettii* and *L. hertigi*, that have not been found to cause infections in humans (Lainson, 1997). Because these species have less clinical significance, their biological and ecological features have not been studied as thoroughly. Little is known about their natural life cycles and taxonomic relationships to other *Leishmania* species are controversial.

The purpose of this investigation was to use polymerase chain reaction (PCR) to screen rodents captured in Texas, New Mexico, Bolivia, and Nicaragua for *Leishmania*, and also examine the reactivity of isolates of *L. enriettii* and *L. hertigi* with *L. mexicana* complex-specific primers and primers specific to *L.* (*Viannia*) to test the specificity of these primers.

LITERATURE REVIEW

Leishmaniasis in the United States, Bolivia, and Nicaragua

Previous studies have shown that species of the genus *Neotoma* (Rodentia: Muridae: Sigmodontinae) have been found to be reservoirs of *Leishmania* (*Leishmania*) *mexicana* in the United States. The parasite has been recorded in *N. micropus* (southern plains woodrat) in southern Texas on a number of occasions (McHugh et al, 1990; Kerr et al., 1995; Raymond et al., 2003). Kerr et al. (1999) also detected *L. mexicana* in the white-throated woodrat (*N. albigula*) in Pima County, an area of southern Arizona. An eastern woodrat (*N. floridana*) collected in Grimes County in east-central Texas was screened by PCR and also found to be infected with this parasite (McHugh et al., 2003). Three *Lutzomyia anthophora* collected in Texas from the nests of *N. micropus* are the only sand flies found naturally infected with *L. mexicana* in North America north of Mexico (McHugh et al., 1993; Kerr et al., 1995).

Leishmaniasis, however, is a rare disease in the United States with a relatively small number of cases having been reported. Including the case reported by Maloney et al. (2002), only 30 autochthonous cases of cutaneous human leishmaniasis, all from Texas, have been reported since 1903 (McHugh et al., 1996). Cases of autochthonous canine visceral leishmaniasis also have been reported in the United States in Texas (Sellon et al., 1993) and Maryland (Eddlestone, 2000), *L.* (*L.*) *donovani* has been implicated in Oklahoma (Anderson et al., 1980) and Ohio (Swenson et al., 1988), and cases attributed to *L. infantum*, a member of the *L. donovani* complex, have been found in New York (Gaskin et al., 2002) and Virginia (Rosypal et al., 2003).

In Bolivia, cutaneous and mucocutaneous leishmaniases are endemic in the Amazon Basin and Andean Highland according to Davies et al. (2000) while visceral leishmaniasis is rare in the country. The disease is widely distributed and found in the departments of La Paz, Beni, Santa Cruz, Pando, and Cochabamba (Grimaldi et al., 1989). Studies have shown a number of species to be the causative agent. Most human cases of leishmaniasis, including reports by Desjeux et al. (1987) and Revollo et al. (1992), have been attributed to *Leishmania* (*Viannia*) *braziliensis* (David et al., 1993). The presence of *Leishmania* (*L.*) *amazonensis*, included in the *L. mexicana* complex, also has been reported. Human infections caused by this species have been reported by Lainson et al. (1994) and Martínez et al. (1998). Bermudez et al. (1993) also isolated *L. amazonensis* from the rice rat (*Oryzomys capito*) (Sigmodontinae), and Tellería et al. (1999) detected the parasite in one *Akodon* sp. (Sigmodontinae) and two *Oligoryzomys* spp. (Sigmodontinae). Recently, *L.* (*V.*) *lainsoni* has been implicated in a cycle of transmission in Bolivia (Martínez et al., 2001; Bastrenta et al., 2002). *Leishmania* (*L.*) *chagasi* also has been isolated and positively identified (Grimaldi et al., 1989), and Le Pont and Desjeux (1985) were able to isolate parasites from several *Lutzomyia longipalpis* that had an enzymatic profile similar to that of *L. chagasi* and *L. infantum*. In 2002, Martínez et al. reported a co-infection by *L. amazonensis* and *L. infantum*/*L. chagasi* from a patient with diffuse cutaneous leishmaniasis. Studies have also found the sand fly *L. nuneztovari anglesi* to be naturally infected with *L. braziliensis* (Tellería et al., 1999; Bastrenta et al., 2002), *L. amazonensis* (Martínez et al., 1999; Tellería et al., 1999), and *L. lainsoni* suggesting

the sand fly could be the main vector of various *Leishmania* species in the region (Bastrenta et al., 2002).

Cutaneous and mucocutaneous leishmaniases are endemic in Nicaragua and pose a major public health problem (Darce et al., 1991; Belli et al., 1994). Leishmaniasis, which was known to exist in the country in 1977 and probably before, was first reported to WHO by the Nicaraguan health authorities in 1980 after the revolutionary war and institution of the National Unified Health System (Missoni and Morelli, 1984). The disease occurs in central forested areas of Nicaragua, and the endemic foci are distributed in the departments of Estelí, Jinotega, Madriz, and Nueva Segovia (in the north), Boaco and Matagalpa (in the central area), and Chontales, Río San Juan, and the former department of Zelaya (in the southeast) (Grimaldi et al., 1989). Studies throughout Nicaragua have found *L. (V.) braziliensis* to be one of the more important causative agents of disease (Missoni and Morelli, 1984; Darce et al., 1991; Belli et al., 1994). *Leishmania (V.) panamensis* has also been found to be quite prevalent in the country according to previous studies (Missoni et al., 1986; Darce et al., 1991; Belli et al., 1994). The isoenzymatic characterization of isolates by Darce et al. (1991) and Belli et al. (1994) also found the presence of a putative *L. panamensis/L. braziliensis* hybrid as a cause of cutaneous forms of leishmaniasis. Atypical cutaneous leishmaniasis caused by *L. (L.) chagasi* has been found in Nicaragua as well (Belli et al., 1999; Convit et al., 2005). In 1994, Duarte et al. reported the first confirmed case of visceral leishmaniasis in the country in a patient from a rural village located in the province of Somotillo, Chinandega.

Leishmania enriettii

Medina first discovered *Leishmania enriettii* in 1946 as an accidental infection in laboratory guinea-pigs (*Cavia porcellus*) in Curitiba, Paraná State, Brazil, and the parasite was later described by Muñiz and Medina in 1948. Muñiz and Medina failed to establish the parasite in humans, rhesus monkeys, golden hamsters, dogs, rabbits, hares, white rats, mice, and the agouti. Most significantly, they also failed to produce skin lesions in the wild guinea-pig, or preá (*Cavia aperea*) (Adler and Halff, 1954; Belehu and Turk, 1976; Machado et al., 1994; Thomaz-Soccol et al., 1996; Lainson, 1997). However, Adler and Halff (1954) were able to produce transient infections by subcutaneous inoculation of promastigotes and amastigotes in suckling mice, and Belehu and Turk (1976) were able to establish self-healing cutaneous leishmaniasis in hamsters by inoculation into the nose.

Further infections were not seen again until 1967 when *L. enriettii* spontaneously reappeared in domestic guinea-pigs from a locality on the outskirts of Curitiba. Twenty-two years later, two adult domestic guinea-pigs were taken to a countryside farm in Capão Bonito, São Paulo State, which is close to remnants of the Atlantic Forest, and were later diagnosed with leishmaniasis due to *L. enriettii* (Machado et al., 1994; Thomaz-Soccol et al., 1996; Lainson, 1997). In a rural area near Curitiba, three more cases of leishmaniasis caused by *L. enriettii* were described by Thomaz-Soccol et al. (1996).

Because the only known vectors of *Leishmania* species are phlebotomine sand flies, it is hard to believe that domestic guinea-pigs, which are sheltered from

sand flies, are the primary natural hosts of *L. enriettii*. Machado et al. (1994) and Lainson (1997) believe that the wild guinea-pig should still be considered the natural host and may acquire only a benign, inapparent infection in the skin. Although the sand fly *Lutzomyia monticola* has been experimentally infected and could be a vector, parasites from the flies failed to produce an infection when inoculated into the skin of a clean guinea-pig. *Leishmania enriettii* has only been found in guinea-pigs in a restricted geographical area of Brazil; however, the wild animal host and sand fly vector remain uncertain at this time (Thomaz-Soccol et al., 1996; Lainson, 1997).

Leishmania hertigi

Leishmania hertigi was found in Panama and first described by Herrer, in 1971, as a new parasite of the tropical porcupine, *Coendou rothschildi*. Although the parasite was found throughout the skin and viscera, there was no indication of any tissue or cell reactions. Attempts to experimentally infect golden hamsters did not cause any gross skin alterations, and the parasites were only recovered from the site of inoculation (Herrer, 1971; Herrer et al., 1973). In a study on cutaneous leishmaniasis among Panamanian forest mammals conducted between April 1965 and September 1974, Herrer et al. (1975) examined 117 tropical porcupines and found 104 (89%) to be infected, all of them cryptically. The high rate of prevalence and the long-lasting, asymptomatic nature of the infection indicate that *L. hertigi* is host-specific and has a well balanced host-parasite relationship that may have evolved into a commensal one (Herrer, 1971; Herrer et al., 1975; Lainson, 1997). *Leishmania hertigi* was later isolated by Zeledón et al. (1977) from one of 25 Costa Rican

porcupines (*C. mexicanum laenatum*) examined, but has not been found in any other mammal species. The sand fly vector of *L. hertigi* remains unknown at the present time (Lainson, 1997; Noyes et al., 1997).

