# EXPLORATION OF POTENTIAL RESERVOIR HOSTS AND VECTORS OF *LEISHMANIA* IN NICARAGUA

A Dissertation

by

RUSSELL WAYNE RAYMOND

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2008

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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August 2008

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# ABSTRACT

Exploration of Potential Reservoir Hosts and Vectors of *Leishmania*
in Nicaragua. (August 2008)
Russell Wayne Raymond, B.S., University of the Incarnate Word;
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Chair of Advisory Committee: Dr. Norman O. Dronen, Jr.

Leishmaniasis is caused by infection with protozoan parasites within the genus *Leishmania* and, in the New World, is transmitted by the bites of female sand flies within the genus *Lutzomyia*. The occurrence of leishmaniasis in rodent species, the geographic distribution of sand fly species in Nicaragua, and environmental factors associated with the distribution of human cases of typical cutaneous leishmaniasis were investigated. Three hundred ninety five rodents representing 17 species were collected from 13 localities from August 2001–March 2006 and screened for *Leishmania* infections. One *Heteromys desmarestianus* and one *Peromyscus mexicanus* were found to be positive for leishmanial infections by PCR. This is the first report of *Leishmania* infections in rodents in Nicaragua. Five hundred fifty six sand flies representing 12 species were collected from 8 localities, including *Lutzomyia hartmanni*, a new record for this species in Nicaragua. The predominant sand fly species captured in western Nicaragua were *Lutzomyia longipalpis* and *Lutzomyia evansi*. The predominant species captured in central and eastern Nicaragua was *Lutzomyia cruciata*. The geographic distribution of sand flies in this study provides additional support to previously-published reports of suspected vectors of *Leishmania* species that cause typical and atypical forms of cutaneous leishmaniasis in Nicaragua.

Distribution data of human cases of typical cutaneous leishmaniasis obtained from the Nicaraguan Ministry of Health, along with GIS and remotely sensed data of elevation, precipitation, temperature, soil types and land use/cover classes, were used to develop predictive logistic regression models for the presence or absence of human cases within 151 municipalities. Mean annual precipitation and land use/cover were determined to be the best environmental variable predictors for the occurrence of typical cutaneous leishmaniasis.

# ACKNOWLEDGEMENTS

A great number of individuals are responsible for the success of this research and manuscript. I would like to express gratitude to my graduate studies committee: Dr. Norman O. Dronen, Jr., Chairman, and members Dr. Thomas M. Craig, Dr. Jimmy K. Olson, and Dr. Markus J. Peterson. Dr. Dronen provided guidance in navigating around the pitfalls associated with a doctoral program. He provided invaluable assistance in the development of a degree plan, the writing of a research proposal and the development of this dissertation. Thank you for taking me under your wing and providing support, encouragement and guidance along the way. Dr. Craig provided an interesting and intensive course in Veterinary Parasitology. I learned skills in his lecture and laboratory that I am certain will serve me well for years to come. Thank you for your input on my research proposal and this dissertation. Dr. Olson's course in Medical and Veterinary Entomology was an incredible introduction to this field. Although I hope that I never have to identify mosquito larvae again; I am reasonably confident that I could. Thank you for your input and guidance along the way. I am perhaps most thankful for Dr. Olson's ability to make me feel like one of the crew. I only spent a short time living in College Station and even though I did not receive my B.S. or M.S. from Texas A&M, he was the one that made me feel like I was an Aggie. Dr. Peterson's class in Research Methods was an invaluable experience. It was one of the most challenging courses that I have ever taken, but I enjoyed it very much. He taught me how the entire research

process comes together, from proposals and funding to data collection, analysis and presentation.

I am indebted to my mentor Dr. Sara F. Kerr, University of the Incarnate Word. Thank you for your years of encouragement and support, without which I am certain that I would have ended my academic studies upon the completion of my B.S. I had no idea that those initial hot afternoons and early mornings spend crawling through the brush at Rothe Ranch and La Copita would lead to hot afternoons and early mornings of crawling through the jungle in Nicaragua. I take pride in explaining to people that I am a professional rat trapper.

To Dr. Chad McHugh, I am well aware of the amount time and effort that you put into identification of sand flies from Nicaragua. Without your expertise in this area, the identification of insect specimens may well have taking forever. I also am appreciative of your advice on improving my writing and organizational skills.

Dr. Robert Bradley and his crew at Texas Tech University provided enormous assistance with identification of rodent species from Nicaragua. Doctora Rafaela Montoya, Nicaraguan Ministry of Health, was my primary point of contact within the country. She took time from her very busy schedule at the hospital in Nueva Guinea to serve as liaison between our crew and numerous individuals and government agencies in Nicaragua. Doctora Montoya's staff in Nueva Guinea as well as the Ministry of Health staffs in Siuna, Matagalpa, Jinotega, Bluefields, Chinendega, and Leon provided hours of help with the identification of trapping localities as well as assistance with field work. Doctora Arlette Vargas traveled with us, on many occasions, to various communities in order to help explain our project to local leaders and to help gather information about "hot spots" of leishmaniasis. Doctor Byron Perez provided much assistance with gaining access to trapping sites in the Matagalpa area and with navigating the paperwork required to export rodents from Nicaragua. Data on the distribution of human cutaneous leishmaniasis was provided by Dr. Juan Jose Amador, Chief Epidemiologist of the Ministry of Health, Managua, Nicaragua. The GIS maps of Nicaragua were provided by the staff at the Nicaraguan Ministerio del Ambiente y los Recursos Naturales.

Diana Kruger was of enormous assistance in a number of areas associated with this project. She provided help with travel plans, reservations, arranged for drivers, graciously allowed us to store mounds of equipment at her home in Managua and provided the best lemonade that I've ever tasted. Most importantly, it was Diana's phone calls to various individuals within the Nicaraguan government that help secure meetings and gain access to Ministry of Health data on the prevalence and distribution of human cases of typical cutaneous leishmaniasis. I also need to thank Reggie Romo with ESRI for showing me a more efficient way to get at the data analysis in GIS and Dr. Paul Messina for much needed assistance with statistical analysis.

Many individuals assisted with the "heavy lifting" associated with specimen collection in sometimes very rugged terrain. Robert Miranda, Christina Salinas, and Sebastian Villarreal (students from the University of the Incarnate Word) made numerous treks carrying equipment into and out of research sites. Ernesto Hernandez and Ariel Hernandez from Chinandega and Waldemar Rios from Siuna provided

countless hours of assistance in everything from specimen collection and specimen processing to driving duties and Spanish/English translation.

I am very grateful to my parents, Ray and Marlene Raymond; thank you for many years of love and support. Finally and most of all, I would like to recognize my wife Carol Armata Raymond; thank you for years of love, encouragement and support. You are an inspiration to me. Without you, I would not have made it this far.

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# INTRODUCTION

## Overview

The leishmaniases are a complex of vector-borne diseases caused by protozoan parasites within the genus *Leishmania* Ross, 1903 (KINETOPLASTIDA: TRYPANOSOMATIDAE). Infection is initiated when the parasites are inoculated into the skin of the host by the bite of an infected sand fly within the genera *Phlebotomus* Rondani and Berte, 1840 and *Lutzomyia* Franca, 1927 (DIPTERA: PSYCHODIDAE). Leishmaniasis is widespread ecologically and geographically and primarily occurs in tropical and subtropical regions on all continents except Antarctica. It is present in at least 88 countries worldwide and is a significant and increasing public health problem, with an estimated 12 million people infected worldwide and 350 million people at risk (WHO, 1990).

Leishmaniasis is endemic in at least 23 nations in the New World and has been reported from all mainland countries with the exceptions of Chile and Uruguay (Grimaldi et al., 1989; Duprey et al. 2006). In the New World, autochthonous human cases have been reported from Texas in the southern United States to Argentina, in South America (Stewart and Pilchard, 1945; Grimaldi et al., 1989; Grogl et al. 1991).

Protozoan parasites of the genus *Leishmania* are responsible for a number of disease syndromes within human and other mammalian hosts. The outcome of infection in humans ranges from mild, self-healing cutaneous lesions to severe and often fatal

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This dissertation follows the style and format of Journal of Parasitology.

visceral involvement (Lainson and Shaw, 1978; WHO, 1984).

## Clinical manifestations of leishmaniasis

The course of disease associated with *Leishmania* infections primarily is dependent on the species of infecting leishmanial parasite, but also may be determined in part by the inoculation size, and the immune status of the host (WHO, 1984; Titus and Ribeiro, 1988; Ashford, 2000; Gangneux et al., 2000). Three primary clinical syndromes characterize the disease manifestations of leishmaniasis: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). All 3 primary forms of leishmaniasis occur in Nicaragua (Missoni et al., 1986; Darce et al., 1991; Belli et al., 1994; Belli et al., 1999).

Cutaneous leishmaniasis often involves mild and self-healing ulcerative skin lesions (Fig. 1), but also can be cosmetically disfiguring (Schonian et al., 1996). Worldwide, there are an estimated 1 to 1.5 million human cases of cutaneous leishmaniasis annually (Desjeux, 2001). Additionally, these ulcerative lesions may predispose infected individuals to bacterial super infections (Fontes et al., 2005).

Mucocutaneous leishmaniasis begins when leishmanial parasites metastasize from skin lesions that occur at the site of inoculation to mucosal tissue, potentially resulting in deformation and massive tissue destruction of the nose, mouth, and throat (David et al., 1993; Almeida et al., 1996). This metastasis of leishmanial parasites from the initial site of inoculation in the skin to mucosal tissue typically occurs years after the cutaneous lesions develop (Almeida et al., 1996).

Visceral leishmaniasis occurs when the parasites travel from the initial site of

Figure 1. Typical cutaneous lesions due to *Leishmania* infection on the arm of a young girl from El Cua, Nicaragua, 2006.

inoculation to lymph nodes, bone marrow and internal organs (Satoskar et al., 1995). Visceral leishmaniasis is a potentially lethal form of the disease involving tissue destruction primarily in the liver and spleen (Ashford, 2000; Heinzel et al., 1989). Additionally, visceral leishmanial parasites within the small intestine may contribute to malabsorption (Muigai et al., 1983).

## Occurrence of clinical forms of leishmaniasis in Nicaragua

Mucocutaneous and visceral leishmaniasis are relatively uncommon in Nicaragua, while cutaneous leishmaniasis occurs with considerable frequency (Belli et al., 1999). Additionally, the human cases of cutaneous leishmaniasis are divided into 2 separate clinical categories, typical and atypical cutaneous leishmaniasis. Typical cutaneous leishmaniasis is characterized by open lesions, while atypical cutaneous leishmaniasis is characterized by the occurrence of non-ulcerative cutaneous nodules (Zeledon et al., 1989). In Nicaragua, approximately 2,000–2,500 human cases of leishmaniasis are reported annually, with approximately 86% of these cases diagnosed as human typical cutaneous leishmaniasis (Ministerio de Salud Nicaragua, 2003).

In addition to the different disease syndromes associated with typical and atypical cutaneous leishmaniasis, the diseases are a result of infections with different species of *Leishmania* parasites. There is a definite spatial segregation to the occurrence of the typical and atypical forms of cutaneous leishmaniasis in Nicaragua, with typical cutaneous leishmaniasis occurring primarily in the central and eastern regions of the country and atypical cutaneous leishmaniasis occurring primarily in western Nicaragua (Missoni et al., 1986; Darce et al., 1991; Belli et al., 1994; Belli et al., 1999). It is likely that the parasites that cause these forms of cutaneous leishmaniasis have different reservoir hosts and different sand fly vectors. In Nicaragua, the natural systems in which the causative agents of human typical cutaneous leishmaniasis are maintained and transmitted are largely unknown.

Reservoir hosts serve in the longterm maintenance of the leishmanial parasite populations, while vectors are responsible for transmission of the parasite among susceptible hosts. A wide variety of mammals other than humans typically serve as reservoir hosts of *Leishmania* species. Humans generally are incidental hosts and become infected while living or traveling within areas where the normal vector–reservoir system is established (Ashford, 1997). Sand flies within the genus *Lutzomyia* serve as the vector for all species of *Leishmania* in the New World. The study described herein was conducted in the Central American country of Nicaragua (Fig. 2) and its objectives were: 1. Identify geographic areas in Nicaragua that have concentrations of human cases of typical cutaneous leishmaniasis; 2. capture and identify rodents near human case residences; 3. screen rodents for *Leishmania* infections using polymerase chain reaction (PCR) and culture techniques; 4. identify complexes and species of *Leishmania*; 5. identify sand fly species in areas of Nicaragua having high prevalence of human cutaneous leishmaniasis; 6. use geographic information systems (GIS) technology, remotely-sensed data, and human incidence data to develop predictive models that identify biotic and abiotic environmental factors that influence the spatial distribution of human typical cutaneous leishmaniasis.

Figure 2. Map of the Central American isthmus showing Nicaragua and surrounding countries. This study took place entirely within the country of Nicaragua.

## Geographic distribution of clinical forms of leishmaniasis in Nicaragua

The CL form of leishmaniasis is the most common in Nicaragua, as well as the rest of Central America, resulting in increasing public health problems and economic loss in terms of disability of infected individuals and the cost of treatment (Carreira et al., 1995). Cutaneous leishmaniasis was first documented in Nicaragua by Doctor Francisco Baltodano near the town of San Juan de Limay in the department of Estelí in 1917 (Ministerio de Salud Nicaragua, 2003) (Fig. 3). It typically is associated with sylvan habitats, with human cases primarily occurring in the northern, central, and eastern regions of the country in the districts of Jinotega, Matagalpa, the North Atlantic Autonomous Region (RAAN), and the South Atlantic Autonomous Regions (RAAS) (Darce et al., 1991).

In addition to typical CL leishmaniasis, which results in ulcerative skin lesions, an atypical form of CL also exists in Nicaragua and several other countries in Central America (Belli et al., 1999; Convit et al., 2005). Atypical CL is characterized by the presence of non-ulcerative cutaneous nodules. The first cases of atypical CL in Nicaragua were identified in 1997, and the highest prevalence occurs in the Pacific plains region in the districts of Chinandega and Leon. There also is a focus of atypical CL in central Nicaragua in the district of Chontales (Belli et al., 1999).