Taxonomy of *L. enriettii* and *L. hertigi*

The taxonomy of the New World species of *Leishmania* has been difficult because of the technical difficulties in understanding their epidemiological cycles, overlap of their distributions, and the diversity of species (Thomaz-Soccol et al., 1993a). These parasites were initially identified and classified based on extrinsic criteria, such as clinical manifestations and geographical and epidemiological features (Cupolillo et al., 1998). *Leishmania* species have generally been divided into the subgenera *L.* (*Leishmania*) which develop in the midgut and foregut of the sand fly (suprapylaria), and *L.* (*Viannia*) which undergo additional development in the hindgut (peripylaria) (Croan and Ellis, 1996; Noyes et al., 1997; Cupolillo et al., 2000; Grimaldi and Schottelius, 2001). Based on experimental sand fly infections, the subgenus *Leishmania* includes both *L. hertigi* (Noyes et al., 1997; Kerr, 2000) and *L. enriettii* (Thomaz-Soccol et al., 1996; Lainson et al., 1997). Appendix B shows the classification of these species according to the World Health Organization (1990).

However, advances in biochemical and molecular biology have helped resolve many issues regarding classification because much of the population heterogeneity has a genetic basis (Cupolillo et al., 1998). Kreutzer et al. (1983) tested 25 enzyme systems to measure the amount of isozyme similarity between 44 *Leishmania* isolates. According to enzyme profiles, there were five major groupings of isolates. These

were designated as *L. mexicana, L. braziliensis, L. donovani, L. tropica,* and *L. hertigi*, which was less than 15% similar to any other. Rioux et al. (1990), using 11 zymodemes (all the strains with the same enzymatic profile), 13 enzymes, and 65 electromorphs, presented a phenogram of New World *Leishmania*. The species *L. deanei*, part of the *L. hertigi* complex, was linked to species of the subgenus *Leishmania*, and the species *L. enriettii* was linked to members of the subgenus *Viannia*, but both with a very low affinity. This did not allow a valid confirmation, therefore, it was best determined that *L. enriettii* and *L. hertigi* should be placed in the subgenus *Leishmania* forming their own complex. Thomaz-Soccol et al. (1993b) analyzed 13 enzymes using 201 isoenzymes and concluded that the *L. enriettii* and *L. hertigi* complexes belong in the subgenus *Leishmania*, although two equally parsimonious trees placed *L. hertigi* near the root, but one with the subgenus *Viannia* and the other with the subgenus *Leishmania.*

Croan et al. (1997) phylogenetically analyzed sequences of the gene encoding the DNA polymerase α catalytic polypeptide along with the RNA polymerase II largest subunit gene of 19 species of *Leishmania* to propose a comprehensive phylogeny. It was reported that the *L. hertigi* complex is more closely related to the genus *Endotrypanum* than to the genus *Leishmania.* This was supported by Noyes et al. (1997) who used restriction fragment polymorphisms and sequences of small subunit ribosomal RNA genes and hybridization studies of kinetoplast DNA to infer that *L. hertigi* is closer to *Endotrypanum* than to *Leishmania.* However, *L. hertigi* develop as amastigotes, rather than trypomastigotes or epimastigotes, which are found

in *Endotrypanum*, therefore, making this species more similar to the genus *Leishmania* on biological grounds (Noyes et al., 1997). Thus, it seems that the *L. hertigi* complex diverged shortly after the *Endotrypanum* and *Leishmania* genera separated. Recently, a revised classification of *Leishmania* was proposed by Cupolillo et al. (2000) based on some of these recent molecular studies that suggest the presence of two major phylogenetic lineages within *Leishmania.* The two lineages, called sections, were named Euleishmania and Paraleishmania. Euleishmania is comprised of species belonging to the subgenera *Leishmania* and *Viannia*, including *L. enriettii.* Section Paraleishmania comprises strains of *Endotrypanum* and those *Leishmania* species closely related to *Endotrypanum*, including *L. heritigi* (Cupolillo et al., 2000; Momen and Cupolillo, 2000; Noyes et al., 2000; Grimaldi and Schottelius, 2001).

Kerr (2000) disagrees with phylogenetic trees presented by Thomaz-Soccol et al. (1993a), Thomaz-Soccol et al. (1993b), Croan et al. (1997), and Noyes et al. (1998), which show that *L.* (*Viannia*) and *Endotrypanum* are ancestral to *L.* (*Leishmania*). Because the trees were un-rooted, they could be inverted, with *L.* (*Leishmania*) at the base and *Endotrypanum* and *L.* (*Viannia*) at the crown, but the taxonomic groupings proposed would be unchanged. Kerr et al. (2000) proposed a well-rooted tree based on the presence or absence of the GP46/M-2 gene family to support the theory of a Palearctic origin of *Leishmania.* The gene family is present in *L. mexicana*, *L. major*, and *L. donovani* but absent in *L.* (*Viannia*), *L. enriettii*, and

Paraleishmania (Kerr et al. 2000). This shows a closer affinity to *L.* (*Viannia*) than to *L. mexicana* for *L. enriettii* and *L. heritigi.*

PCR

Conventional methods of leishmaniasis diagnosis rely on direct parasitologic tests, including microscopic smear examination or *in vitro* culture of parasites from biopsy materials, but these methods have variable success rates, lack sensitivity, and can be time consuming. Serological tests also have been used, including enzyme linked immunosorbent assay (ELISA), Leishmanin dermal test (LDT), and indirect fluorescent antibody test (IFAT). However, these methods cannot distinguish between past and present infection and cross-react with antibodies against other pathogens (Bhattacharyya et al., 1996). A molecular approach capable of detecting nucleic acids unique to the parasite and designed for effective field use could help resolve some of the setbacks of conventional methodologies. In recent years PCR has proven to be a highly sensitive and specific tool in the rapid detection of *Leishmania* DNA in epidemiological studies (Singh et al., 1999; Brecelj et al., 2000; Oliveira et al., 2005).

Members of the order Kinetoplastida, including *Leishmania*, possess a unique DNA-containing structure in the mitochondrion of the cell, called the kinetoplast (Rodgers et al., 1990). Kinetoplast DNA (kDNA) is composed of two components, maxicircle and minicircle kDNA. Maxicircle kDNA is 20-40 kilobase (kb) long, present as 10-50 copies per cell, and carries the genes encoding the mitochondrial enzymes. Minicircles are usually 1 kb in length, present as 10,000 -100,000 copies per cell, but have no known function (Rogers et al., 1988; Rogers et al., 1990).

Sequence comparison of minicircles from different *Leishmania* species revealed a region of approximately 200 base pairs (bp) which is conserved between species, while the remaining sequence varies between species and subspecies (Rogers et al., 1990). Probes using cloned minicircle fragments have been used to determine organisms at the species, subspecies, and strain levels.

PCR is a diagnostic DNA probe method that uses a thermostable DNA polymerase and a set of oligonucleotide primers to direct amplification of the hybridization target sequence, which can be amplified a millionfold through 30 cycles of a series of priming-elongation-denaturation cycles (Rogers et al., 1990). The primer sequences 13A (5'-GTG GGG GAG GGG CGT TCT-3'), which includes a 10-bp sequence conserved in several kinetoplastid minicircles, and 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3'), which contains a 12-bp sequence believed to be the origin of replication of kinetoplastid minicircles, are specific at the genus level and are oriented to amplify a 120-bp portion of the conserved region (Rogers et al., 1990). Gel electrophoresis can detect minute quantities of kDNA in the original sample following PCR amplification.

A number of scientists have since developed many sets of primers designed to target and amplify various minicircle kDNA sequences to identify *Leishmania* at the genus, subgenus, complex, and species levels. Other primer pairs that are genus-specific include LSUC/LSUL and primer A/primer B. Primers LSUC (5'-CAA ACT GGG GGT TGG TGT AA-3'), present in the minicircles of all the kinetoplastid parasites, and LSUL (5'-TTT TGA ACG GGG TTT CTG-3'), present in the

minicircles of only the *Leishmania* parasites, are 37 bp apart with the resulting product full length minus approximately 37-bp portion of the minicircles (Bhattacharyya et al., 1996). Primer A (5'-(G/C)(G/C)(C/G) CC(A/C) CTA T(A/T)T TAC ACC AAC CCC-3') and primer B (5'-GGG GAG GGG CGT TCT GCG AA-3') produce a 120-bp amplification product (Silva et al., 2001; Oliveira et al., 2005).

Others have gone on to develop more specific kDNA primers for New World *Leishmania* parasites. Disch et al. (2005) used the forward genus primer A and replaced the reverse primer with the *Viannia* subgenus-specific reverse primer (5'-GAA CGG GGT TTC TGT ATG C-3') which coincides with the one used by López et al. (1993). The genus-specific sense primer and the *Viannia* subgenus-specific reverse primer produce a 51-bp fragment for kDNA from parasites belonging to this subgenus. Primer pair B1 (5'-GCC GTT GGT GTA ATA TAG TGG-3') and B2 (5'-CTA ATT GTG CAC GGG G-3'), developed by De Bruijn and Barker (1992), is specific to parasites of *L.* (*Viannia*) and produces a band approximately 750 bp in size (Alexander et al., 1998; Weigle et al., 2002; Brandão-Filho et al., 2003; Oliveira et al., 2005). Brecelj et al. (2000) designed a set of primers specific to the subgenus *Viannia* as well. B1(+) (5'-GGA ATT CCC GAC ATG CCT CTG GGT A-3') and B2(-) (5'-CGA ATT CAC TAT ATT ACA CCA ACC CCT A-3') amplify a 131-bp fragment of the conserved region of kDNA. A set of primers that amplify a conserved kDNA sequence of parasites belonging to the *L. braziliensis* complex include the primers MP1L and MP3H. The extension primers MP1L (5'-TAC TCC CCG ACA TGC CTC TG-3') and MP3H (5'-GAA CGG GGT TTC TGT ATG C-3')

produce a single amplification product 70 bp in length (López et al., 1993). Brenière et al. (1999) also developed primers specific to the *L. braziliensis* complex. L1 (5'-CCT ACC CAG AGG CCT GTC GGG-3') and L2 (5'-TAA TAT AGT GGG CCG CGC AC-3') produce an amplification product of approximately 610 bp. Belli et al. (1998) developed a multiplex reaction using primers 13A, 13B, and MP3H to produce a 54-bp fragment from members of the *L. braziliensis* complex and a 120-bp fragment from all other *Leishmania* complexes. The primers M1 (5'-CCA GTT TCG AGC CCC GGA G-3') and M2 (5'-GGT GTA AAA TAG GGG CGG ATG CTC TG-3') are specific to the *L. mexicana* complex. The kDNA bands are approximately 700-800 bp in length (Eresh et al., 1994; Oliveira et al., 2005).