Mucocutaneous leishmaniasis occurs in Nicaragua to a much lesser extent than does cutaneous leishmaniasis, with only a few dozen cases reported each year. In 2002 there were 59 cases reported, and in the first 3 months of 2003, there were 21 reported

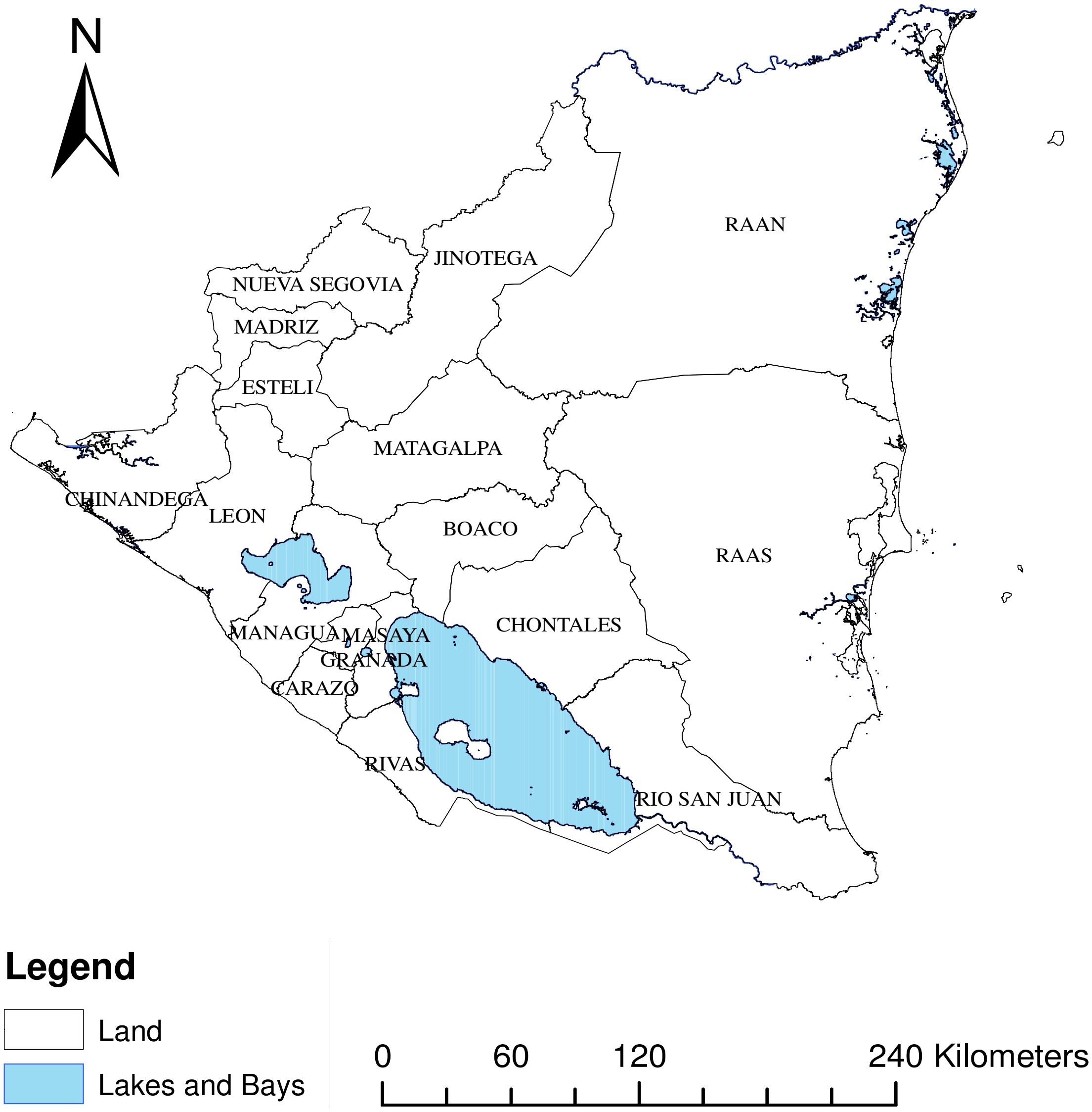


Figure 3. Map of Nicaragua showing political departments, land areas and freshwater lakes and bays.

cases of MCL (Ministerio de Salud Nicaragua, 2003). Human cases of MCL appear to be concentrated in the districts of Rio San Juan and Chontales in the south central part of the country.

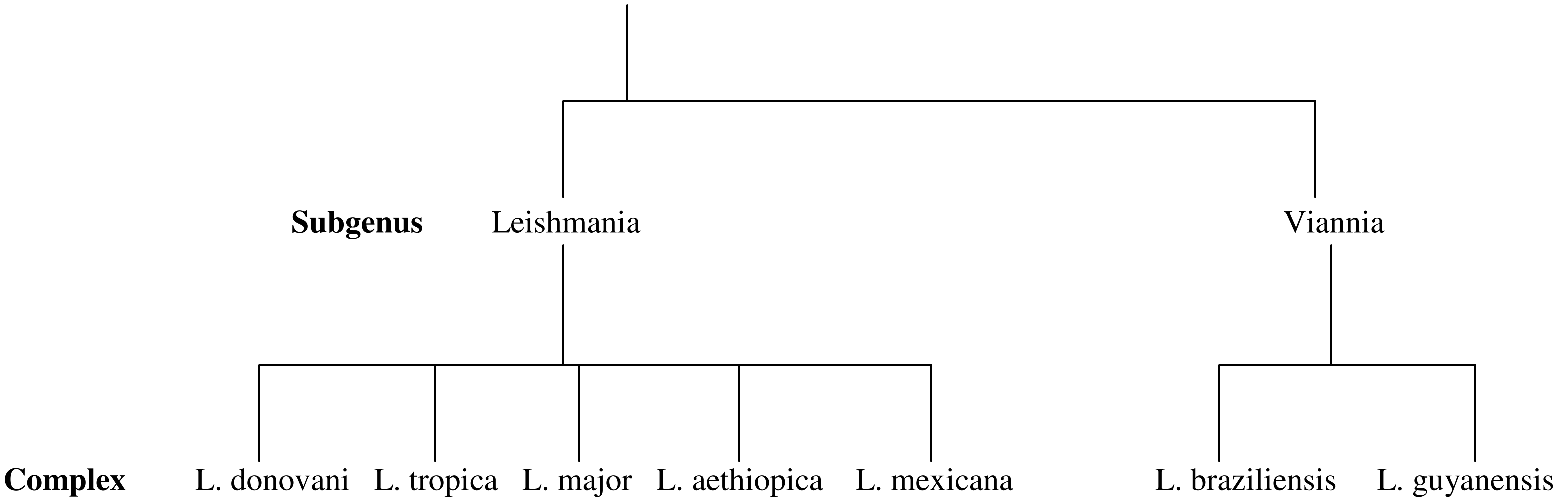
Although visceral leishmaniasis is the most severe form of disease associated with *Leishmania* infection, it occurs infrequently in Nicaragua (Belli et al., 1999). The first confirmed case of VL in Nicaragua occurred in a patient from the Island of Zapatera on Lake Nicaragua in 1988 (Ministerio de Salud Nicaragua, 2003). The geographic distribution of VL in Nicaragua mirrors the distribution of cases of atypical CL (Belli et al., 1999). The distribution of these cases is pertinent because spatial distribution is one of the factors considered when placing leishmanial parasites into taxonomic groups.

## Taxonomy of *Leishmania* parasites

Parasites within the genus *Leishmania* (KINETOPLASTIDA: TRYPANOSOMATIDAE) typically have been placed into taxonomic groups based on geographic distribution, preference of vertebrate host, manifestation of disease symptoms and site of promastigote development within the gut of the arthropod vector (Table 1). The genus *Leishmania* is divided into 2 subgenera, *Leishmania* (*Viannia*) Lainson and Shaw, 1987 and *Leishmania* (*Leishmania*) Ross, 1903 based primarily upon the parasite's site of attachment and development within the sand fly vector. Within the subgenus *Leishmania* (*Viannia*), the flagellated promastigote forms of the parasites undergo development within the sand fly hind gut with subsequent migration to the midgut and foregut before transmission by bite. Parasites within the subgenus *Leishmania* (*Viannia*) are found only in Neotropical regions of the New World.

Table 1. General classification of leishmanial parasites.

| | |
|---|---|
| **Domain** | Eukarya |
| **Kingdom** | Protista |
| **Phylum** | Sarcomastigophora |
| **Class** | Zoomastigophora |
| **Order** | Kinetoplastida |
| **Family** | Trypanosomatidae |
| **Genus** | *Leishmania* |



The subgenus *Leishmania* (*Leishmania*) includes those parasites that undergo attachment and development solely within the foregut and midgut of the sand fly vector. This group also is transmitted by bite of an infected sand fly and includes species found in both the Old and New Worlds (Lainson and Shaw, 1987). A third group of closely related parasites undergo development limited to the hindgut of the sand fly. Transmission of this group is potentially via the ingestion of the sand fly or sand fly feces by the vertebrate host. These are thought to be more primitive parasites of Old World lizards and are placed in the separate genus, *Sauroleishmania* Saf'janova, 1982 (Lainson and Shaw, 1979).

There is disagreement as to the mode of transmission and the phylogenetic placement of species within the genus *Sauroleishmania*. Noyes and Chance (1998) maintained the mode of transmission between the sand fly vector and the lizard host still is unproven. They hypothesize that, since *Sauroleishmania* are lizard blood parasites that potentially evolved from mammalian blood parasites, transmission by the bite of the sand fly is the most likely method of transmission. Recent comparisons of small subunit ribosomal DNA (rDNA) sequences among species within the genus *Sauroleishmania* and mammalian species within the genus *Leishmania* indicate a close phylogenetic relationship (Briones et al., 1992). The small subunit rDNA gene sequences studied by Briones et al. (1992) showed a greater than 99% similarity between *Sauroleishmania tarentolae* Wenyon, 1921 and *Leishmania donovani* Laveran and Mesnil, 1903. Additionally, analysis of the mitochondrial minicircle DNA has shown sequences that are conserved among *Sauroleishmania gymnodactyli* Khodukin and Sofiev, 1940, *S.*

*guliki* Saf'janova, 1982, *S. taraentolae* and the Old World mammalian parasite *Leishmania infantum* Nicolle, 1908 (Fu and Kolesnikov, 1994). Croan and Ellis (1996) and Croan et al. (1997) provided analysis of the gene sequences that encode DNA polymerase catalytic polypeptide and the RNA polymerase II largest subunit gene of 19 species of *Leishmania* and *Sauroleishmania*, showing increased evidence for inclusion of the lizard species within the genus *Leishmania*.

More recently, extensive biochemical and molecular tests have led to suggestions for revision of the classification of leishmanial parasites. Cupolillo et al. (2000) proposed that the genus *Leishmania* be subdivided into two distinct lineages, the Sections Euleishmania and Paraleishmania. Section Euleishmania would include all the parasites currently placed within the subgenera *Leishmania* (*Leishmania*) and *Leishmania* (*Viannia*), while Section Paraleishmania would include those parasites thought to be more closely related to the genus *Endotrypanum* Mesnil and Brimont, 1908 than to other species of *Leishmania*. The Section Paraleishmania would include those species of *Leishmania* that are not pathogenic to man and are primarily parasites of sloths and porcupines. Under the classic classification system, these leishmanial parasites had been placed under the subgenus *Leishmania* but had been difficult to place within a defined complex (Cupolillo et al., 2000).

### ***Leishmania* complexes and disease distribution**

Typical CL primarily is associated with parasites within the complexes *Leishmania* (*Leishmania*) *tropica* Wright,1903, *L.* (*L.*) *major*, Yakimoff and Schokhor, 1914 and *L.* (*L.*) *aethiopica*, Ashford & Bray, 1973 in the Old World and within the complexes *L.* (*L.*) *mexicana* Biagi, 1953, *L.* (*Viannia*) *braziliensis* Vianna, 1911, and *L.* (*V.*) *guyanensis* Floch, 1954 in the New World (WHO, 1984). The atypical CL form of the disease occurs only in the New World and has been associated with the species *L.* (*L.*) *chagasi* Cunha and Chagas, 1937*,* within the complex *L. donovani* (Belli et al,. 1999). *L.* (*L.*) *chagasi* is the same species that primarily is responsible for VL (Lainson and Shaw, 1987).

Mucocutaneous leishmaniasis in the New World most often is associated with the species *L.* (*V.*) *braziliensis* within the complex *L.* (*V.*) *braziliensis*. Visceral leishmaniasis principally is associated with the complex *Leishmania donovani* in both the Old and New World. The primary species associated with VL in the Old World are *L.* (*L.*) *donovani* and *L.* (*L.*) *infantum*. In the New World, VL most often is associated with the species *L.* (*L.*) *chagasi* (Lainson and Shaw, 1987).

Parasites within the complexes *L.* (*L.*) *mexicana* and *L.* (*V.*) *braziliensis* are the most common etiological agents of typical CL in the New World (Grimaldi et al., 1989). In Nicaragua, 2 species of *Leishmania* parasites have been isolated from typical CL lesions in human patients; *L.* (*V.*) *braziliensis* and *L.* (*V.*) *panamensis* Lainson and Shaw, 1972 (Missoni et al., 1986; Belli et al., 1994). Additionally, a putative *L.* (V.) *braziliensis* - *L.* (V.) *panamensis* hybrid may be the causative agent of some CL in Nicaragua (Belli et al., 1994). This putative *L.* (*V.*) *braziliensis* - *L.* (*V.*) *panamensis* hybrid from Nicaragua has been questioned and may have been due to contamination of the original sample (Uliana et al., 2000).

The known geographic distribution of the *L.* (*L.*) *mexicana* complex extends from Arizona and Texas in the western United States to southern Brazil, South America (Kerr et al., 1995; Kerr et al., 1999; Uliana et al., 2000). The known geographic distribution of *L.* (*V.*) *braziliensis* complex extends from Mexico (Canto-Lara et al., 1999), El Salvador, and Belize in Central America, to southern Brazil (Lainson and Shaw, 1987).

The ranges of complexes *L.* (*L.*) *mexicana* and *L.* (V.) *braziliensis* overlap in Central America indicating that CL in Nicaragua potentially may be a result of infection with parasites from either complex. The species *Leishmania (L.) mexicana* has a geographic range from the western United States to northern Columbia and Venezuela (Uliana et al., 2000). *Leishmania* (*L.*) *mexicana* has not been isolated from either humans or wild mammalian hosts in Nicaragua; however, it has been isolated from at least 9 human cases of cutaneous leishmaniasis in Honduras near the border with Nicaragua (Noyes et al., 1997). Other species within the complex *Leishmania* (*L.*) *mexicana*, that are known to infect man, have not been shown to be distributed farther north than Panama (Uliana et al., 2000). The research described herein concentrated on identifying actual or potential reservoir hosts of *Leishmania* species that are known to cause human typical CL and the associated sand flies in Nicaragua.

## ***Leishmania* species associated with clinical forms of leishmaniasis in Nicaragua**

*Leishmania* (*L.*) *chagasi* has been demonstrated to be the causative agent of human visceral and atypical cutaneous leishmaniasis in Nicaragua (Belli et al., 1999). *Leishmania* (*V.*) *braziliensis* is the primary species that has been isolated from human mucocutaneous lesions and *L.* (*L.*) *panamensis* has been demonstrated to be the primary species isolated from human typical cutaneous lesions. Although, *L.* (*V.*) *braziliensis* has been isolated from typical cutaneous lesions and *L.* (*L.*) *panamensis* has been isolated from mucocutaneous lesions (Belli et al., 1994).

## General life cycle

*Leishmania* species are obligate heteroxenous parasites that are transmitted to a vertebrate host through the bite of an infected sand fly vector (Fig. 4). Two morphologically-distinct forms of the parasite exist, immobile rounded amastigotes and mobile promastigotes. The round to ovoid amastigote stage found within the vertebrate host is strictly intracellular and replicates primarily within macrophage phagolysosomes. Amastigotes are small, typically 3–6.5 µm in diameter and are among the smallest known eukaryotic cells (Lainson and Shaw, 1978; Roberts and Janovy, Jr., 2005). The elongate promastigote stage possesses a single anterior flagellum and is the extracellular form of the parasite that replicates within the alimentary canal of the sand fly. The promastigote forms are slender and typically are 5–25 µm in length (Roberts and Janovy, Jr., 2005)

The genus *Leishmania* is divided into subgenera based primarily on the site of development within the sand fly vector. Suprapylarian leishmanal parasites, subgenus *Leishmania* (*Leishmania*), attach and replicate within the sand fly midgut, while Peripylarian leishmanial parasites, subgenus *Leishmania* (*Viannia*), initially attach and replicate within the sand fly hindgut, with subsequent migration to the midgut and foregut. Hypopylarian leishmanial species, genus *Sauroleishmania*, undergo strictly posterior station hind gut development and are restricted to reptilian hosts.

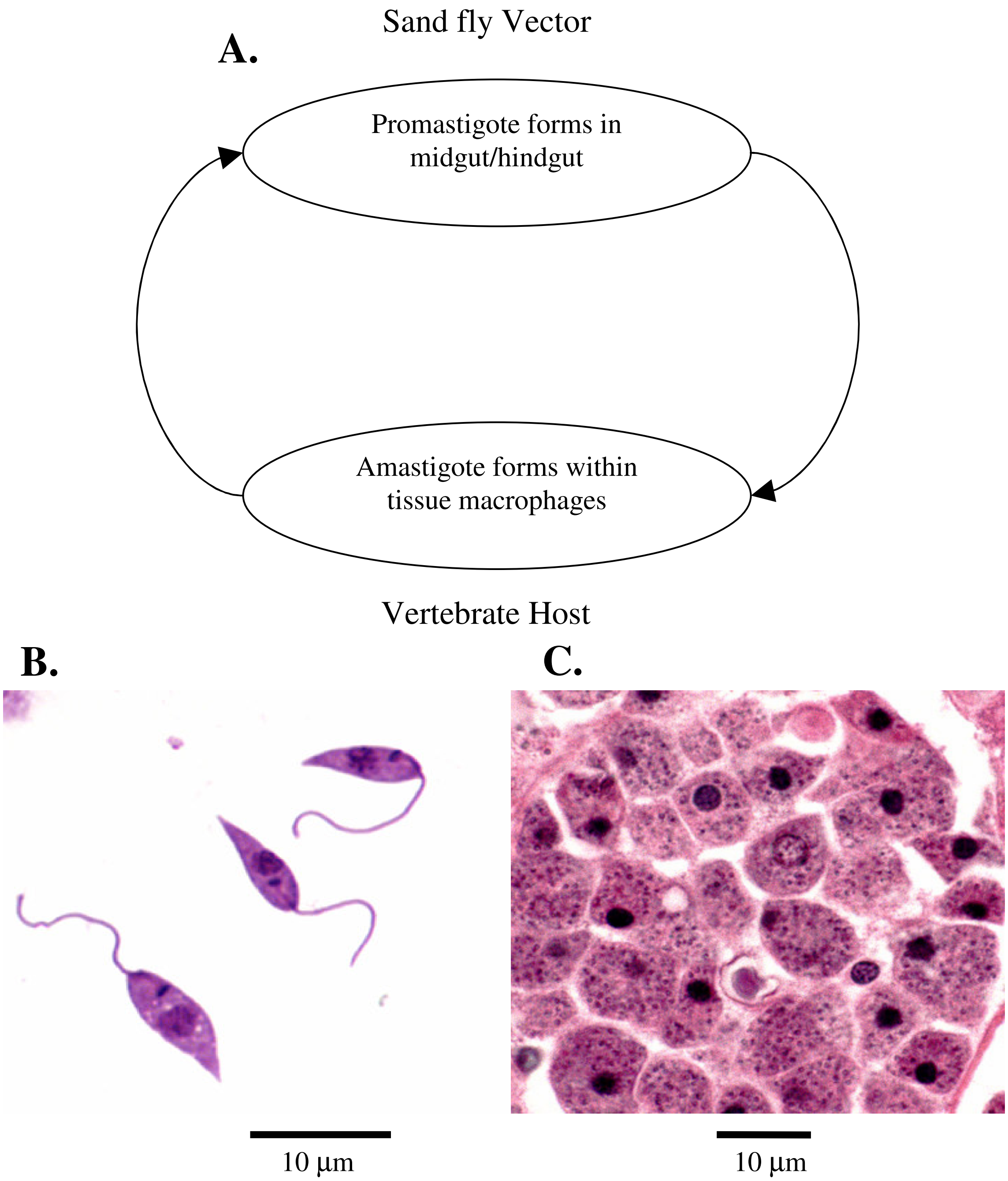


Figure 4. Transmission cycle and morphological forms of *Leishmania*. (A) general transmission cycle, (B) promastigotes, and (C) amastigotes within hamster liver macrophages; larger darkly-stained bodies within the cells are the host cell nuclei. *Leishmania* amastigotes are seen as smaller darkly-stained bodies within the cytoplasm (Sullivan, 2004).

## Life cycle stages within the sand fly vector

Sand flies within the family Psychodidae serve as biological vectors for all species of *Leishmania*. Sand flies of the genus *Lutzomyia* serve as the vectors in the New World, while Old World *Leishmania* species are transmitted by sand flies of the genus *Phlebotomus* Rondani and Berte, 1840 (Killick-Kendrick, 1990). Sand flies have scissor-like cutting mouthparts and are blood pool feeders. Upon ingestion of amastigotes by a female sand fly feeding on an infected reservoir host, the amastigotes transform and complete part of their life cycle as elongated procyclic promastigotes that replicate by binary fission within the gut of the insect vector. The procyclic promastigotes attach to the wall of the midgut or hindgut to avoid elimination with the digested blood meal from the sand fly digestive tract (Killick-Kendrick, 1990).

Pimenta et al. (1994) demonstrated that promastigote survival within the sand fly was predicated on the ability of the procyclic forms of the parasite to attach to the gut wall of the vector. This attachment is mediated by modified surface polysaccharides of the glycocalyx that bind with receptors on epithelial cells of the sand fly midgut or hindgut (Turco and Sacks, 2003). The primary parasite surface polysaccharide is lipophosphoglycan (LPG), and variations within the side chains of LPG among different species of *Leishmania* are believed to be an important factor in determining the ability of the parasite to attach and establish infection within a particular species of sand fly. It is this variation within parasite surface LPG molecules and variation within receptors on the epithelium of the sand gut that leads to specificity in vector competence (Pimenta et al., 1994; McConville et al., 1995).

The extrinsic incubation period is approximately two weeks; the procyclic promastigotes then transform into metacyclic promastigotes that detach from the gut wall and migrate to the sand fly proboscis and are the forms that are infective to the vertebrate host (Molyneux and Killick-Kendrick, 1987; Turco and Sacks, 2003). The detachment of the metacyclic promastigotes from the epithelial cells of the sand fly gut wall is mediated by structural changes to the LPG molecules of the glycocalyx resulting in the loss of binding domains (Sacks et al., 1995). Transmission to susceptible vertebrate hosts then occurs upon subsequent blood-meal feeding by the female sand fly.

### The role of sand fly saliva in transmission and infectivity

Transmission to a susceptible vertebrate host occurs when parasites are inoculated into the bite wound by the female sand fly. Sand flies typically inoculate relatively small numbers of leishmanial parasites into the host, usually ranging between 10–1,000 parasites (Warburg and Schlein, 1986). Sand fly saliva that is inoculated into the bite wound contains anticoagulants and vasodilators that, along with aiding the arthropod vector in acquiring a blood meal, also appear to enhance the ability of leishmanial parasites to establish infection by suppression of the host immune response (Titus and Ribeiro, 1988; Theodos et al., 1991). Titus and Ribeiro (1988) infected laboratory mice with either *Leishmania major* parasites alone or *Leishmania major* parasites along with salivary gland homogenate from the sand fly *Lutzomyia longipalpis* Lutz and Neiva, 1912. They reported that mice injected with parasites and salivary gland homogenate developed cutaneous lesions that were consistently much larger and contained up to 5,000 times more parasites than mice that were injected with parasites alone. The infectivity-enhancing properties of the sand fly saliva were more pronounced when small numbers of parasites were inoculated.

Hall and Titus (1995) investigated the infection-enhancing capabilities of sand fly saliva from *Phlebotomus papatasi* on *Leishmania major* infections in mice microphages. Sand fly saliva that is injected into the bite wound leads to increased infectivity through its ability to inhibited nitric oxide (NO) production by macrophages in response to Interferon gamma (IFN-γ) produced by host T cells. Titus and Ribeiro (1988) also demonstrated that saliva from other species of arthropods, including *Aedes aegypti* Linnaeus 1762, *Rhodnius prolixus* Stal, 1859, and *Ixodes dammini* Say, 1821 did not enhance infection. Theodos et al. (1991) demonstrated similar results while conducting experiments using different species of sand flies and different species of *Leishmania*. Additionally, prior exposure to sand fly saliva through bites of uninfected flies may result in the production of anti-saliva antibodies by the host immune system which may decrease the virulence-enhancing effects of sand fly saliva on leishmanial infections. This may suggest that individuals that have not been previously exposed to sand fly saliva, such as the very young or those from nonendemic locales, may be at greater risk for infection with leishmanial parasites (Belkaid et al., 1998b).

## Life cycle stages within the vertebrate host

Once inoculated into a susceptible vertebrate host by the sand fly and prior to phagocytosis by host cell macrophages, the metacyclic promastigotes are able to avoid lyses by the host’s innate immune response due to a thickening of the LPG layer (Brittingham et al., 1995). Additionally, the parasite surface glycoprotein, gp63, plays a role in avoiding complement mediated lyses of the metacyclic promastigotes by degrading proteins associated with the complement activation pathway. The metacyclic promastigotes are phagocytized by macrophages within hours and transform into oval non motile amastigotes (Brittingham et al., 1995).

The amastigotes reproduce by binary fission within phagolysosomes. This is remarkable in that the phagolysosomes are the organelles within eukaryotic cells that function in the destruction of intracellular pathogens. Sand fly saliva, inoculated into the bite wound along with leishmanial parasites, has been demonstrated to reduce intracellular killing of parasites by inhibiting the ability of cytokine Interferon gamma (IFN-γ) to stimulate macrophage production of nitric oxide and hydrogen peroxide during early phases of infection (Hall and Titus, 1995). Metacyclic promastigotes, as well as amastigotes, also are believed to be able to survive macrophage-mediated killing by inhibiting the macrophage's ability to produce interleukin-12 (IL-12). Interleukin-12 up regulates the production of IFN-γ by *Leishmania* antigen-activated Th1 cells, which in turn stimulates macrophages to kill intracellular pathogens (Carrera et al., 1996; Belkaid et al., 1998a; Weinheber et al., 1998). Carrera et al. (1996) demonstrated that mouse bone marrow-derived macrophages infected with *Leishmania major* displayed an inability to synthesize IL-12 mRNA and to secrete IL-12.

Reproduction of the amastigotes by binary fission occurs until rupture of the host cell. The released amastigotes then are phagocytized by other macrophages where they again reproduce by binary fission. Female sand flies ingest the amastigotes when taking a blood meal. The amastigotes transform into the procyclic promastigote forms and continue to replicate by binary fission within the midgut or hindgut of the sand fly, completing the life cycle (Bray and Alexander, 1987). Although sand flies of the genus *Lutzomyia* are known to serve as vectors for all species of *Leishmania* in the New World, no proven vectors have been described in Nicaragua.

### ***Lutzomyia* species in Nicaragua**

Vectors are those organisms that are responsible for the transmission of parasites from their reservoirs to susceptible hosts. Over 400 species of sand flies have been named in the New World, of which 14 have been proven to be vectors for leishmaniasis and another 25 species being suspected vectors (Young and Duncan, 1994; Killick-Kendrick, 1999). Among all of those, twenty-nine (29) species of sand flies previously have been reported in Nicaragua (Table 2).