Oligonucleotide primers have also been designed to detect kDNA of Old World parasites. In 1999, Singh et al. designed a set of primers that are highly specific to parasites of the *L. donovani* complex. Using these primers, designated NM12F (5'-GTG GTC GTG GCG CTT ATG TG-3') and NM12R (5'-AAG CAC CGT ACA AAA TCA CGT AC-3'), results in a 204-bp product. Cortes et al. (2004) developed primers on the basis of a complete DNA sequence of the *L. infantum* kinetoplast minicircle. MC1 (5'-GTT AGC CGA TGG TGG TCT TG-3') and MC2 (5'-CAC CCA TTT TTC CGA TTT TG-3') are sensitive only to members of the *L. donovani* complex and produce an amplification product of 447 bp. Primers Lsmc1 (5'-CGT TCT GCA AAA TCG GG-3') and Lsmv1 (5'-TAT TGC TTT ATT CCT AT-3') amplify a 461-bp fragment from the kDNA of cutaneous species only (Bhattacharyya et al., 1993). Hu et al. (2000) developed a set of primers that is

specific to *L. donovani*. The species-specific primer I (5'-TAG GAC GTG TGT GTG GGT GG-3') and primer II (5'-CTG TAT GAG GGA TGA ATA AGC G-3') amplify a minicircle kDNA fragment 297 bp in length. Another set of PCR primers specific at the species level to *L. donovani* is the LdI primers. The LdI primers (5'-AAA TCG GCT CCG AGG CGG GAA AC-3' and 5'-GGT ACA CTC TAT CAG TAG CAC-3') amplify a fragment of approximately 600 bp (Salotra et al., 2001).

MATERIALS AND METHODS

Field methods

Field procedures followed are the methods described by Kerr et al. (1999). Rodents were collected using Sherman live traps (Sherman Live Traps, Inc., Tallahassee, Florida). The traps were baited with oatmeal, sunflower seeds, or fresh fruit. They then were placed near rodent pathways or den openings in the late afternoon and collected the following morning. Captured rodents were transferred to a cotton stockinette to be weighed with a precision spring scale (Avinet USA, Dryden, New York). They then were transferred to a wire-mesh cone to be sexed and identified. Tissue samples were taken from each ear using a sterile, disposable, 2 mm biopsy punch (Miltex Instrument Co., New York). Tissue samples were also collected from the base of the tail of animals using a sterile scalpel blade.

Tissue samples

Ear tissue samples were taken for testing from 74 *Neotoma micropus* captured during 338 trap nights (total traps set during study) in the fall of 2004 at Medina Annex, part of Lackland Air Force Base, located in Bexar County, Texas. Tissue samples were collected from 42 rodents trapped in various localities in Nicaragua during 767 trap nights from December 2004 through March 2006. Ear tissue was collected from all animals as well as a separate tail sample from 27 of them (69 total samples) for screening. Twenty-five tail tissue samples were collected by Dr. Louise Emmons in Bolivia from two localities in Noël Kempff Mercado National Park, Santa Cruz: El Refugio and Los Fierros. The samples, stocked in 95% ethanol, were air-

dried prior to testing. Eighty-four tissue samples (all from ear), collected by Dr. Nelson Powers from study sites located in El Dorado, Santa Fe County, New Mexico were also tested by PCR. A summary of the sample information is found in Table I. Appendix A lists detailed information on all mammals screened.

TABLE I. Summary of tissue samples.

Country	Location	Samples	Sample source
Bolivia	El Refugio	24	Tail
	Los Fierros	1	
Nicaragua	El Cuá	18	Ear (42) Tail (27)
	La Esperanzita	2	
	La Fonseca	10	
	Nueva Guinea	2	
	El Paraisito	18	
	La Sardina	2	
	Selva Negra	17	
New Mexico, USA	El Dorado	84	Ear
Texas, USA	Medina Annex	74	Ear

Tissue preparation procedures

Tissue samples were processed by the method used by Rogers et al. (1990) and Kerr et al. (1999). The samples were placed on a sterile alcohol pad and scraped with a sterile scalpel blade to remove contaminants and excess hair. The DNA was extracted by placing the tissue sample in 40µl of lysis buffer (10mM Tris/ 10mM EDTA) and incubated for 30 min at 95 C in a water bath. The lysate was then stored at -20 C until PCR could be performed.

Parasite culture methods

Isolates, obtained from the American Type Culture Collection, of *L. enriettii* (ATCC® 30035), *L. hertigi* (ATCC® 30286), *L.* (*L.*) *amazonensis* (ATCC® 50131), and *L.* (*V.*) *guyanensis* (ATCC® 50126) were cultured in M199 medium with Earle's salts (GIBCO-BRL, Gaithersburg, Maryland) supplemented with 5% (v/v) heat-inactivated fetal bovine serum (GIBCO), 100X penicillin/streptomycin (GIBCO), 1M Hepes buffer (Sigma Chemical Co., St. Louis, Missouri), 0.25% (v/v) bovine hemin (Sigma), and 10mM hypoxanthin (Sigma) in addition to 5 ml of human urine cleared at 600 g for 20 min in a centrifuge. The medium was then passed through a 0.2 μm filter (Nalge Nunc International, Rochester, New York) for sterilization. The pH was adjusted to 7.4 using 1M sodium hydroxide (Fisher Scientific Company, Fair Lawn, New Jersey). The promastigotes, cultured in 5 ml of medium in tissue culture flasks (25 cm^2) (Corning Inc., Corning, New York), were maintained in a 25 C incubator, monitored using an inverted phase contrast microscope (Olympus Optical Company, Japan), and passed weekly to fresh medium.

Parasite kDNA

To isolate parasite kDNA from cultured promastigotes, they were first counted using a hemacytometer (Hausser Scientific, Horsham, Pennsylvania) to estimate the concentration of parasite culture. The desired amount of parasite culture containing 10^6 parasites was aliquoted to a microcentrifuge tube and centrifuged at 3000 g for 15 min. After removing the supernatant, the pellet was resuspended in sufficient Delbecco's phosphate buffered saline (DPBS) (Mediatech, Inc., Herndon,

Virginia) to wash the parasites. The parasites in DPBS solution were again centrifuged at 3000 g for 15 min and the supernatant discarded. The pellet was resuspended in 25 µl of lysis buffer and incubated for 30 min at 95 C in a water bath. The solution was then centrifuged at 2000 g for 5 min. The pellet, containing parasite kDNA, was resuspended in DNase/RNase-free water which was serially diluted to create desirable parasite concentrations for PCR use.

Primers

To amplify DNA from *Leishmania* species by PCR, the forward primer was the genus-specific primer 13A (5'-GTG GGG GAG GGG CGT TCT-3'), and the reverse primer was the genus-specific primer 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3') (Rogers et al., 1990). The forward primer 13A and the reverse primer M1.1 (5'-CCA GTT TCG ACC GCC GGA GC-3'), which target a kDNA sequence uniquely conserved in the *L. mexicana* complex, were used to detect and amplify DNA from this complex. To detect DNA from *L.* (*Viannia*) spp., the forward primer specific to *L.* (*Viannia*), B4 (5'-TCG TAC TCC CCG ACA TGC CTC-3'), and the genus-specific reverse primer 13B were used (Melby, unpublished data).

***Leishmania* kDNA amplification**

The minicircle kDNA was amplified by PCR in a master mix containing concentrations of 1× PCR reaction buffer (Roche-Mannheim, Germany), 0.2 mM dNTP mix (Roche-Mannheim), 0.2 µM forward and reverse primers, and 3U/100 µl Taq polymerase (Roche-Mannheim). Twenty-three µl of master mix along with 2 µl of the DNA isolate was added to each reaction tube. The reaction tubes were then

placed in a thermal cycler (Perkin Elmer, Norwalk, Connecticut) for amplification consisting of an initial hold at 94 C for 5 min, followed by 30 repeated cycles of a denaturation step at 94 C for 30 sec, primer annealing at 55 C for 30 sec, and chain elongation at 72 C for 30 sec, concluded by a final hold at 72 C for 7 min.

Detection of amplified kDNA

The PCR product was detected using a gel electrophoresis apparatus. A 2.0% agarose gel composed of 0.5X TBE, agarose, and ethidium bromide (0.1µl /1ml TBE) was used. Two µl of loading dye was added to each reaction tube with PCR product. Ten µl of each reaction tube was then added to a gel well. Also added to a well was a positive control, negative control (DNase/RNase-free water), and a 50-2,000-bp ladder (Sigma). The electrophoresis apparatus was run for 50 min at 100V. Amplification products were detected with ethidium bromide staining under UV light. The genus-specific primers 13A/13B gave a ~120-bp amplification product for all *Leishmania* strains, the 13A/M1.1 primers gave a ~105-bp product for *L. mexicana* complex kDNA, and the B4/13B primers left a ~135-bp product for *L.* (*Viannia*) kDNA. The transilluminated gel was photographed with a Polaroid camera to record the results.

RESULTS

PCR analysis of tissue samples

All tissue samples were tested by the PCR method previously described. Four of the 25 (16%) samples from Bolivia (2 *Oryzomys acritus* and 2 *Oryzomys nitidus*) were positive with the genus-specific primer pair 13A/13B. Tissue samples 654 (Figure 1), 692 (Figure 2), 729 (Figure 2), and 806 then were found to be positive when tested using *L. mexicana* complex-specific primers (13A/M1.1) and produced negative results when tested with *L.* (*Viannia*) primers (B4/13B). Results are summarized in Table II and Table IV.