In 1953, Fairchild and Hertig (1959, 1961a, 1961b) collected 16 species of sand flies from 5 localities in Nicaragua. They captured 13 species of sand flies from the village of Villa Somosa (since renamed Villa Sandino) in the district of Chontales including: *Lutzomyia* (*Lu.*) *aclydifera* Fairchild and Hertig, 1952*, Lu. bispinosus* Fairchild and Hertig, 1951*, Lu. cruciatus* Coquillett, 1907*, Lu. gomezi* Nitzulescu, 1931*, Lu. panamensis* Shannon, 1926*, Lu. shannoni* Dyar, 1919*, Lu. trinidadensis* Newstead, 1922*, Lu. vesicifera* Fairchild and Hertig, 1947*, Lu. ylephiletor* Fairchild and Hertig, 1952 and 4 previously unnamed species. The 4 unnamed species later were described as *Lu. serrana* Domasceno and Arouck, 1949*, Lu. dasymera* Fairchild and Hertig, 1961*, Lu. odax* Fairchild and Hertig, 1961, and *Lu. nicaraguensis* Fairchild and Hertig, 1961. However, Young (1979) considered *Lu. nicaraguensis* not to be a new species but a

Table 2. Known *Lutzomyia* species of Nicaragua.

| Species | Year Collected | Reference |
|---|---|---|
| *Lu. aclydiferus* | 1953 | Fairchild and Hertig 1959 |
| *Lu. barettoi* | 1953 | |
| *Lu. bispinosus* | 1953 | |
| *Lu. cruciatus* | 1953 | |
| *Lu. evansi* | 1953 | |
| *Lu. gomezi* | 1953 | |
| *Lu. ovallesi* | 1953 | |
| *Lu. panamensis* | 1953 | |
| *Lu. shannoni* | 1953 | |
| *Lu. trinidadensis* | 1953 | |
| *Lu. vesiciferus* | 1953 | |
| *Lu.ylephiletor* | 1953 | |
| *Lu. serrana* | 1953 | Fairchild and Hertig 1961 |
| *Lu. dasymera* | 1953 | |
| *Lu. odax* | 1953 | |
| *Lu. nicaraguensis* | 1953 | |
| *Lu. sanquinaria* | 1977 | Zeledon and Murillo 1983 |
| *Lu. trapidoi* | 1977 | |
| *Lu. geniculata* | 1977 | |
| *Lu. olmeca* | 1977 | |
| *Lu. vespertilioniis* | 1977 | |
| *Lu. carpenteri* | 1988–94 | Valle and Rivera 1995 |
| *Lu. cayennensis* | 1988–94 | |
| *Lu. chiapanensis* | 1988–94 | |
| *Lu. longipalpis* | 1988–94 | |
| *Lu. zeledoni* | 1988–94 | Le Pont 1987 |
| *Lu. legerae* | 1988–94 | Le Pont 1995 |
| *Lu. caprini* | 1995 | Collantes and Martinez-Ortega 1997 |
| *Lu. camposi* | 1995 | |

subspecies of *Lu. hirsuta* Mangabeira, 1942. Two species of sand flies were captured in the Upper Valley of the Rio Pantasma in the District of Jinotega; *Lu. cruciatus* and *Lu. ovallesi* Oritz, 1952. Three sand fly species were captured near the community of Guapinolar (near the capitol city of Managua), *Lu. barettoi* Mangabeira, 1942*, Lu. cruciatus,* and *Lu. evansi* Nunez Tovar, 1942. *Lutzomyia barettoi* also was captured in the District of Carrazo. *Lutzomyia. cruciatus* and *Lu. shannoni* also were were captured near the community of Casa Colorado near Managua, (Fairchild and Hertig, 1959, 1961a, 1961b)

In February of 1977, Zeledon and Murillo (1983) collected 11 species near the community of Boca San Carlos in the district of Rio San Juan located on the southern border with Costa Rica. Six species were among those previously collected in Nicaragua by Fairchild and Hertig (1959, 1961a, 1961b); however, their collections did include 5 previously unreported species*:* *Lu. sanguinaria* Fairchild and Hertig, 1957, *Lu. trapidoi* Fairchild and Hertig, 1952*, Lu. geniculata* Mangabeira, 1941, *Lu. olmeca olmeca* Vargas and Najera, 1959, and *Lu. vespertilionis* Fairchild and Hertig, 1947.

Between 1988 and 1994, Valle and Rivera (1995) collected 12,556 *Lutzomyia* specimens representing 17 species at 16 different localities, including 4 previously unreported species (Table 3). *Lu. carpenteri* Fairchild and Hertig, 1953*, Lu. cayennensis* Floch and Abonnenc, 1941*, Lu. chiapanensis* Dampf, 1947, and *Lu. longipalpis* Lutz and Neiva, 1912 were reported as new records from Nicaragua. Although this was a large collection of sand flies, 5 species comprised nearly 90% of the total individuals captured: *Lu. ylephiletor* (~ 35%)*, Lu. panamensis* (~ 24 %),

Table 3. Summary of *Lutzomyia* species captured in Nicaragua by Valle and Rivera, 1995.

| Species | Pacific Region | Central Highlands and Atlantic Regions | Totals |
|---|---|---|---|
| *Lu. aclydifera* | 0 | 49 | 49 |
| *Lu. barretoi majuscula* | 0 | 66 | 66 |
| *Lu. carpenteri* | 0 | 127 | 127 |
| *Lu. cayannensis* | 12 | 0 | 12 |
| *Lu. chiapanensis* | 143 | 1 | 144 |
| *Lu. cruciata* | 4 | 2,097 | 2,101 |
| *Lu. evansi* | 733 | 192 | 925 |
| *Lu. gomezi* | 2 | 9 | 11 |
| *Lu. longipalpis* | 691 | 0 | 691 |
| *Lu. ovallesi* | 1 | 219 | 220 |
| *Lu. panamensis* | 0 | 3,016 | 3,016 |
| *Lu. sanguinaria* | 0 | 63 | 63 |
| *Lu. serrana* | 0 | 41 | 41 |
| *Lu. shannoni* | 0 | 111 | 111 |
| *Lu. trapidoi* | 0 | 590 | 590 |
| *Lu. vesiscifera* | 37 | 3 | 40 |
| *Lu. ylephyletor* | 0 | 4,349 | 4,349 |
|  | 1,623 | 10,933 | 12,556 |

*Lu. cruciata* (~ 17%), *Lu. evansi* (~ 7%) and *Lu. longipalpis* (~ 6%). Valle and Rivera (1995) divided the country into 2 large regions based on distribution of sand fly species. *Lutzomyia evansi* and *Lu. longipalpis* were the dominant species collected in the Pacific plains region. No specimens of *Lu. longipalpis* were collected from any other region of Nicaragua. *Lutzomyia ylephiletor, Lu. panamensis* and *Lu. cruciata* were the dominant species collected in the central highlands and Atlantic lowland plains regions of Nicaragua. No specimens of *Lu. ylephiletor* or *Lu. panamensis,* and only few specimens of *Lu. cruciata* were collected from the Pacific plains region of western Nicaragua. Le Pont et al. (1987, 1995) described the capture of 2 additional species of sand flies in Nicaragua, *Lu. zeledoni* Young and Murillo, 1984 and *Lu. legerae* Le Pont et al. 1995. *Lutzomyia zeledoni* specimens were collected in the District of Chinandega, near the Honduras border as well as near Juigalpa in the District of Chontales. The *Lu. legerae* specimens were collected near the towns of Juigalpa and Santo Tomas in the District of Chontales.

Collantes and Martinez-Ortega (1997) collected 79 sand fly specimens, representing 10 species from 10 localities in 1995, including 2 new records for Nicaragua, *Lu. camposi* Rodriguez, 1952 and *Lu. caprina* Osorno-Mesa et al. 1972. Two *Lu. camposi* were captured near the town of El Castillo in the District of Rio San Juan and 2 *Lu. caprina* were captured between Matagalpa and Jinotega in the district of Matagalpa.

Of the 29 species of *Lutzomyia* reported from Nicaragua, 4 have been proven to be vectors of *Leishmania* species in other Central and South American countries: *Lu. ovallesi* vector of *L.*(*V.*) *braziliensis*, *Lu. trapidoi* vector of *L.*(*V.*) *panamensis*, *Lu. olmeca olmeca* vector of *L.* (*L.*) *mexicana*, and *Lu. longipalpis* vector of *L.* (*L.*) *chagasi*. Three additional reported species, *Lu. evansi, Lu. gomezi,, Lu. panamensis* have been identified as probable vectors elsewhere in Central or South America (Killick-Kendrick 1999).

## Dispersion patterns of *Lutzomyia*

Leishmaniasis tends to be a highly focal disease in its geographic distribution. A primary reason for this distribution pattern may be the limited flight ranges of the sand fly vectors. Several studies of Neotropical sand fly species have shown that *Lutzomyia* are poor flyers and tend not to disperse over great distances. During mark-release-recapture studies most species of *Lutzomyia* were found to disperse no greater than 200m from their initial site of release (Chaniotis et al., 1974; Alexander, 1987). In fact, the vast majority of individuals released during these studies were recaptured within 100m of the point of release. *Lutzomyia longipalpis*, a vector of the causative agent of visceral leishmaniasis *L.* (*L.*) *chagasi*, has been shown to disperse slightly farther than other Neotropical sand fly species (Dye et al., 1991; Morrison, 1993). During a mark-release-recapture study conducted in Brazil, most individuals of *Lu. longipalpis* were recaptured at or within 20m of the site of release; however, 1 individual male dispersed as far as 700m from its release point (Dye et al., 1991). An additional mark-release-recapture study of *Lu. longipalpis* in Colombia demonstrated that this species was capable of dispersing up to 960m (Morrison et al., 1993). Although Morrison et al. (1993) reported that *Lu.longipalpis* was capable of dispersing over "considerable" distances; the average distance traveled by male sand flies was reported to be less than 100m, with females traveling significantly less. I think that this is important to note because only female sand flies feed on a vertebrate host in order to acquire a blood meal; therefore, only females are responsible for the transmission of *Leishmania* among hosts.

### Reservoir hosts

Reservoir hosts are those responsible for the sustained maintenance of a parasite population in a natural system (Ashford, 1996). The leishmaniases are primarily zoonoses, with mammals other than man serving as reservoir hosts. Canids, rodents and sloths usually serve as the reservoirs of *Leishmania* species in the New World (Grimaldi et al., 1989). Canids are an important reservoir of parasites within the *Leishmania donovani* complex and rodents have been indicated as the primary reservoirs of the *Leishmania mexicana* complex (Shaw and Lainson, 1987). A wide variety of forest mammals have been identified as potential reservoirs for parasites within the *Leishmania braziliensis* complex, including rodents, marsupials, canids, and sloths.

A number of mammals, primarily rodents, have been incriminated as hosts and potential reservoirs of the *Leishmania mexicana* complex. Humans typically serve as incidental hosts and are not a significant component of the natural transmission cycle (Ashford, 1996). The big-eared climbing rat, *Ototylomys phyllotis* Merriam, 1901 was reported to be the primary reservoir host for *Leishmania* (*L.*) *mexicana* in Belize, with the spiny pocket mouse, *Heteromys desmarestianus* Gray, 1868, the vesper rat *Nyctomys sumichrasti* Saussure, 1860, and the hispid cotton rat, *Sigmodon hispidus* Say and Ord, 1825 also implicated (Disney, 1964; Lainson and Strangways-Dixon, 1964). In Mexico, *Leishmania* (*L.*) *mexicana* has been isolated from the deer mouse, *Peromyscus yucatanicus* J. A. Allen and Chapman, 1897, the black-eared rice rat, *Oryzomys melanotis* Thomas, 1893*, Ototylomys phyllotis* and *Sigmodon hispidus* (Chable-Santos et al., 1995; Canto-Lara et al., 1999). In the United States, the southern plains woodrat, *Neotoma micropus* Baird, 1855 has been implicated as a reservoir host of *Leishmania* (*L.*) *mexicana* in Texas (McHugh et al., 1990; Kerr et al., 1995). It also has been isolated from a domestic cat, *Felis catus* Linaeus, 1758 in Texas (Craig et al., 1986); the white-throated woodrat *Neotoma albigula* Hartley, 1894 in Arizona (Kerr et al., 1999); and identified in the eastern woodrat, *Neotoma floridana* Ord, 1818 by polymerase chain reaction (PCR) and microscopic examination of histological sections in Texas (McHugh et al., 2003). In Guatemala, *Leishmania* (*L.*) *mexicana* has been identified in the genus *Ototylomys* (Grimaldi et al., 1989). In Panama, *Leishmania* (*L.*) *mexicana* has been identified in the large-headed rice rat *Oryzomys capito* Olfers, 1818, the spiny rat *Proechimys semispinosus* Tomes, 1860, and the spotted paca *Agouti paca* Linnaeus, 1766*,* as well as in the brown murine opossum *Marmosa robinsoni* Bangs, 1898 (Herrer et al., 1973).

Rodents within the genus *Proechimys* J. A. Allen, 1899 are the primary hosts for *Leishmania* (*L.*) *amazonensis*, an etiological agent of CL in Brazil. However, the rice rats, *Oryzomys capito*, *O. concolor* Wagner, 1845, *O. macconnelli* Thomas, 1910, the bristly mouse, *Neacomys spinosus* Thomas, 1882, the South American water rat, *Nectomys squamipes* Brants, 1827, the black rumped agouti, *Dasyprocta prymnolopha*

Wagler, 1831, and a number of marsupials also have been implicated as hosts (Lainson and Shaw, 1987).

*Leishmania* (*V.*) *braziliensis* is the most geographically-widespread of the parasites that cause human cutaneous leishmaniasis in the New World and it has been isolated from a number of mammalian hosts including rodents, edentates, primates, marsupials, equines, and carnivores. The rodent host records of *Leishmania* (*V.*) *braziliensis* include: *Oryzomys capito*, *Orysomys concolor*, *Oligoryzomys nigripes* Desmarest, 1819, the grass mouse, *Akodon arviculoids*, *Proechimys* species, the white-footed climbing mouse, *Rhipidomys leucodactylus* Tschudi, 1844, and the house rat, *Rattus rattus* Fischer de Waldheim, 1803 (Lainson and Shaw, 1987; Grimaldi et al., 1989). Edentate hosts include the two-toed sloths, *Choloepus didactylus* Linnaeus 1758 (Lainson and Shaw, 1987) and *Choloepus hoffmanni* Peters 1858 (Herrer et al., 1973). Primate hosts include the three striped night monkey, *Aotus trivirgatus* Humboldt, 1811 and the marmoset, *Saguinus geoffroyi* Pucheran, 1845 (Herrer et al., 1973). Marsupial hosts include the opossum, *Didelphis marsupialis* Linnaeus, 1758 (Lainson and Shaw, 1987). *L. (V.) braziliensis* parasites also have been isolated from the equines, *Equus asinus* Linnaeus, 1758 and *Equus caballus* Linnaeus, 1758 (Grimaldi et al., 1987). Carnivore host records include *Canis familiaris* Linnaeus, 1758 (Grimaldi et al., 1989), the ring-tailed coati, *Nasua nasua* Linnaeus, 1766, the kinkajou, *Potos flavus* Schreber, 1774, and the olingo, *Bassaricyon gabbii* J. A. Allen, 1876 (Herrer et al., 1973).