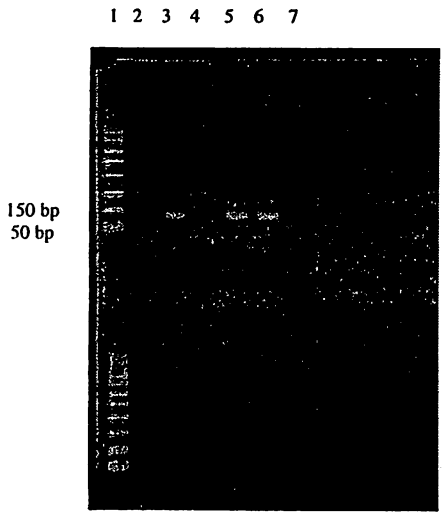


FIGURE 1. Results of tissue samples from Bolivia using primers 13A/M1.1 and B4/13B, part 1.

Row 1 (13A/M1.1): lane 1, bp ladder; lane 2, 642; lane 3, 654; lane 4, 639; lane 5, *L. mexicana* 838 positive control (10^2 parasites/µl); lane 6, *L. mexicana* 838 positive control (10 parasites/µl); lane 7, negative control. Row 2 (B4/13B): lane 1, bp ladder; lane 2, 642; lane 3, 654; lane 4, 639; lane 5, *L.* (*Viannia*) 3164 positive control (10^2 parasites/µl);lane 6, *L.* (*Viannia*) 3164 positive control (10 parasites/µl); lane 7, negative control.

FIGURE 2. Results of tissue samples from Bolivia using primers 13A/M1.1 and B4/13B, part 2.

Row 1 (13A/M1.1): lane 1, bp ladder; lane 2, 677; lane 3, 686; lane 4, 692; lane 5, 729; lane 6, *L. mexicana* Py4 positive control (10^2 parasites/µl); lane 7, negative control. Row 2 (B4/13B): lane 1, bp ladder; lane 2, 677; lane 3, 686; lane 4, 692; lane 5, 729; lane 6, *L.* (*Viannia*) 3164 positive control (10^2 parasites/µl); lane 7, negative control.

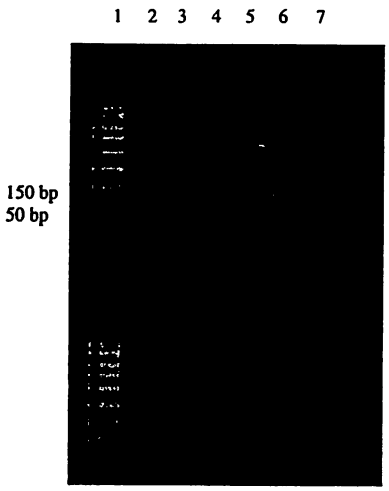


TABLE II. PCR analysis of samples from Bolivia using genus-specific primers.

Species	Positive	Negative	Total
Akodon dayi	0	1	1
Necromys lenguarum	0	1	1
Oecomys bicolor	0	2	2
Oryzomys acritus	2	4	6
Oryzomys nitidus	2	11	13
Proechimys longicaudatus	0	2	2
Total	4	21	25

One of 74 (1.4%) ear samples from Texas tested positive by PCR. A *Neotoma micropus* (ID number 447D7E712C) was found positive for primer pairs 13A/13B and 13A/M1.1 and was negative for primer set B4/13B. Results are summarized in Table III and Table IV.

TABLE III. PCR analysis of samples from Texas using genus-specific primers.

Species	Positive	Negative	Total
Neotoma micropus	1	73	74

TABLE IV. PCR analysis of samples using subgenus and complex-specific primers.

Species	Identification	Country	Location	Primers	PCR result
Oryzomys acritus	654	Bolivia	El Refugio	13A/13B	+
				13A/M1.1	+
				B4/13B	-
Oryzomys acritus	692	Bolivia	El Refugio	13A/13B	+
				13A/M1.1	+
				B4/13B	-
Oryzomys nitidus	729	Bolivia	El Refugio	13A/13B	+
				13A/M1.1	+
				B4/13B	-
Oryzomys nitidus	806	Bolivia	El Refugio	13A/13B	+
				13A/M1.1	+
				B4/13B	-
Neotoma micropus	447D7E712C	Texas, USA	Medina Annex	13A/13B	+
				13A/M1.1	+
				B4/13B	-

None of the samples from Nicaragua or New Mexico tested positive for *Leishmania* using PCR. The results are summarized in Table V and Table VI. All positive controls were created from isolates provided by Dr. Peter Melby of University of Texas Health Science Center at San Antonio (UTHSCSA).

TABLE V. PCR analysis of samples from New Mexico using genus-specific primers.

Species	Positive	Negative	Total
Neotoma micropus	0	68	68
Neotoma albigula	0	14	14
Dipodomys ordii	0	1	1
Peromyscus leucopus	0	1	1
Total	0	84	84

TABLE VI. PCR analysis of samples from Nicaragua using genus-specific primers.

Species	Positive	Negative	Total
Proechimys semispinosus	0	16	16
Mus musculus	0	4	4
Peromyscus nudipes	0	14	14
Sigmodon hirsutus	0	2	2
Oryzomys cousei	0	1	1
Melanomys caliginosus	0	2	2
Oryzomys alfaroi	0	3	3
Total	0	42	42

PCR analysis of *L. enriettii* and *L. hertigi* isolates

Leishmania enriettii (ATCC® 30035) and *L. hertigi* (ATTC® 30286) isolates were tested by the PCR method previously described. *Leishmania enriettii*, *L. heritigi*, and *L. amazonensis* (ATTC® 50131) (positive control) were tested using primers 13A/M1.1. Isolates were tested at concentrations of 10^4 to 10 parasites/µl. *Leishmania enriettii* and *L. hertigi* were positive at 10^4 and 10^3 parasites/µl and negative at 10^2 and 10 parasites/µl. *Leishmania amazonensis* produced positive results at all concentrations (Table VII, Figure 3).

TABLE VII. PCR analysis of *Leishmania* isolates using primers 13A/M1.1.

parasites/µl	10^4	10^3	10^2	10
parasites/reaction	20,000	2,000	200	20
L. enriettii	Positive	Positive	Negative	Negative
L. hertigi	Positive	Positive	Negative	Negative
L. amazonensis	Positive	Positive	Positive	Positive

FIGURE 3. Analysis of *L. enriettii* and *L. hertigi* isolates using primers 13A/M1.1.

Lane 1, bp ladder; lane 2, *L. enriettii* 10^4 parasites/µl; lane 3, 10^3 parasites/µl; lane 4, 10^2 parasites/µl; lane 5, 10 parasites/µl; lane 6, *L. hertigi* 10^4 parasites/µl; lane 7, 10^3 parasites/µl; lane 8, 10^2 parasites/µl; lane 9, 10 parasites/µl; lane 10, *L. amazonensis* 10^4 parasites/µl; lane 11, 10^3 parasites/µl; lane 12, 10^2 parasites/µl; lane 13, 10 parasites/µl; lane 14, negative control.

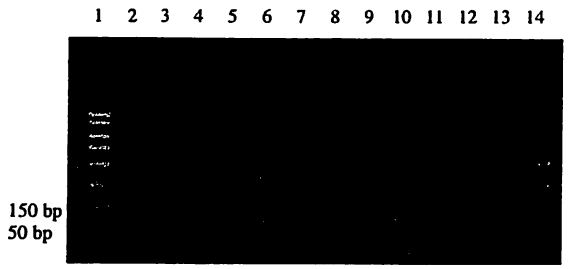
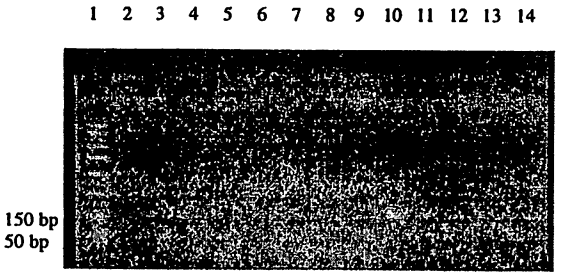


FIGURE 4. Analysis of *L. enriettii* and *L. hertigi* isolates using primers B4/13B.

Lane 1, bp ladder; lane 2, *L. enriettii* 10^4 parasites/µl; lane 3, 10^3 parasites/µl; lane 4, 10^2 parasites/µl; lane 5, 10 parasites/µl; lane 6, *L. hertigi* 10^4 parasites/µl; lane 7, 10^3 parasites/µl; lane 8, 10^2 parasites/µl; lane 9, 10 parasites/µl; lane 10, *L. guyanensis* 10^4 parasites/µl; lane 11, 10^3 parasites/µl; lane 12, 10^2 parasites/µl; lane 13, 10 parasites/µl; lane 14, negative control.



Leishmania enriettii, *L. hertigi*, and *L. guyanensis* (ATCC® 50126) (positive control) were tested using primers B4/13B. Isolates were tested at concentrations of 10^4 to 10 parasites/µl. *Leishmania enriettii* and *L. hertigi* produced negative results at all concentrations, and *L. guyanensis* was positive at 10^4 and 10^3 parasites/µl but negative at 10^2 and 10 parasites/µl (Table VIII, Figure 4).