Control of leishmaniasis requires an understanding of the reservoir-vector transmission system. Few of these systems have been adequately described anywhere in the world. Complete objective incrimination of an animal species as a reservoir host of a parasite is difficult and in many cases impossible (WHO, 1990). The World Health Organization has outlined criteria for the incrimination of reservoirs hosts; among these are that the percentage of individuals within a host population that become infective is large and may be greater than 20% (WHO, 1990). In Nicaragua, no proven reservoirs or sand fly vectors have been identified. This study identified rodent and sand fly species that were associated with areas that were known to be foci of human typical CL in order to discern their possible role as reservoirs and vectors, and to compare species distribution with rodents and sand flies collected in areas where atypical CL is the predominant form of leishmaniasis.

### Study area

Nicaragua is located in the center of the Central American Isthmus and is bordered by Honduras to the north and Costa Rica to the south. It is bounded by the Atlantic Ocean on the east and the Pacific Ocean on the west. The topography of Nicaragua previously has been described to be divided into 3 general geographic regions: the Pacific plains, the central highlands, and the Atlantic lowland plains (Taylor, 1963) (Fig. 5).

The Pacific plains region predominantly is characterized by mean annual temperatures between 25.5–27.5° C; however a series of volcanoes is located on a roughly north-south line through the center of this region and mean annual temperatures surrounding the peaks are considerably lower (Fig. 6). The Pacific plains region has a mean annual rainfall between approximately 1,000–1,800 mm (Fig. 7),

Figure 5. Map of the general geographic regions of Nicaragua as described by Taylor, 1963. This study included rodent and sand fly collections from each of these regions.

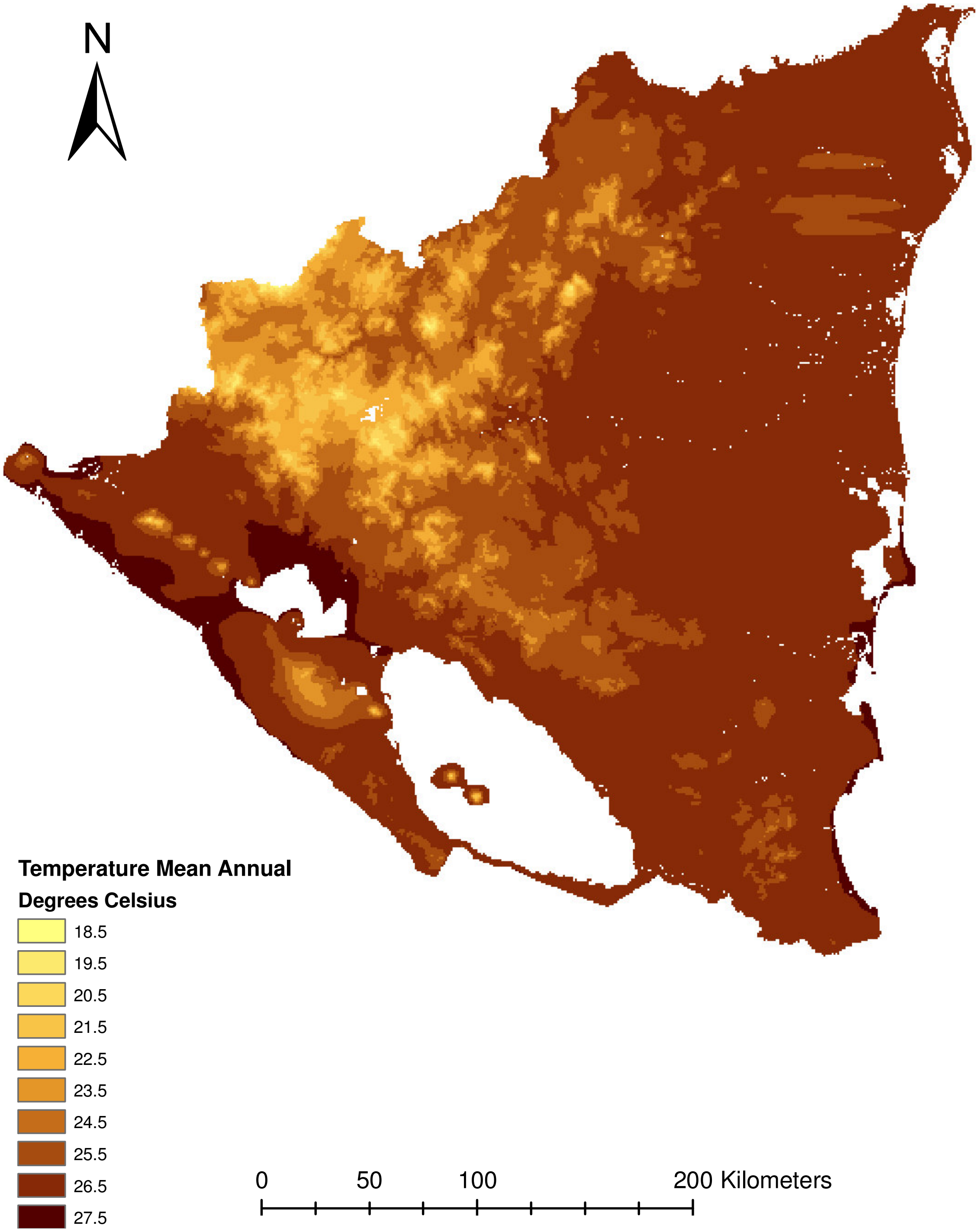


Figure 6. Map of the mean annual temperature zones of Nicaragua.

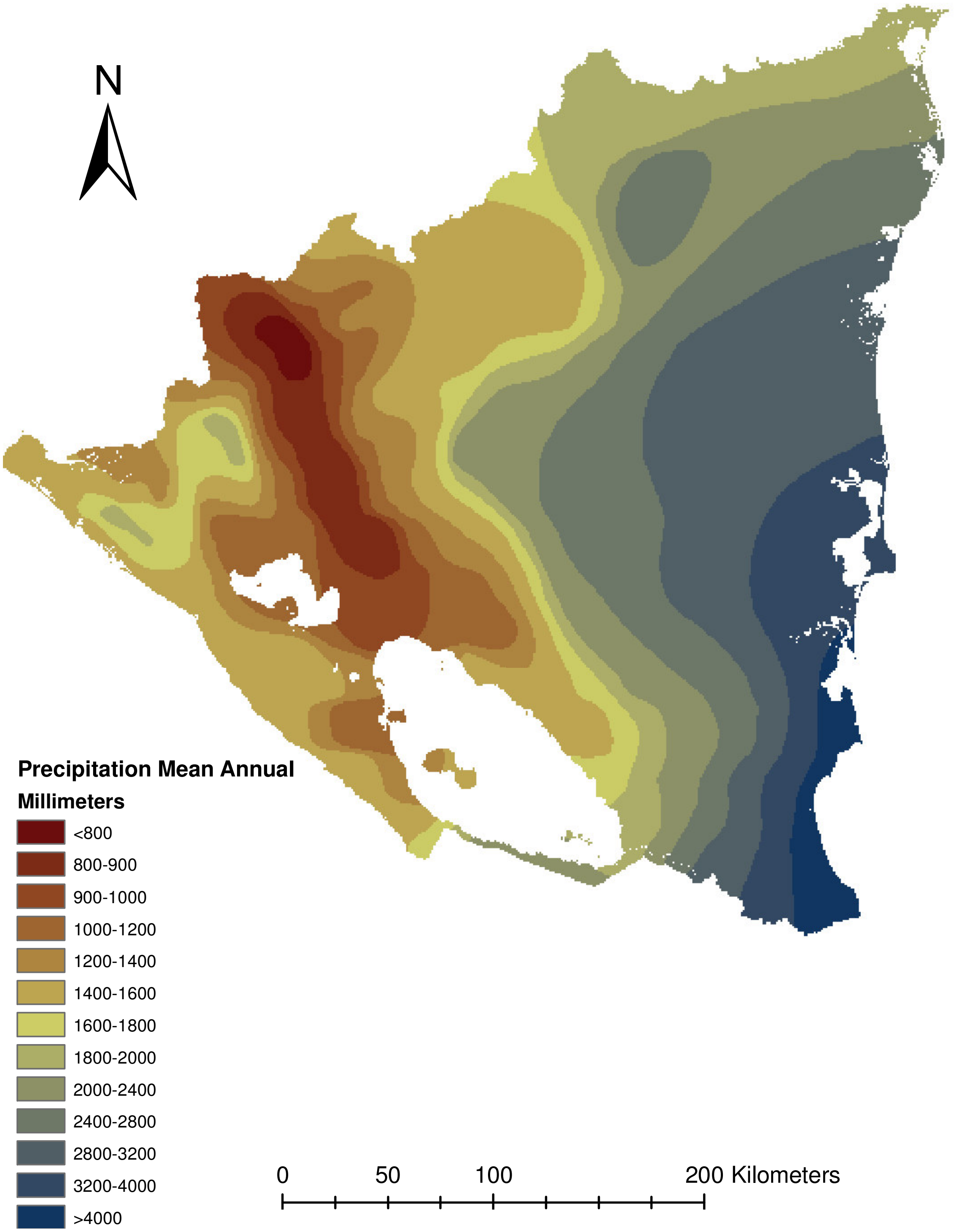


Figure 7. Map of the mean annual precipitation zones of Nicaragua.

but has pronounced wet and dry seasons (Taylor, 1963). The dry season occurs roughly from December until May or June and the wet season lasts from June until November. The Pacific plains region has elevations that predominantly range from sea level to approximately 200 m above mean sea level (MSL). Elevations of the volcanoes range to nearly 1,600 m above MSL (Fig. 8). The soils of the Pacific plains are typically sandy or sandy-loam to loam, with areas of clay soils scattered throughout (Fig. 9) (Taylor, 1963 and MARENA 2004). This region of Nicaragua is the most populous and has extensive agricultural usage and natural vegetation cover consisting primarily of deciduous forest or deciduous shrub land is generally less that 25% (Fig. 10) (MARENA, 2004).

The central highlands region has a subtropical climate with mean annual temperatures typically ranging between 18.5–24.5° C (MARENA, 2004). This is the driest region of the country with mean annual rainfall between 800–1,600 mm (Taylor, 1963; MARENA, 2004). The central highlands also have distinct wet and dry seasons; however the dry season is generally shorter than that of the Pacific plains region, typically lasting for approximately 3–5 months in areas of seasonal evergreen rain forest and 3 months in lower montane rain forest (Taylor, 1963). The central highlands region is characterized by the Segoviana, Isabelia, and Dariense mountain ranges with elevations varying from 600 m to 2,000 m above MSL. The soils of the western portion of the central highlands are predominantly clay-loam, while clay soils dominate the eastern two-thirds of this region (MARENA, 2004). Land usage is extremely varied,

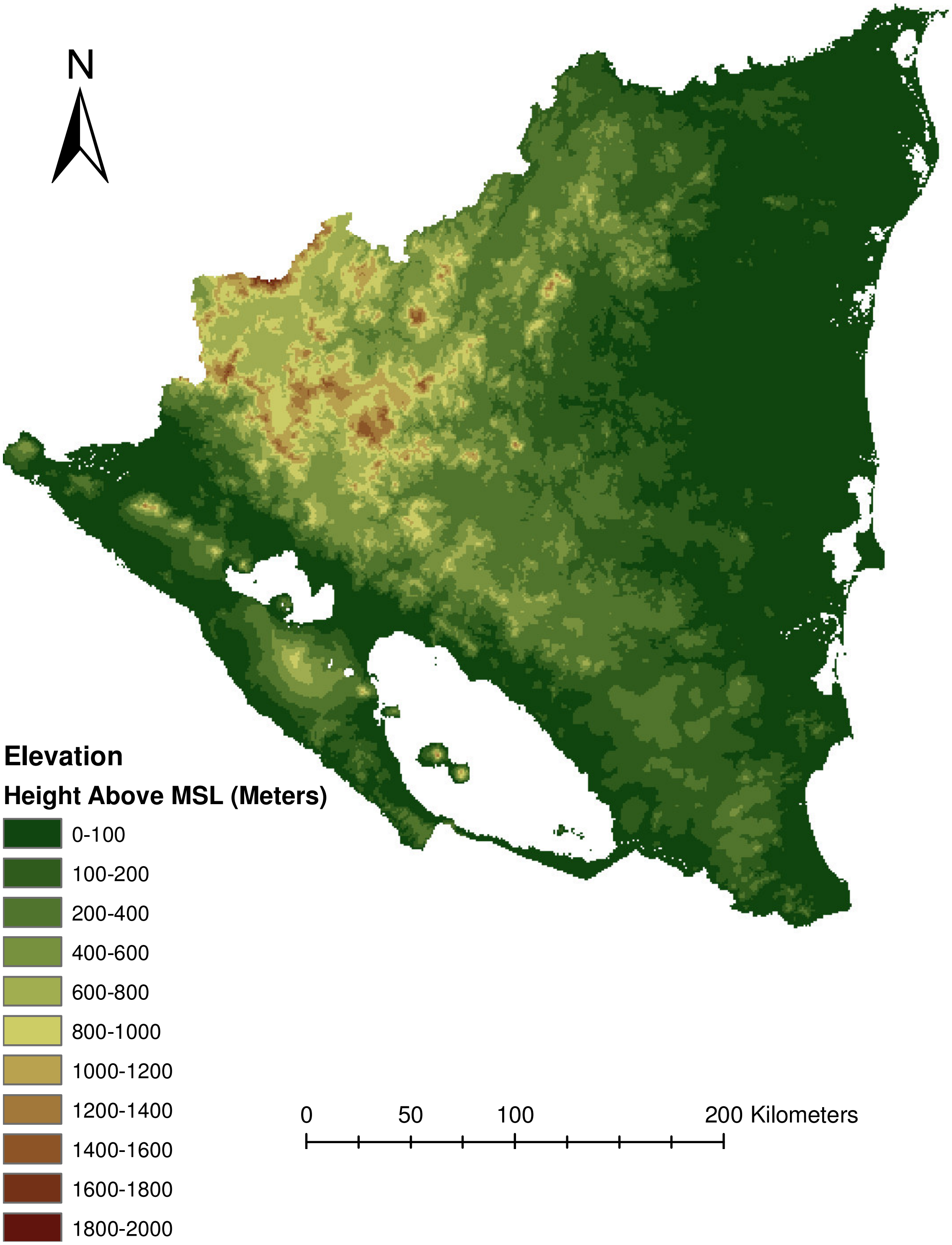


Figure 8. Map of the elevation zones of Nicaragua.

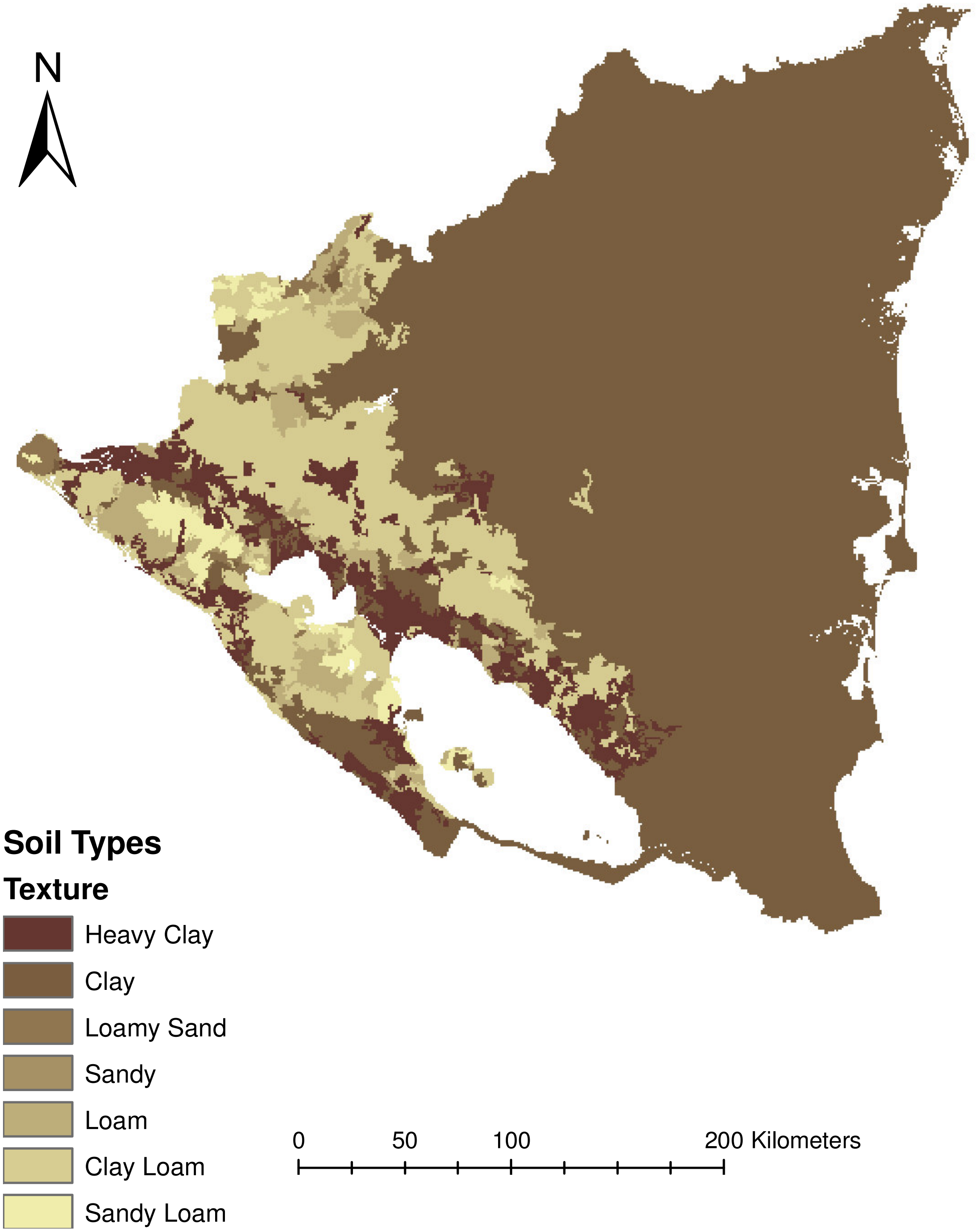


Figure 9. Map of the soil type zones of Nicaragua.

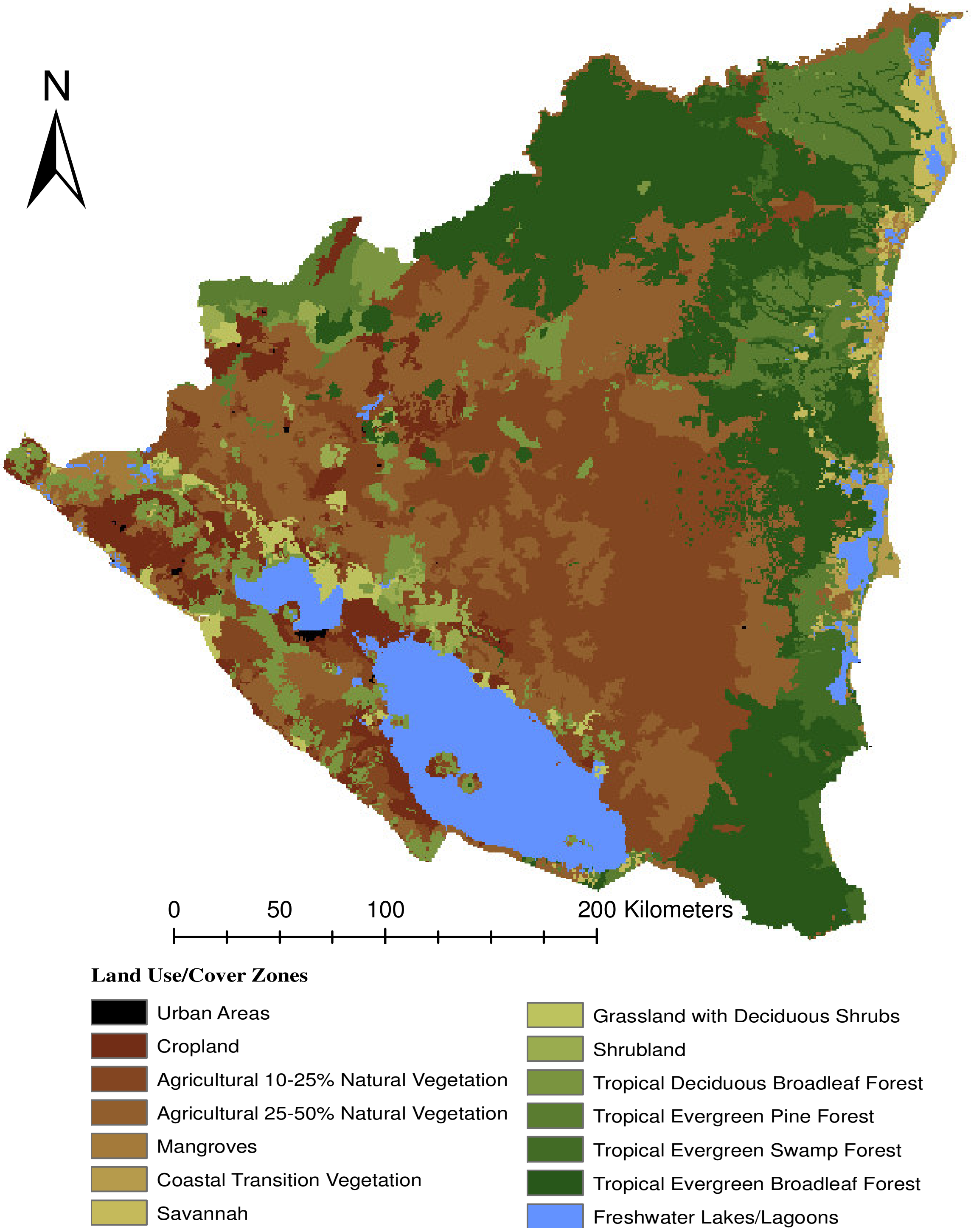


Figure 10. Map of the land use/cover zones of Nicaragua.

ranging from areas of extensive agricultural usage to areas of relatively undisturbed seasonal evergreen rain forest and lower montane tropical rain forest.

The Atlantic lowland plains region is predominantly characterized by a mean annual temperature of 26.5° C. This region is the wettest in Nicaragua with mean annual rainfall ranging from approximately 1,800 mm to greater than 4,000 mm, with areas near the Atlantic coast receiving the most precipitation (Taylor, 1963). There are distinct wet and dry seasons; however, the dry season is relatively short and lasts approximately 3 months from February to April (Taylor, 1963). Elevations throughout most of this region are below 200 m above MSL. However, the very southern extreme of the mountain ranges that run through the center of the country extend into the Atlantic lowland plains with altitudes in some areas to 600 m above MSL. Land use and coverage of the Atlantic lowland plains varies considerably, with areas of extensive agricultural use and 10–15% natural vegetation coverage to areas dominated by tropical evergreen broadleaf forest (Taylor, 1963; MARENA, 2004). This area also has seen the greatest amount of timber harvesting and conversion from forest to farm and ranchland within the last 25 years (MARENA, 2004).

### Diagnostic screening techniques for *Leishmania* in tissue

A number of diagnostic techniques have been used to identify the presence *Leishmania* parasites within tissues of mammalian hosts. The most common among these have been: staining and direct microscopic examination of histological sections, staining and microscopic examination of prepared tissue smears, Enzyme Linked Immunosorbent Assays (ELISA), in vitro culture, and PCR.

## In vitro culture

Rearing of live promastigotes of *Leishmania* in cell culture medium is one diagnostic test for the presence of parasites within tissue biopsies. The culture medium is used to simulate the biochemical environment found within the sand fly vector. Within the culture medium, the amastigotes within the mammalian host tissue transform and reproduce as promastigotes. Schneider's *Drosophila* medium supplemented with fetal bovine serum has been used, as well as blood-based media for the cultivation of promastigotes (Hendricks and Wright, 1979). Hendricks and Wright (1979) reported a successful cultivation rate of 67% in Schneider's *Drosophila* medium compared to a success rate of 15% in blood-based media. Rodriquez and others (1994) indicated a successful in vitro cultivation rate of 42% and indicated that the poor success rate may be due to secondary contamination with bacteria and fungi. Promastigotes also have been successfully cultured using supplemented M199 medium (Kerr et al., 1999).

## Polymerase chain reaction (PCR)

The use of PCR is highly sensitive as a diagnostic tool in testing for the presence of parasites within tissue (Rodgers et al., 1990; Bensoussan et al., 2006). Protozoan parasites of the genus *Leishmania* possess a kinetoplast, consisting of approximately 10,000 copies of DNA minicircles per parasite. A number of different types of PCR have been used to confirm the presence of leishmanial parasites within host tissue. Among the most widely used PCR methods are assays which test for the presence of either genomic or kinetoplast DNA. Three of the most commonly used PCR assays are kinetoplast DNA (kDNA) PCR, internal transcribed spacer 1 region of the small subunit rRNA genes (ITS1) PCR, and splice leader mini-exon (SLME) PCR of the gp63 gene locus (Bensoussan et al., 2006).

In a direct comparison of these 3 commonly used diagnostic PCR methods, Bensoussan et al. (2006) demonstrated kDNA PCR to be the most sensitive method (98.7%) for screening for the presence of leishmanial parasites within host tissue. ITS1 PCR showed a sensitivity of 91%, while SLME PCR demonstrated a sensitive of 53.8%. Bensoussan et al. (2006) reported that parasite culture detected 62.8% of the positive specimens while microscopy accurately detected 74.4% of the positives.

Rodriquez et al. (1994) reported a positive kDNA PCR test in 98% of patients that had been clinically diagnosed with leishmaniasis, while in vitro culture and microscopy identified 42% and 64% of the positive cases, respectively. Belli et al. (1998) indicated a sensitivity of 100% using kDNA PCR to test dermal scrapings from human patients diagnosed with cutaneous leishmaniasis. Thus, kDNA PCR was determined to be significantly more sensitive in the detection of *Leishmania* species within host tissue than in vitro culture or microscopy.

Within the kDNA minicircles, there is a region of approximately 200 base pairs that is highly conserved among species, while the remaining region of kDNA may vary significantly (Rodgers et al., 1990). It is within this conserved region that a 120 base pair sequence of kDNA can be amplified by PCR to identify the presence of parasites within the tissue sample. The genus specific primers, 13A (5'– GTGGGGGAGGGGCGTTCT –3') and 13B (5'– ATTTTACACCAACCCCCAGTT –

3') hybridize to a sequence within the kDNA and promote chain elongation at those sites during PCR (Rodgers et al., 1990, Rodriquez et al. 1994, Belli et al.1998).

## Geographic Information System (GIS) and remote sensing

Ecological components influence the spatial and temporal distribution of populations of *Leishmania* species as well as its sand fly vectors and reservoir hosts. Regionally-variable ecological conditions can add complexity to the understanding of those factors that help to determine the geographic distribution of species (Cromley, 2003). An understanding of the specific environmental conditions that serve as the best predictors for the distribution of *Leishmania* species would be useful in determining the potential geographic range of the parasite as well as providing useful information to be applied in control (Molyneux, 2001).