TABLE VIII. PCR analysis of *Leishmania* isolates using primers B4/13B.

parasites/µl	10^4	10^3	10^2	10
parasites/reaction	20,000	2,000	200	20
L. enriettii	Negative	Negative	Negative	Negative
L. hertigi	Negative	Negative	Negative	Negative
L. guyanensis	Positive	Positive	Negative	Negative

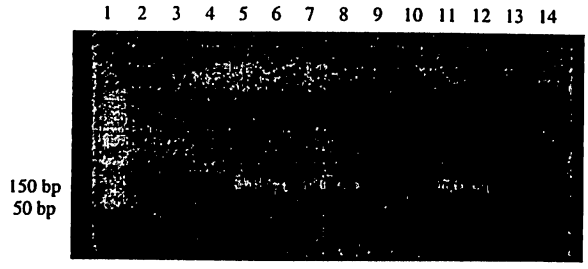
Leishmania enriettii, *L. hertigi*, *L. guyanensis*, and *L. amazonensis* isolates were tested at concentrations of 10^4 to 10^2 parasites/µl using primer pair 13A/13B. *Leishmania hertigi* and *L. amazonensis* were positive for all concentrations, *L. enriettii* was negative for all concentrations, and *L. guyanensis* was positive for 10^4 but negative for 10^3 and 10^2 parasites/µl (Table IX, Figure 5).

TABLE IX. PCR analysis of *Leishmania* isolates using primers 13A/13B.

parasites/µl	10^4	10^3	10^2
parasites/reaction	20,000	2,000	200
L. enriettii	Negative	Negative	Negative
L. hertigi	Positive	Positive	Positive
L. guyanensis	Positive	Negative	Negative
L. amazonensis	Positive	Positive	Positive

FIGURE 5. Analysis of *Leishmania* isolates using primers 13A/13B.

Lane 1, bp ladder; lane 2, *L. enriettii* 10^4 parasites/µl; lane 3, 10^3 parasites/µl; lane 4, 10^2 parasites/µl; lane 5, *L. hertigi* 10^4 parasites/µl; lane 6, 10^3 parasites/µl; lane 7, 10^2 parasites/µl; lane 8, *L. guyanensis* 10^4 parasites/µl; lane 9, 10^3 parasites/µl; lane 10, 10^2 parasites/µl; lane 11, *L. amazonensis* 10^4 parasites/µl; lane 12, 10^3 parasites/µl; lane 13, 10^2 parasites/µl; lane 14, negative control.



DISCUSSION

Previous studies have demonstrated the reliability of the genus-specific primers 13A and 13B developed by Rogers et al. (1990) in detecting *Leishmania* spp. using polymerase chain reaction (Kerr et al., 1995; Belli et al., 1998; Kerr et al., 1999; Kerr et al., 2003; Raymond et al., 2003). Recently, Dr. Melby (UTHSCSA) developed a forward primer, B4 (paired with 13B), and a reverse primer, M1.1 (paired with 13A), that are specific to *L.* (*Viannia*) and *L. mexicana* complex, respectively. The objectives of this investigation were to use PCR to screen rodents collected in New Mexico, Texas, Bolivia, and Nicaragua for *Leishmania*, and to analyze the specificity of *L. mexicana* complex primers and subgenus *Viannia* primers.

Human leishmaniasis in Bolivia has reportedly been attributed to *L. braziliensis* (Desjeux et al., 1987; Revollo et al., 1992; David et al., 1993), *L. amazonensis* (Lainson et al., 1994; Martínez et al., 1998), and *L. lainsoni* (Martínez et al., 2001; Bastrenta et al., 2002), as well as a case caused by a co-infection of *L. amazonensis* and *L. infantum/L. chagasi* (Martínez et al., 2002). *Leishmania amazonensis*, the only one of the above species found in *L. mexicana* complex, was also detected in an *Oryzomys capito* (Bermudez et al., 1993), one *Akodon* sp., and two *Oligoryzomys* spp. (Tellería et al., 1999). Of the 25 tissue samples from Bolivia screened in this study, four tested positive for *Leishmania*. All four of the samples, from the locality of El Refugio, were also positive using the *L. mexicana* complex primers 13A/M1.1. Two of the samples, 654 and 692, were identified as being

Oryzomys acritus while samples 729 and 806 were confirmed to be *Oryzomys nitidus*. Liu (2003) also reported *L. amazonensis* in an *O. nitidus* from Los Fierros and in what was considered an *O. perenensis*, but now identified as the newly named species *O. acritus*, from El Refugio. Thus, the four rodents from this study were likely infected with *L. amazonensis* which is consistent with previous reports of this *Leishmania* species in the department of Santa Cruz, Bolivia (Martínez et al, 1998). The results of this study further implicate species of the genus *Oryzomys* as possible reservoirs of *L. amazonensis* in the region.

Leishmania braziliensis, *L. panamensis*, and *L. chagasi* have been reported as causative agents of leishmaniasis in various regions throughout Nicaragua (Missoni and Morelli, 1984; Missoni et al., 1986; Darce et al., 1991; Belli et al., 1994; Belli et al., 1999; Convit et al., 2005). In 2003, Liu reported a *Peromyscus mexicanus* and a *Heteromys desmarestianus* collected from Selva Negra, located in the department of Matagalpa, as positive using the genus-specific primer pair 13A/13B. However, little is known about the natural vectors and reservoirs in the country. Results of this study were negative for all tissue samples screened. Although no *Leishmania* was detected during the study, more research and a larger sample size may have increased the likelihood of finding the parasite since leishmaniasis is endemic in the country. Future studies are necessary to determine the natural cycles of *Leishmania* in Nicaragua.

One *N. micropus* (ID number 447D7E712C) of 74 collected in Texas was positive for *Leishmania* and additionally tested positive for *L. mexicana* complex.

The prevalence rate of 1.4% is lower than the 14.7% overall prevalence rate of the 19-month mark-release-recapture study conducted by Raymond et al. (2003) at the same locality. The authors did, however, report a seasonal prevalence rate ranging from 3.8 to 26.7% indicating that prevalence varies from season to season. The presence of *L. mexicana* in *N. micropus* at Medina Annex, Bexar County is consistent with previous reports of the parasite in this mammal species and supports the hypothesis that it is a reservoir host of *L. mexicana* in Texas (McHugh et al., 1990; Kerr et al., 1995; McHugh et al., 1996; Raymond et al., 2003).

All rodents screened from New Mexico tested negative for *Leishmania*. This is consistent with current information that no *Leishmania* has been reported in the state. However, the parasite has been detected west of New Mexico in *N. albigula* collected in Pima County, Arizona (Kerr et al., 1999). Additional research will be needed to determine if the range of enzootic leishmaniasis extends into New Mexico.

Although PCR has proven to be a sensitive tool in the detection of *Leishmania*, the rapid and simple method of preparing tissue biopsies for PCR amplification used can decrease sensitivity between 10 and 100-fold (Rogers et al., 1990). Therefore, the possibility of false negatives exists if an infection was below the detection limit of PCR, or if localized infections were not sampled when biopsies were taken.

The neotropical species *L. enriettii* and *L. hertigi*, are not pathogenic to man making them less significant clinically. Because of this, little is known about their epidemiological cycles or their taxonomic relationships to other members of

Leishmania. Molecular studies conducted by Rioux et al. (1990), Thomaz-Soccol et al. (1993a), and Thomaz-Soccol et al. (1993b) place *L. enriettii* and *L. hertigi* in the subgenus *Leishmania* which contains *L. mexicana* complex. On the other hand, Kerr (2000) demonstrated a closer affinity to subgenus *Viannia* for both species. The latest research has shown that *L. hertigi* is more closely related to members of the genus *Endotrypanum* than to members of *Leishmania* (Croan et al, 1997; Noyes et al., (1997); Cupolillo et al., 2000; Momen and Cupolillo, 2000; Noyes et al., 2000; Grimaldi and Schottelius, 2001). This investigation attempted to clarify some of these relationships.

Isolates *L. enriettii* (ATCC® 30035) and *L. hertigi* (ATCC® 30286) were tested by PCR using *L. mexicana* complex-specific primers 13A/M1.1 with *L. amazonensis* (ATCC® 50131) used as a positive control. Both species produced positive results at concentrations of 10^4 and 10^3 parasites/µl, but not at 10^2 and 10 parasites/µl. *Leishmania hertigi* produced an amplification product near the expected 105 bp region for these primers. There are two explanations for these results. It indicates possible similarities between kDNA of *L. hertigi* and members of *L. mexicana* complex, or it shows the primer pair 13A/M1.1 is not specific to *L. mexicana* complex. *Leishmania enriettii* also was positive with these primers, but amplified a fragment of approximately 75 bp which is smaller than the 105 bp product of *L. mexicana* complex kDNA. These results also question the validity of this primer pair. However, according to Melby (personal communication), the low

annealing temperature of 55 C could hybridize other kDNA and produce false positive results.

Leishmania enriettii (ATCC® 30035) and *L. hertigi* (ATCC® 30286) isolates were also tested using the *L. Viannia* subgenus primer pair B4/13B with *L. guyanensis* (ATCC® 50126) used as the positive control. *Leishmania enriettii* and *L. hertigi* were negative at all concentrations tested. This signifies that neither species contains the conserved region of kDNA found in *L.* (*Viannia*) spp. amplified by this set of primers.

To further enhance the results of this study, the *L. enriettii* and *L. hertigi* isolates were then tested with the genus-specific primer pair 13A/13B. *Leishmania hertigi* produced positive results at all concentrations tested (10^4 to 10^2 parasites/μl), but surprisingly, *L. enriettii* tested negative at all concentrations. The results of *L. hertigi*, previously untested with this primer pair, indicate that this species has some commonalities in kDNA structure with other members of the genus *Leishmania*, although previous studies have indicated a closer relationship to *Endotrypanum*. The negative results produced by *L. enriettii* are puzzling. Rogers et al. (1990) tested an isolate of *L. enriettii* using this set of primers and were able to amplify the target sequence, albeit a weak positive result. Possible explanations for the difference in results include variable methods in isolation of kDNA from cultured promastigotes, creating PCR reaction mixture, and PCR settings. Genetic differences between the two *L. enriettii* isolates also may have influenced the outcome of PCR testing. More research is required to determine the dependability of the results from the current

study. Additional isolates of both *L. enriettii* and *L. hertigi* should be tested with the three primer pairs to confirm the above results. Various PCR settings, especially annealing temperature, should be used as well to reinforce the study outcomes.