GIS and remote sensing capabilities are increasingly being used in epidemiological research as tools for displaying and interpreting the ecological factors that affect the distribution of vector-borne diseases (Hendrickx et al., 2001). Environmental data and georeferenced earth surveillance data are being used in conjunction with GIS to develop maps that help to describe the spatial and temporal distribution of disease-causing agents, reservoir hosts, and vectors (Molyneux, 2001; Rushton, 2003). These GIS databases and generated maps can be useful tools in understanding and communicating the temporal and spatial environmental processes associated with vector-borne diseases, like the leishmaniases.

Previous studies have employed the use of GIS and free and readily-available environmental data from remote sensing sources to explain and predict the distribution of leishmaniasis and other vector-borne diseases as well as develop risk models for exposure to the parasites (Yilma and Malone, 1998; Bavia et al., 2001; Fuentes et al., 2001; Kristensen et al., 2001; Malone et al., 2001; Elnaiem et al., 2003; Gebre-Michael et al., 2004; King et al., 2004; Klinkenberg et al., 2004). Distribution of species is a result of environmental influences, such as rainfall, temperature, soil type, elevation, and vegetative land cover, have been identified in these studies as useful predictors for geographic distribution of parasites. In addition, several recent studies have used GIS and remote sensing technology to identify biotic and abiotic factors that influenced the distribution of the sand fly vectors associated with leishmaniasis (Thomson et al., 1999; Thomson and Conner, 2000).

King et al. (2004) used GIS and remote sensing data to evaluate and predict the risk of CL in Colombia using incidence data obtained from the Colombian Ministry of Health, digital elevation models (DEM) from the U.S. Geological Service's Earth Resources Observation System (USGS-EROS), and land cover data obtained from the National Oceanic and Atmospheric Administration's Advanced Very High Altitude Radiometer satellite imagery (NOAA-AVHRR). Satellite images from a 1 year period were classified into 25 land cover zones based on spectral characteristics. The DEM and land cover data was overlaid in GIS software over maps of the distribution of human cases. The authors evaluated the predictive power of datasets containing disease distribution and elevation data only, disease distribution and land cover data only, and disease distribution along with both elevation and land cover data. They determined that land cover was a better predictor of areas where leishmaniasis occurred than elevation.

However, combining the two datasets provided increased accuracy of the predictive value of their maps.

El Naiem et al. (2003), used GIS to map and develop risk models in an attempt to explain the environmental factors that influenced the geographic distribution of human cases of visceral leishmaniasis in Sudan, East Africa between the years 1996–2000. They investigated the effect of numerous environmental factors that influenced the spatial distribution of the sand fly vectors, the reservoirs, and human hosts including: average rainfall, vegetation, soil type, altitude, and the distance from rivers. Regression analyses of environmental variables in the study by El Naiem et al. (2003) indicated that the best predictors for the presence of visceral leishmaniasis were the average rainfall and elevation, with average rainfall being the single most important variable in affecting the spatial distribution. El Naiem et al. (2003) indicated that the sand fly vectors were found to inhabit areas with annual rainfall between 400–1,200 mm and elevations less than approximately 500 m above mean sea level. The authors suggested that these variables were the most likely to influence the habitat distribution of the known sand fly vector, *Phlebotomus orientalis*, within the region.

To date, no studies have been done attempting to define the biotic or abiotic environmental factors that affect the spatial distribution of *Leishmania* species in the Nicaragua.

# MATERIALS AND METHODS

## Overview

All collecting of rodent and sand fly specimens was accomplished after required permissions were obtained from the Nicaraguan Ministry of Health. In fact a long series of meetings were required to gain access to trapping sites. In Managua, the Minister of Health was briefed on the objectives and scope of this research in order to gain approval to operate a health-related research study within Nicaragua. To conduct research operations at each of the study sites, meetings were arranged in order to discuss the details of our project with the Ministry of Health representative at the department and municipality levels, and finally permission from the individual community leaders and land owners was obtained. This process of progressing through a hierarchy of permission granting entities often was time consuming. The time spent in meetings at many different government levels cut considerably into the time available for conducting field research, and it was quickly learned that extra days needed to be budgeted on each trip in order to account for the necessary briefings and coordination with local entities.

In most instances, this research was conducted while working closely with the local hospital or health clinic and I often was accompanied by an employee of the Ministry of Health, usually a representative of the vector control program. Rodent trapping and sand fly collections were conducted near localities that were identified as having concentrations of human CL. These localities were identified using records obtained from hospitals or clinics and by direct conversation with public health officials and community leaders. Many of the trapping sites were fairly remote and accessible only by four wheel drive truck or on foot. Additionally, much of the trapping was done during the rainy season, which added to the difficulty of gaining site access.

**Study areas**

Rodent collections were accomplished at 13 localities within 9 municipalities and 7 districts in Nicaragua between August 2001 and March 2006 and included 3,638 trap nights (Fig. 11). These collection localities were widespread throughout the country and included areas where human typical CL did and did not occur. The 13 rodent trapping localities (Table 4) included areas within each of the general geographic regions of the country: the Pacific plains, the central highlands, and the Atlantic lowland plains.

Sites within the Pacific plains region included Bella Vista and San Cristobal in the district of Chinandega and Las Marias and San Jacinto in the District of Leon. Trapping sites within the central highlands region included El Tigre and Selva Negra in the district of Matagalpa; Los Mangos in the District of Madriz; and El Cua in the District of Jinotega. Collection localities within the Atlantic lowland plains region included El Balsamo and Rosa Grande in the North Atlantic Autonomous Region (RAAN) and Nueva Guinea, La Esperanzita 2 and La Fonseca in the South Atlantic Autonomous Region (RAAS). Sand fly traps also were placed at these localities and in the city of Bluefields, located on the Caribbean coast.

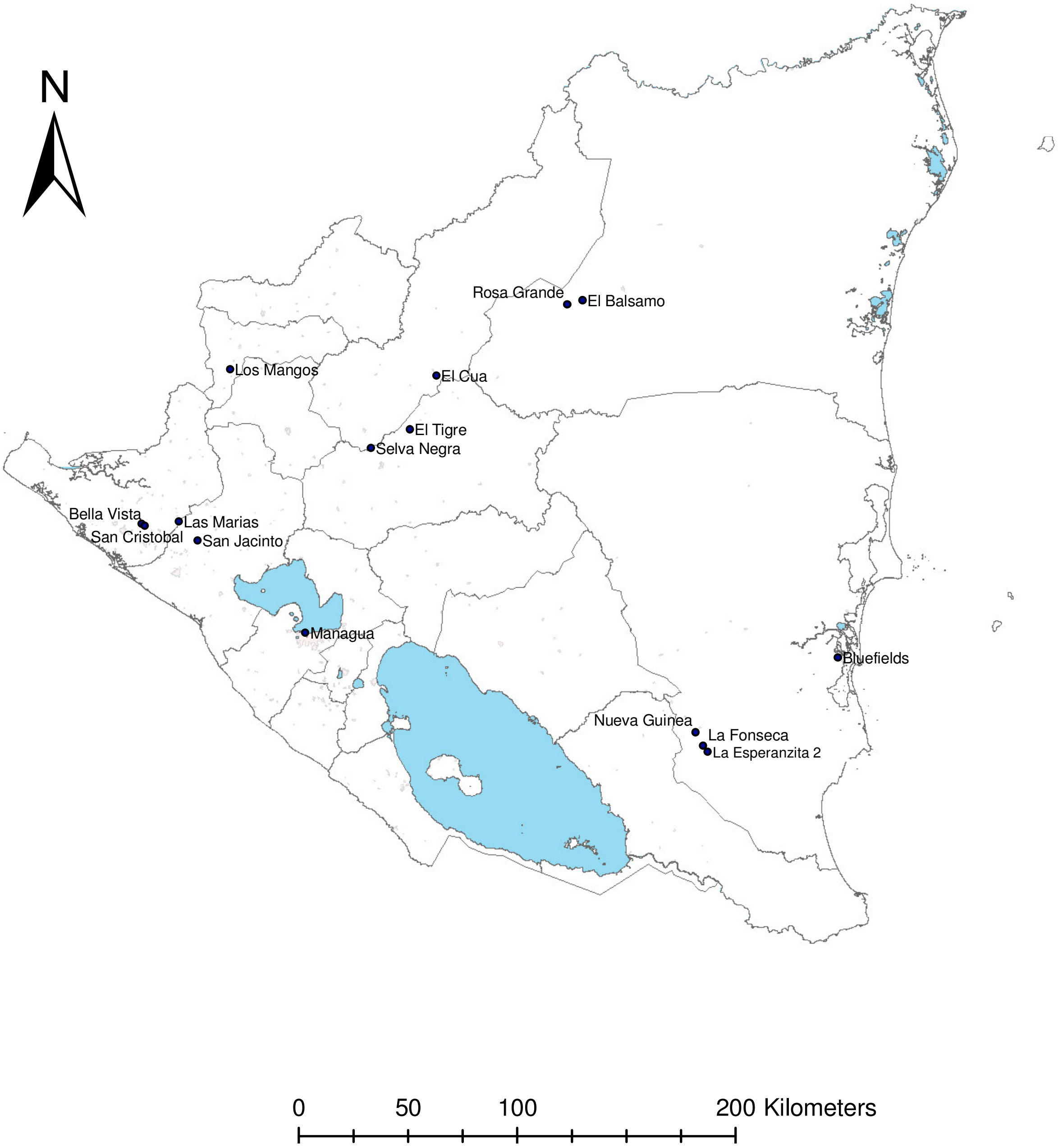


Figure 11. Map of the rodent and sand fly collecting localities in Nicaragua during August 2001–March 2006.

Table 4. Rodent collection sites in Nicaragua during August 2001–March 2006.

| Locality | Municipality | Department | Lat/Long |
|---|---|---|---|
| Bella Vista | Chichigalpa | Chinandega | 12 39’N 086 57’W |
| El Balsamo | Siuna | RAAN[1] | 13 39’N 084 58’W |
| El Cua | Cua-Bocay | Jinotega | 13 20’N 085 39’W |
| El Paraisito | Nueva Guinea | RAAS[2] | 11 41’N 084 24’W |
| El Tigre | El Tuma-La Dalia | Matagalpa | 13 04’N 085 45’W |
| La Esperanzita 2 | Nueva Guinea | RAAS[2] | 11 32’N 084 20’W |
| La Fonseca | Nueva Guinea | RAAS[2] | 11 34’N 084 22’W |
| Las Marias | Telica | Leon | 12 37’N 086 48’W |
| Los Mangos | San Lucas | Madriz | 13 26’N 086 35’W |
| Rosa Grande | Siuna | RAAN[1] | 13 38’N 085 08’W |
| San Jacinto | Telica | Leon | 12 35’N 086 45’W |
| San Cristobal | Chinandega | Chinandega | 12 40’N 087 02’W |
| Selva Negra | Matagalpa | Matagalpa | 12 59’N 085 54’W |

[1] Regíon Autónoma Atlántico Sur (South Atlantic Autonomous Region)
[2] Regíon Autónoma Atlántico Norte (North Atlantic Autonomous Region)

### Study areas within the Pacific plains region

This region of Nicaragua experiences relatively few cases of human typical CL and rodent and sand fly collection were made to compare with regions where the prevalence of human typical CL is high. This region of Nicaragua does, however, have the highest prevalence of human atypical CL. The trapping sites within this region included Bella Vista, Las Marias, San Cristobal and San Jacinto. The Bella Vista site (12° 39’N, 086° 57’W) is located within the municipality of Chichigalpa. The trapping site was located on the western slopes of the Casita volcano and at an elevation of approximately 700 m above MSL. This area receives between 1,600–1,800 mm of rainfall per year and has a mean annual temperature of approximately 23.5° C. The soil predominantly is sandy loam but has numerous rocks. The trapping locations within this site were located in or near an area that is heavily planted in coffee with large trees scattered throughout.

The Las Marias (12° 37’N, 086° 48’W) and San Jacinto (12° 35’N, 086° 45’W) study sites are located within the municipality of Telica. The Las Marias site has an elevation of approximately 175 m above MSL and a mean annual precipitation between 1,600–1,800 mm. The mean annual temperature is approximately 26.5° C. The soil is predominantly sandy loam with some scattered rocks; and this area mostly is farm and grazing land with smaller trees and brush primarily in hedgerows. The San Jacinto site is located at an elevation of approximately 175 m above MSL and has a mean annual rainfall of 1,400–1,600 mm. The mean annual temperature is approximately 26.5° C.

The soil at this site is sandy loam with scattered small rocks. This area appeared to be primarily cattle grazing land and was covered with scattered small scrub brush.

The San Cristobal (12° 40’N, 087° 02’W) study site is located within the municipality of Chinandega. It is located on the western slope of the San Cristobal volcano and has an elevation of approximately 460 m above MSL and a mean annual rainfall between 1,600–1,800 mm. Mean annual temperature at this site is approximately 25.5° C. The soil in this area is sandy loam and trapping was conducted in areas that had extensive coffee plantations with large trees scattered throughout.

### Study areas within the central highlands region

Although there are few cases of human typical CL in the north and western areas of the central highlands region, the center and eastern sections contain those municipalities that reported the greatest number of human cases. Trapping sites within this region included El Cua, El Tigre, Los Mangos and Selva Negra. El Cua (13° 20’N, 085° 39’W) is located within the municipality of Cua-Bocay. This region of Nicaragua is rather mountainous and the elevations at this site ranged between 700–800 m above MSL. The mean annual rainfall is between 1,400–1,600 mm. Mean annual temperature is approximately 23.5° C. Clay soils dominate the region and land usage and coverage varies considerably. Much of the region is dominated by agricultural use including coffee and banana plantations. However parts of this municipality are covered with semideciduous broadleaf tropical forest. The trapping sites near El Cua were located in woody areas near the edges of coffee and bean fields.

El Tigre (13° 04’N, 085° 45’W) is located within the municipality of El Tuma - La Dalia. The trapping site has an elevation of approximately 450 m above MSL and has a mean annual precipitation rate of 1,200–1,400 mm. The mean annual temperature is approximately 24.5° C. The soils predominantly are clay and the area consists mainly of small farms with scattered trees primarily along hedgerows and river banks.

Los Mangos (13° 26’N, 086° 35’W) is located in the northwestern part of the central highlands near the Honduras border. This trapping site has an elevation of approximately 980m above MSL and a mean annual rainfall of 1,000–1,200 mm. The mean annual temperature is approximately 22.5° C. The soil in this area is predominantly clay loam but has large rocks scattered throughout. This is very rugged, mountainous terrain and the trapping site was located in an area that had scattered evergreen pines and deciduous oak trees with small hillside farms scattered throughout.