Based on the results of this study, I conclude that the primer pairs 13A/M1.1 and B4/13B can reliably differentiate between *L.* (*L.*) *mexicana* complex and *L.* (*Viannia*), respectively, but the primers may not be as specific as previously thought.

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APPENDIX A: Raw data

Bolivia mammal data

	Date	ID Number	Sex	Species	Location	PCR Date	PCR result
1	9/14/2003	639	F	*Akodon dayi*	El Refugio	Aug-16-04	Neg
2	9/14/2003	642	F	*Oryzomys nitidus*	El Refugio	Aug-16-04	Neg
3	9/14/2003	646	F	*Oryzomys nitidus*	El Refugio	Aug-16-04	Neg
4	9/15/2003	650	M	*Oecomys bicolor*	El Refugio	Aug-16-04	Neg
5	9/16/2003	654	F	*Oryzomys acritus*	El Refugio	Aug-16-04	POS
6	9/17/2003	657	M	*Oryzomys acritus*	El Refugio	Aug-16-04	Neg
7	9/30/2003	701	M	*Necromys lenguarum*	Los Fierros	Aug-16-04	Neg
8	9/8/2004	677	F	*Proechimys longicaudatus*	El Refugio	Nov-24-04	Neg
9	9/8/2004	686	F	*Oryzomys nitidus*	El Refugio	Nov-24-04	Neg
10	9/9/2004	692	F	*Oryzomys acritus*	El Refugio	Nov-24-04	POS
11	9/11/2004	729	M	*Oryzomys nitidus*	El Refugio	Nov-24-04	POS
12	9/7/2005	776	F	*Proechimys longicaudatus*	El Refugio	Jan-26-06	Neg
13	9/9/2005	788/789	F	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
14	9/9/2005	794	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
15	9/9/2005	795	F	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
16	9/10/2005	796	F	*Oryzomys acritus*	El Refugio	Jan-26-06	Neg
17	9/10/2005	800	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
18	9/11/2005	902	F	*Oecomys bicolor*	El Refugio	Jan-26-06	Neg
19	9/12/2005	903	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
20	9/12/2005	904	F	*Oryzomys acritus*	El Refugio	Jan-26-06	Neg
21	9/13/2005	802	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
22	9/13/2005	804	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
23	9/13/2005	806	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	POS
24	9/14/2005	812	M	*Oryzomys nitidus*	El Refugio	Jan-26-06	Neg
25	9/15/2005	821	F	*Oryzomys acritus*	El Refugio	Jan-26-06	Neg

Nicaragua mammal data

	Date	ID Number	Sex	Weight (g)	Species	Location	Site	Culture Results	PCR Date	PCR Results
1	12/16/04	Nica 368	F	408	*Proechimys semispinosus*	La Sardina		Neg	Mar-8-05	Neg
2	12/17/04	Nica 369	M	175	*Proechimys semispinosus*	El Paraisito		Neg	Mar-8-05	Neg
3	12/17/04	Nica 370	M	253	*Proechimys semispinosus*	El Paraisito		Neg	Mar-8-05	Neg
4	12/18/04	Nica 371	F	145	*Proechimys semispinosus*	El Paraisito		Neg	Mar-8-05	Neg
5	12/18/04	Nica 372	M	250	*Proechimys semispinosus*	El Paraisito		Neg	Mar-8-05	Neg
6	12/19/04	Nica 373	F	295	*Proechimys semispinosus*	El Paraisito		Neg	Mar-8-05	Neg
7	12/21/04	Nica 374	F	290	*Proechimys semispinosus*	La Esperanzita #2		Neg	Mar-8-05	Neg
8	12/22/04	Nica 375	F	315	*Proechimys semispinosus*	La Fonseca		Neg	Mar-8-05	Neg
9	12/22/04	Nica 376	F	395	*Proechimys semispinosus*	La Fonseca		Neg	Mar-8-05	Neg
10	12/23/04	Nica 377	M	233	*Proechimys semispinosus*	La Fonseca		Neg	Mar-8-05	Neg
11	12/23/04	Nica 378	M	130	*Proechimys semispinosus*	La Fonseca		Neg	Mar-8-05	Neg
12	12/23/04	Nica 379	M	397	*Proechimys semispinosus*	La Fonseca		Neg	Mar-8-05	Neg
13	3/16/05	Nica 380		360	*Proechimys semispinosus*	El Paraisito		N/A	Dec-1-05	Neg
14	3/17/05	Nica 381	F	10	*Mus musculus*	El Paraisito	farmer's house	N/A	Dec-1-05	Neg
15	3/18/05	Nica 382		385	*Proechimys semispinosus*	El Paraisito	woodlot	N/A	Dec-1-05	Neg
16	5/12/05	Nica 382 B	F	325	*Proechimys semispinosus*	El Paraisito	farm	Neg	Dec-1-05	Neg
17	5/13/05	Nica 383	M	510	*Proechimys semispinosus*	Nueva Guinea	ranch	Neg	Dec-1-05	Neg
18	3/12/06	Nica 384	M	34	*Peromyscus nudipes*	Selva Negra	Bavaria	Neg	Mar-22-06	Neg
19	3/12/06	Nica 385		44	*Peromyscus nudipes*	Selva Negra	Bavaria	Neg	Mar-22-06	Neg
20	3/12/06	Nica 386	M	40	*Peromyscus nudipes*	Selva Negra	Bavaria	Neg	Mar-22-06	Neg
21	3/12/06	Nica 387	M	40	*Peromyscus nudipes*	Selva Negra	Bavaria	Neg	Mar-22-06	Neg
22	3/12/06	Nica 388	M	45	*Peromyscus nudipes*	Selva Negra	Bavaria	Neg	Mar-22-06	Neg
23	3/12/06	Nica 389	M	38	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-22-06	Neg
24	3/12/06	Nica 390	F	50	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-22-06	Neg
25	3/12/06	Nica 391	F	45	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-23-06	Neg
26	3/12/06	Nica 392	M	45	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-23-06	Neg
27	3/12/06	Nica 393	F	45	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-23-06	Neg
28	3/12/06	Nica 394	M	50	*Peromyscus nudipes*	Selva Negra	Atajo	Neg	Mar-23-06	Neg

29	3/14/06	Nica 395	M	70	*Sigmodon hirsutus*	El Cuá	bean field	Neg	Mar-23-06	Neg
30	3/14/06	Nica 396		70	*Sigmodon hirsutus*	El Cuá	bean field	Neg	Mar-23-06	Neg
31	3/14/06	Nica 397	M	40	*Peromyscus nudipes*	El Cuá	bean field	Neg	Mar-24-06	Neg
32	3/14/06	Nica 398	M	50	*Oryzomys cousei*	El Cuá	bean field	Neg	Mar-24-06	Neg
33	3/14/06	Nica 399	M	30	*Melanomys caliginosus*	El Cuá	bean field	Neg	Mar-23-06	Neg
34	3/14/06	Nica 400	M	7	*Mus musculus*	El Cuá	bean field	Neg	Mar-24-06	Neg
35	3/14/06	Nica 401	M	45	*Melanomys caliginosus*	El Cuá	bean field	Neg	Mar-24-06	Neg
36	3/14/06	Nica 402	F	10	*Mus musculus*	El Cuá	farmer's house	Neg	Mar-24-06	Neg
37	3/14/06	Nica 403	M	5	*Mus musculus*	El Cuá	farmer's house	Neg	Mar-24-06	Neg
38	3/15/06	Nica 404	M	26	*Oryzomys alfaroi*	El Cuá	Galope	Neg	Mar-24-06	Neg
39	3/15/06	Nica 405	M	50	*Peromyscus nudipes*	El Cuá	Galope	Neg	Mar-24-06	Neg
40	3/15/06	Nica 406	M	35	*Oryzomys alfaroi*	El Cuá	Galope	Neg	Mar-24-06	Neg
41	3/15/06	Nica 407	F	45	*Peromyscus nudipes*	El Cuá	Galope	Neg	Mar-24-06	Neg
42	3/15/06	Nica 408	M	35	*Oryzomys alfaroi*	El Cuá	Galope	Neg	Mar-24-06	Neg

New Mexico, USA mammal data

	Date	ID Number	Sex	Weight (g)	Species	Location	PCR Date	PCR Result
1	17-Aug-04	PJR2904	F	60	*Dipodomys ordii*	El Dorado	1-Sep-04	Neg
2	17-Aug-04	PJR2905	M	205	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
3	17-Aug-04	PJR2907	M	145	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
4	17-Aug-04	PJR2908	M	26	*Peromyscus leucopus*	El Dorado	1-Sep-04	Neg
5	17-Aug-04	PJR2910	F	210	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
6	17-Aug-04	PJR2912	F	190	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
7	17-Aug-04	PJR2919	M	215	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
8	17-Aug-04	PJR2920	F	155	*Neotoma micropus*	El Dorado	1-Sep-04	Neg
9	29-Sep-04	PJR2957	F	150	*Neotoma albigula*	El Dorado	27-Oct-04	Neg
10	29-Sep-04	PJR2958	M	200	*Neotoma micropus*	El Dorado	27-Oct-04	Neg
11	29-Sep-04	PJR2959	F	180	*Neotoma micropus*	El Dorado	27-Oct-04	Neg
12	29-Sep-04	PJR2961	F	150	*Neotoma albigula*	El Dorado	27-Oct-04	Neg
13	29-Sep-04	PJR2964	M	135	*Neotoma albigula*	El Dorado	27-Oct-04	Neg
14	13-Apr-05	PJR2981	M	150	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
15	13-Apr-05	PJR 2982	F	270	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
16	13-Apr-05	PJR2983	F	200	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
17	13-Apr-05	PJR2990	F	215	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
18	13-Apr-05	PJR2991	M	200	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
19	13-Apr-05	PJR2992	F	160	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
20	13-Apr-05	PJR2994	F	250	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
21	13-Apr-05	PJR2995	F		*Neotoma micropus*	El Dorado	28-Mar-06	Neg
22	13-Apr-05	PJR2997	F	225	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
23	14-Apr-05	PJR3001	M		*Neotoma micropus*	El Dorado	28-Mar-06	Neg
24	14-Apr-05	PJR3005	M	230	*Neotoma micropus*	El Dorado	28-Mar-06	Neg
25	14-Apr-05	PJR3007	M	41	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
26	14-Apr-05	PJR3013	F	175	*Neotoma albigula*	El Dorado	29-Mar-06	Neg
27	14-Apr-05	PJR3016	F	200	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
28	14-Apr-05	PJR3017	M	170	*Neotoma micropus*	El Dorado	29-Mar-06	Neg

29	14-Apr-05	PJR3020	F	170	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
30	14-Apr-05	PJR3021	M	330	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
31	14-Apr-05	PJR3023	F	155	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
32	14-Apr-05	PJR3027	F	135	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
33	14-Apr-05	PJR3032	M	95	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
34	14-Apr-05	PJR3034	M	95	*Neotoma micropus*	El Dorado	29-Mar-06	Neg
35	17-May-05	PJR3058	M	115	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
36	17-May-05	PJR3060	M	130	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
37	17-May-05	PJR3062	F	115	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
38	17-May-05	PJR3065	M	230	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
39	17-May-05	PJR3069	M	115	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
40	17-May-05	PJR3071	M	300	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
41	17-May-05	PJR3073	F	215	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
42	17-May-05	PJR3074	M	145	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
43	17-May-05	PJR3075	M	145	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
44	17-May-05	PJR3079	F	87	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
45	17-May-05	PJR3086	M	235	*Neotoma micropus*	El Dorado	30-Jan-06	Neg
46	17-May-05	PJR3088	M	125	*Neotoma albigula*	El Dorado	31-Jan-06	Neg
47	17-May-05	PJR3089	F	215	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
48	18-May-05	PJR3094	F	155	*Neotoma albigula*	El Dorado	31-Jan-06	Neg
49	18-May-05	PJR3097	F	180	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
50	18-May-05	PJR3117	M	120	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
51	15-Jun-05	PJR3172	M	130	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
52	15-Jun-05	PJR3173	F	265	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
53	15-Jun-05	PJR3174	M	125	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
54	15-Jun-05	PJR3180	M	310	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
55	15-Jun-05	PJR3184	M	105	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
56	15-Jun-05	PJR3188	F	165	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
57	16-Jun-05	PJR3208	F	180	*Neotoma albigula*	El Dorado	31-Jan-06	Neg
58	16-Jun-05	PJR3211	M	145	*Neotoma micropus*	El Dorado	31-Jan-06	Neg
59	16-Jun-05	PJR3212	F	195	*Neotoma albigula*	El Dorado	31-Jan-06	Neg

60	16-Jun-05	PJR3215	M	160	*Neotoma albigula*	El Dorado	1-Feb-06	Neg
61	16-Jun-05	PJR3218	F	110	*Neotoma albigula*	El Dorado	1-Feb-06	Neg
62	16-Jun-05	PJR3220	M	145	*Neotoma albigula*	El Dorado	1-Feb-06	Neg
63	17-Aug-05	PJR3251	M	105	*Neotoma micropus*	El Dorado	1-Feb-06	Neg
64	17-Aug-05	PJR3253	M	95	*Neotoma micropus*	El Dorado	1-Feb-06	Neg
65	17-Aug-05	PJR3254	F	125	*Neotoma micropus*	El Dorado	1-Feb-06	Neg
66	17-Aug-05	PJR3262	M	105	*Neotoma micropus*	El Dorado	1-Feb-06	Neg
67	17-Aug-05	PJR3264	M	210	*Neotoma micropus*	El Dorado	1-Feb-06	Neg
68	17-Aug-05	PJR3269	F	100	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
69	16-Aug-05	NM-2	F	235	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
70	16-Aug-05	NM-3	F	255	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
71	16-Aug-05	NM-5	F	170	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
72	16-Aug-05	NM-6	F	95	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
73	16-Aug-05	NM-7	F	190	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
74	16-Aug-05	NM-9	F	200	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
75	16-Aug-05	NM-11	F	255	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
76	16-Aug-05	NM-14	F	180	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
77	16-Aug-05	NM-21	F	115	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
78	16-Aug-05	NM-22	M	170	*Neotoma micropus*	El Dorado	2-Feb-06	Neg
79	16-Aug-05	NM-23	F	205	*Neotoma albigula*	El Dorado	7-Feb-06	Neg
80	16-Aug-05	NM-24	F	165	*Neotoma micropus*	El Dorado	7-Feb-06	Neg
81	16-Aug-05	NM-25	F	130	*Neotoma albigula*	El Dorado	7-Feb-06	Neg
82	16-Aug-05	NM-30	M	150	*Neotoma albigula*	El Dorado	7-Feb-06	Neg
83	16-Aug-05	NM-31	M	225	*Neotoma micropus*	El Dorado	7-Feb-06	Neg
84	16-Aug-05	NM-33	M	115	*Neotoma micropus*	El Dorado	7-Feb-06	Neg

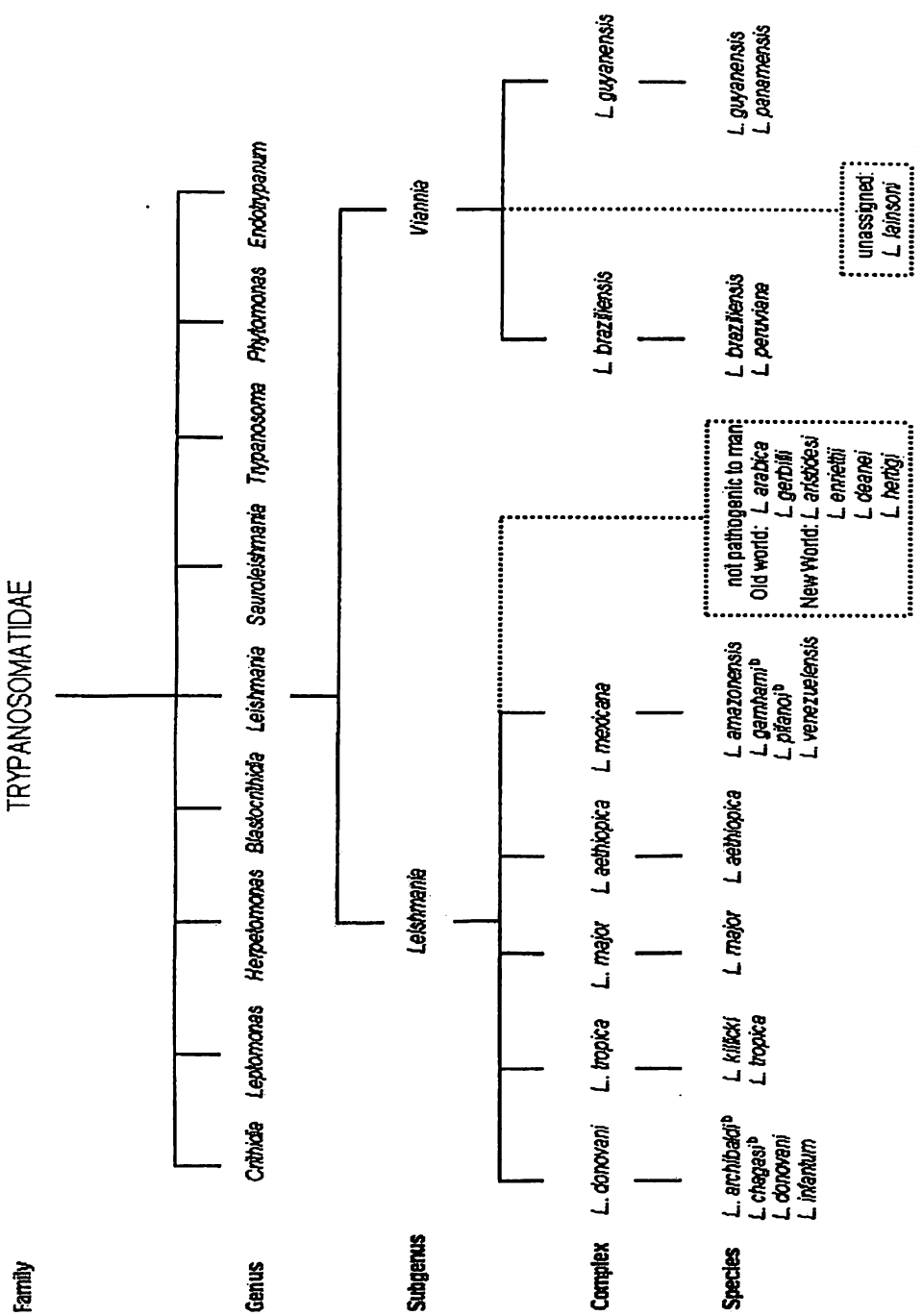
Texas, USA mammal data

	Date	ID Number	Sex	Weight (g)	Species	Location	Site	Status	PCR Date	PCR Results
1	29-Sep-04	44776F4D08	F	355	*Neotoma micropus*	Medina Annex	M73	New	20-Oct-04	Neg
2	29-Sep-04	4161522E67	M	380	*Neotoma micropus*	Medina Annex	M71	New	20-Oct-04	Neg
3	29-Sep-04	4507466278	M	290	*Neotoma micropus*	Medina Annex	M6	New	20-Oct-04	Neg
4	29-Sep-04	426C534B6B	F	290	*Neotoma micropus*	Medina Annex	M75	Recapture	20-Oct-04	Neg
5	29-Sep-04	426D4A296E	F	365	*Neotoma micropus*	Medina Annex	M87	Recapture	20-Oct-04	Neg
6	29-Sep-04	4503675828	F	295	*Neotoma micropus*	Medina Annex	M86	New	20-Oct-04	Neg
7	29-Sep-04	450344031A	M	335	*Neotoma micropus*	Medina Annex	M74	New	20-Oct-04	Neg
8	12-Oct-04	4506250679	M	170	*Neotoma micropus*	Medina Annex	M141	New	20-Oct-04	Neg
9	12-Oct-04	4477737C61	M	125	*Neotoma micropus*	Medina Annex	M81	New	20-Oct-04	Neg
10	12-Oct-04	4505523C00	F	290	*Neotoma micropus*	Medina Annex	M94	New	20-Oct-04	Neg
11	12-Oct-04	450A47063	F	190	*Neotoma micropus*	Medina Annex	M29	New	20-Oct-04	Neg
12	12-Oct-04	450731275D	F	290	*Neotoma micropus*	Medina Annex	M35	New	20-Oct-04	Neg
13	12-Oct-04	426D606261	M	365	*Neotoma micropus*	Medina Annex	M108	Recapture	20-Oct-04	Neg
14	12-Oct-04	42192E3A2D	F	315	*Neotoma micropus*	Medina Annex	M140	Recapture	20-Oct-04	Neg
15	12-Oct-04	426C6A341C	F	310	*Neotoma micropus*	Medina Annex	M142	Recapture	20-Oct-04	Neg
16	19-Oct-04	447D7E712C	F	265	*Neotoma micropus*	Medina Annex	M180	New	14-Nov-04	POS
17	19-Oct-04	4503633521	F	325	*Neotoma micropus*	Medina Annex	M17	New	5-Nov-04	Neg
18	19-Oct-04	4477772C6A	M	315	*Neotoma micropus*	Medina Annex	M168	New	5-Nov-04	Neg
19	19-Oct-04	426A4F7B0A	F	310	*Neotoma micropus*	Medina Annex	M107	Recapture	5-Nov-04	Neg
20	19-Oct-04	441A526442	F	375	*Neotoma micropus*	Medina Annex	M122	Recapture	14-Nov-04	Neg
21	19-Oct-04	4507022B09	F	330	*Neotoma micropus*	Medina Annex	M131	New	5-Nov-04	Neg
22	26-Oct-04	45046C136B	F	300	*Neotoma micropus*	Medina Annex	M41	New	5-Nov-04	Neg
23	26-Oct-04	450373023D	F	285	*Neotoma micropus*	Medina Annex	M193	New	14-Nov-04	Neg
24	26-Oct-04	441C395A2E	M	410	*Neotoma micropus*	Medina Annex	M102	Recapture	5-Nov-04	Neg
25	26-Oct-04	426E1A3C37	F	360	*Neotoma micropus*	Medina Annex	M128	Recapture	14-Nov-04	Neg
26	26-Oct-04	450B7B0E3C		410	*Neotoma micropus*	Medina Annex	M186	Recapture	14-Nov-04	Neg
27	3-Nov-04	4506551110	M	180	*Neotoma micropus*	Medina Annex	M199	New	14-Dec-04	N/A
28	3-Nov-04	441F7B535E	F	310	*Neotoma micropus*	Medina Annex	M200	Recapture	14-Dec-04	N/A
29	3-Nov-04	450A113D74	M	345	*Neotoma micropus*	Medina Annex	M189	New	14-Dec-04	N/A
30	3-Nov-04	441F0F4D5E	F	320	*Neotoma micropus*	Medina Annex	M204	Recapture	14-Dec-04	N/A

31	3-Nov-04	447235512F	F	330	*Neotoma micropus*	Medina Annex	M199	New	14-Dec-04	N/A
32	3-Nov-04	441E2A5C68	M	380	*Neotoma micropus*	Medina Annex	M187	Recapture	14-Dec-04	N/A
33	3-Nov-04	441E701367	M	110	*Neotoma micropus*	Medina Annex	M181	New	14-Dec-04	N/A
34	10-Nov-04	45063C1C5A	M	285	*Neotoma micropus*	Medina Annex	M76	New	14-Dec-04	Neg
35	10-Nov-04	45036c0928	M	365	*Neotoma micropus*	Medina Annex	M104	New	14-Dec-04	Neg
36	10-Nov-04	4506557570	F	325	*Neotoma micropus*	Medina Annex	M30	New	14-Dec-04	Neg
37	10-Nov-04	426C6A341C	F	295	*Neotoma micropus*	Medina Annex	M109	Recapture	14-Dec-04	Neg
38	10-Nov-04	450717094A	M	365	*Neotoma micropus*	Medina Annex	M112	New	14-Dec-04	Neg
39	7-Dec-04	45062F4404		360	*Neotoma micropus*	Medina Annex	M146	Recapture	14-Dec-04	Neg
40	7-Dec-04	45070C7140	M	260	*Neotoma micropus*	Medina Annex	M82	New	14-Dec-04	Neg
41	7-Dec-04	447973201B	M	245	*Neotoma micropus*	Medina Annex	M4	New	14-Dec-04	Neg
42	7-Dec-04	44796E3923	F	280	*Neotoma micropus*	Medina Annex	M145	New	14-Dec-04	Neg
43	7-Dec-04	447C425F03	M	410	*Neotoma micropus*	Medina Annex	M138	New	14-Dec-04	Neg
44	7-Dec-04	441F200649	M	365	*Neotoma micropus*	Medina Annex	M173	New	14-Dec-04	Neg
45	7-Dec-04	441E7A0733	M	410	*Neotoma micropus*	Medina Annex	M83	New	14-Dec-04	Neg
46	7-Dec-04	4503366707	M	310	*Neotoma micropus*	Medina Annex	M89	New	14-Dec-04	Neg
47	7-Dec-04	450719325A	M	190	*Neotoma micropus*	Medina Annex	M144	New	14-Dec-04	Neg
48	7-Dec-04	426E1D4D6F		335	*Neotoma micropus*	Medina Annex	M5	Recapture	14-Dec-04	Neg
49	7-Dec-04	450A6C2866	F	210	*Neotoma micropus*	Medina Annex	M143	New	14-Dec-04	Neg
50	7-Dec-04	441E701244	F	245	*Neotoma micropus*	Medina Annex	M147	New	14-Dec-04	Neg
51	7-Dec-04	441F05152F		415	*Neotoma micropus*	Medina Annex	M4	Recapture	14-Dec-04	Neg
52	8-Dec-04	4506222E43	F	315	*Neotoma micropus*	Medina Annex	M172	New	14-Dec-04	Neg
53	8-Dec-04	441F200649	M	365	*Neotoma micropus*	Medina Annex	M171	Recapture	14-Dec-04	Neg
54	8-Dec-04	441C383B63		285	*Neotoma micropus*	Medina Annex	M159	Recapture	14-Dec-04	Neg
55	8-Dec-04	441F206D62	M	145	*Neotoma micropus*	Medina Annex	M1	New	14-Dec-04	Neg
56	8-Dec-04	426C6F776A		335	*Neotoma micropus*	Medina Annex	M160	Recapture	14-Dec-04	Neg
57	8-Dec-04	441C2C375F	F	295	*Neotoma micropus*	Medina Annex	M160	New	14-Dec-04	Neg
58	8-Dec-04	441C591170	F	315	*Neotoma micropus*	Medina Annex	M163	New	14-Dec-04	Neg
59	8-Dec-04	441E721F5C		380	*Neotoma micropus*	Medina Annex	M157	Recapture	14-Dec-04	Neg
60	8-Dec-04	441E3A4E4E		345	*Neotoma micropus*	Medina Annex	M161	Recapture	14-Dec-04	Neg
61	8-Dec-04	441E47723A	F	325	*Neotoma micropus*	Medina Annex	M149	New	14-Dec-04	Neg
62	8-Dec-04	441C506C48	M	365	*Neotoma micropus*	Medina Annex	M155	New	14-Dec-04	Neg
63	8-Dec-04	45032B3434	M	395	*Neotoma micropus*	Medina Annex	M155	Recapture	14-Dec-04	Neg

64	8-Dec-04	447973201B		230	*Neotoma micropus*	Medina Annex	M172	New	14-Dec-04	Neg
65	8-Dec-04	44742B4D76	F	200	*Neotoma micropus*	Medina Annex	M156	New	14-Dec-04	Neg
66	8-Dec-04	441E2B2210	F	330	*Neotoma micropus*	Medina Annex	M1	New	14-Dec-04	Neg
67	8-Dec-04	441FC187F	M	325	*Neotoma micropus*	Medina Annex	M167	New	14-Dec-04	Neg
68	15-Dec-04	450744621F	M	345	*Neotoma micropus*	Medina Annex	M223	New	22-Dec-04	Neg
69	15-Dec-04	44771C422A	M	340	*Neotoma micropus*	Medina Annex	M221	New	22-Dec-04	Neg
70	15-Dec-04	45046C553C	M	230	*Neotoma micropus*	Medina Annex	M224	New	22-Dec-04	Neg
71	15-Dec-04	450437286E	F	290	*Neotoma micropus*	Medina Annex	M213	New	22-Dec-04	Neg
72	15-Dec-04	441F1D3517		420	*Neotoma micropus*	Medina Annex	M176	Recapture	22-Dec-04	Neg
73	15-Dec-04	441E701367		150	*Neotoma micropus*	Medina Annex	M202	Recapture	22-Dec-04	Neg
74	15-Dec-04	441E63414B		330	*Neotoma micropus*	Medina Annex	M229	Recapture	22-Dec-04	Neg

APPENDIX B: Taxonomy of *Leishmania*



[a]The classification of genera and subgenera is based on extrinsic characters and that of the complexes mainly on intrinsic characters (isoenzymes).
[b]Some workers do not consider these to be separate species

Cited from WHO 1990