The trapping site at Selva Negra (12° 59’N, 085° 54’W) is located within the northeast section of the municipality of Matagalpa. This trapping site also is quite mountainous and has elevations ranging from 1,200 to 1,600 m above MSL. The mean annual precipitation is between 1,200–1,400 mm and the mean annual temperature is 20.5° C. The soils within the area predominantly are clay and clay loam. This trapping site is covered in submontane evergreen tropical forest with scattered coffee plantations.

### Study areas within the Atlantic lowland plains region

This region contains several municipalities with high numbers of reported cases of human typical CL. Trapping sites within this region included El Paraisito, La Esperanzita 2, La Fonseca located in the southern region of the Atlantic lowland plains and El Balsamo and Rosa Grande located in the northern Atlantic lowland plains. This region is the wettest in the country with some areas receiving greater than 4,000 mm of mean annual rainfall.

El Balsamo (13° 39’N, 084° 58’W) and Rosa Grande (13° 38’N, 085° 08’W) are located within the municipality of Siuna. The elevation at both localities is approximately 300 m above MSL and the mean annual precipitation in both localities is 1,400–1,600 mm. The mean annual temperature is approximately 25.5° C and the soils in this region are predominantly reddish clay.

El Paraisito (11° 41’N, 084° 24’W), La Esperanzita 2 (11° 32’N, 084° 20’W) and La Fonseca (11° 34’N, 084° 22’W) are located in the municipality of Nueva Guinea. The average elevation at the El Paraisito study site is approximately 200 m above MSL and the mean annual precipitation is between 2,000–2,400 mm. The mean annual temperature is approximately 26.5° C. The soil here is predominantly brown clay. Much of the area surrounding the El Paraisito site is dotted with cattle ranches and dairy farms with 10–25% natural vegetation coverage. Areas with large trees are scattered and exist primarily along hedge rows and river banks.

The average elevation at the La Fonseca study site is approximately 175m above MSL and the mean annual precipitation is between 2,000–2,400 mm. The mean annual temperature is approximately 26.5° C. The soil predominantly is brown clay. The area around La Fonseca also contains numerous farms, cattle ranches, and dairies; but, there is considerably more natural vegetation coverage than at the El Paraisito site. It is classified as primarily agricultural usage with 25–50% natural vegetation coverage.

The La Esperanzita 2 study site is located near the southern extreme of the Nueva Guinea municipality and has an average elevation of approximately 100 m above MSL. The mean annual precipitation is between 2,400–2,800 mm and the mean annual temperature is approximately 26.5° C. The soil also is predominantly brown clay and the area is classified as evergreen broadleaf tropical forest.

### Rodent trapping

Trapping localities for rodent collections were chosen after consultation with individuals within the Nicaragua Ministry of Health and were selected based on proximity to reported cases of human leishmaniasis. Rodents were collected using 12.5 x 12.5 x 38.0 cm Sherman® live traps (H. B. Sherman Traps Inc., Tallahassee, FL). Traps were baited with a mixture of locally-obtained fruit and/or oatmeal. Traps were placed near obvious rodent den openings, along easily-distinguishable runways of occupied rodent dens, or at the base of large trees (Fig. 12). Live-animal traps were set in the evening and collected early the next morning to reduce the exposure of rodents to heat, cold or rain. All animal work was conducted under Texas A&M University approved animal use protocol AUP #2005–75.

### Tissue collection

Conditions for aseptic processing of rodent tissue were less than ideal. Rodents were transported in the traps to a central processing location which, in many cases, was the tailgate of the truck. They then were transferred from the traps into a cotton stockinette (McKesson General Medical, Richmond, VA) and weighed using a precision

Figure 12. Sherman live mammal traps placed at Selva Negra research site in the municipality of Matagalpa, Nicaragua.

spring scale. Once restrained in the cotton stockinette, the rodents then were euthanized on site by thoracic compression and immediately processed for leishmaniasis screening. After the rodents were euthanized, they then were sexed and identified to species. Two tissue samples were collected from each ear and the base of the tail of all rodents using a sterile, disposable, 2 mm biopsy punch (Sklar Instruments, West Chester, PA). The ears and tail of the rodents generally have less hair coverage and provide the most likely sight for female sand flies to feed. When lesions were present, biopsies were taken from the lesion and surrounding area. The tissue biopsies then were either placed in culture media or frozen until analysis by PCR could be accomplished. All rodents were placed in plastic storage bags and kept in a portable freezer until transfer to an appropriately-labeled ice chest containing frozen gel packs and, ultimately transported to the United States and the research laboratory at the University of the Incarnate Word in San Antonio, Texas. The United States Centers for Disease Control and Prevention issued a permit (PHS Permit No. 2004–04–021) for the import and transfer of etiological agents and vectors of human disease pertaining to this project.

All collected rodents then were shipped to Dr. Robert Bradley at Texas Tech University, Lubbock, Texas. Dr. Bradley confirmed, or corrected the field identification of rodent species and all rodents were prepared as museum skins and deposited in the Recent Mammals Collection. Samples of muscle, heart, liver, spleen, and kidney tissue were deposited in the Vital Tissues Collection of the museum at Texas Tech University (Appendix A).

### Tissue biopsy preparation and screening using culture medium

Tissue biopsies were processed in Nicaragua according to the method described by Kerr et al. (1995). The biopsy tissue was rinsed with isopropyl alcohol and wiped with alcohol swabs to remove dirt and other contaminants. The tissue then was scraped with a sterile scalpel blade to remove excess hair before freezing or placement in cell culture medium. However, there was no access to a laminar flow safety cabinet during the preparation of tissue biopsies and cultures, so bacterial and fungal contamination was a consistent problem. One biopsy from each ear and the tail was stored in a -20° C freezer until analysis by PCR. One biopsy from each ear and the tail was placed in a 500 µl microcentrifuge tube, immersed in 70% isopropyl alcohol and shaken in a vortex mixer for 15 seconds to aid in the removal of contaminants. The tissue samples then were removed from the alcohol and placed in a culture tube containing modified medium M199 (Gibco-BRL, Gaithersburg, MD) supplemented with 20% (v/v) heat-inactivated fetal bovine serum (Summit Biotechnology, Fort Collins, CO), 10mM adenine, 0.25% (v/v) bovine hemin, 50 mM HEPES buffer, 50 IU/ml penicillin, and 50 µg/ml streptomycin (pH 7.4). The tissue samples in culture medium then were stored at room temperature in Nicaragua and monitored using an inverted phase contrast microscope daily for the presence of promastigotes of *Leishmania*. After the culture samples were returned to the laboratory at the University of the Incarnate Word in San Antonio, they were stored in a 25° C incubator and examined a minimum of twice weekly using an inverted phase contrast microscope.

## Tissue biopsy screening using polymerase chain reaction (PCR)

Tissue screening by PCR was accomplished at the laboratory in San Antonio, Texas. Tissue biopsies were prepared for PCR by placing the thawed tissue biopsy samples in 40 µl of lysing buffer (10 mM Tris / 10 mM EDTA, pH 8.0) and incubating at 95° C for 30 minutes (Rodgers et al., 1990). The samples then were stored at -20° C until PCR could be performed on the lysate.

The PCR master mix was prepared using final concentrations of 1X PCR reaction buffer (Boehringer-Mannheim, Germany), 0.2 mM nucleotides (Boehringer-Mannheim), 1.0 µM forward primer 13A (5'–GTGGGGGAGGGGCGTTCT–3') and reverse primer 13B (5'–ATTTTACACCAACCCCCAGTT–3') (Rogers et al., 1990), RNase free water, and 0.75 U/25 µL Taq polymerase (Boehringer Mannheim). Twenty-three microliters of the master mix were added to individual reaction tubes and 2 µl of the tissue lysate then was added to each tube. The reaction tubes then were placed in a thermal cycler (Perkin Elmer, Norwalk, CT) and the PCR reactions run for 35 cycles.

Analysis of the PCR product was conducted through gel electrophoresis. A 2% ultrapure DNA grade agarose gel (Bio-Rad, Hercules, CA) was prepared using 0.5X Tris-Borate-EDTA (TBE), agarose, and ethidium bromide (0.1µl / 1ml TBE). Two microliters of loading buffer were added to each reaction tube containing the PCR product. After completion of PCR, 10 µl from each reaction tube then was placed into the wells of the gel. A positive control, negative control ($H_2O$), and a 100 base pair ladder also were loaded into the gel. The positive control was prepared from known concentrations of parasites reared in culture medium. The electrophoresis apparatus was run at 96 Volts for 1 hour. A band in the 120 base pair region indicated a positive sample.

### Sand fly trapping

Insects were collected using Hock new standard miniature light traps (John W. Hock, Gainsville, FL) placed in areas near human case residences and mammal trapping sites (Fig. 13). Sand flies were killed by placing the collection receptacles in a freezer overnight and then the specimens were placed in a covered Petri dish between 2 pieces of tissue for transport. Much of the specimen collection effort took place during the rainy season and although the sand fly traps were equipped with rain hoods, often the insects would become very wet. In those instances, the collection containers were placed in a dark, dry room for a day or two prior to placing them in the freezer to kill any insects that remained alive. Sand flies were identified to species using the guide of Young and Duncan (1994). The United States Centers for Disease Control and Prevention issued a permit (PHS Permit No. 2004–04–021) for the import and transfer of etiological agents and vectors of human disease pertaining to this project.

### Geographic Information System (GIS) and remote sensing

Geographic information systems, remote sensing technology, and prevalence data for the distribution of *Leishmania* species in Nicaragua were used to build GIS maps and predictive statistical models for the presence or absence of human typical CL. Prevalence data on the distribution of human cases of typical CL were obtained from Dr. Juan Jose Amador, Director of Epidemiology for the Nicaragua Ministry of Health. These data were of human typical CL reported by various health clinics within the

Figure 13.  Insect trap near El Cua in the municipality of El Cua-Bocay, Nicaragua.

country. No data were available about travel history of individuals and it is unknown as to whether the leishmaniasis was contracted in the same municipality as reported. It is possible, and even probable, that some of the reported cases of CL were acquired at locations outside of the reporting municipality. However, assuming that most individuals spend the vast majority of their time near locations were they live and work, it also is likely that most cases were acquired and reported within an individual's home municipality.

GIS was used to investigate relationships between the environmental factors such as mean annual precipitation, elevation, land use and coverage, soil type, mean annual temperature and the prevalence of *Leishmania* infections. GIS maps of environmental variables including precipitation, land cover zones, mean annual temperature and soil types were obtained from Nicaraguan Ministry of Atmosphere and Natural Resources. Digital elevation data used for this analysis were acquired from a raster data set prepared by Earth Systems Research Institute Inc. (ESRI). The original remote sensing data for this elevation raster data set was compiled by the United States Geological Service's Earth Resources Observation System (USGS-EROS) digital elevation model (DEM) GTOP030 data sets of North America. Resolution of the raster data is approximately 1 $km^2$.

The GIS data obtained by the Nicaraguan Ministry of Climate and Natural Resources were provided in the form of ArcGIS shapefiles. These shapefile data were converted to raster data sets with a cell size of 1 $km^2$. This was done to match the resolution of the DEM and provide consistency during data analysis. The shapefile data included a base map of political boundaries, soil types, mean annual precipitation, mean annual temperature and a land use and coverage map.

The soil map of Nicaragua provided the geographic distribution of 7 categories of soil types. These included heavy clay, clay, loamy sand, sand, loam, clay loam and sandy loam. The precipitation map included 13 different zones of mean annual precipitation ranging from less than 800mm to greater than 4,000mm. The temperature map included 9 different zones of mean annual temperature ranging from 18.5° C to 27.5° C. The land use and coverage map acquired from the Nicaragua Ministry of Climate and Natural Resources included 70 ecological zones. To aid in statistical analysis and to reduce the possibility of any one category being statistically significant by random chance, similar land use/coverage classes were combined into a total of 13 classes (Table 5). The DEM was reclassified to include 11 elevation ranges from 0 to 2,000 meters above mean sea level.

Table 5. Nicaragua land use/cover classes used for statistical analysis

| Class Description |
|---|
| Urban Area |
| Cropland |
| Agricultural Systems with 10–25% Natural Vegetation |
| Agricultural Systems with 25–50% Natural Vegetation |
| Mangroves |
| Coastal Transition Vegetation |
| Savannah |
| Grassland with Deciduous Shrubs |
| Shrubland |
| Tropical Deciduous Broadleaf Seasonal Forest |
| Tropical Evergreen Pine Seasonal Forest |
| Tropical Evergreen Swamp Forest |
| Tropical Evergreen Broadleaf Seasonal Forest |

Data of human cases of typical CL were entered into the attribute table of the GIS base map of Nicaragua municipalities and displayed as numbers of cases per municipality. The shapefiles for 151 of the 152 municipalities within Nicaragua then were converted to raster format prior to analysis. The municipality of Corn Island (approximately 9 km$^2$) was excluded from GIS analysis because environmental data were unavailable. This base map then was overlaid with the raster data sets of elevation, mean annual precipitation, mean annual temperature, soil types, and land use and coverage. Since the municipalities varied considerably in size and population, leishmaniasis case data also were normalized to municipality population and municipal area (km$^2$) prior to analysis.

Distribution and analysis maps were developed using ArcView 9.0 and ArcGIS Spatial Analyst 8.2 software (ESRI, Redlands, CA). The zonal statistics tool of ArcGIS Spatial Analyst was used to identify the area (km$^2$) of each of the different zones of environmental data that fell within the geographic area representing the distribution of human cases of human typical CL. The geographic coordinate system used for all GIS maps is WGS 1984 and the projected coordinate system is WGS 1984 UTM Zone 16N and the map projection is Transverse Mercator.

### Statistical analysis

Binary logistic regression analysis was performed to determine the correlation of the distribution of cases of human typical CL with different environmental variables using SPSS 14.0 (Chicago, IL). Generated statistical models to predict the dichotomous outcome variable, the presence or absence of human typical CL, within a geographic area were based upon all georeferenced data in order to determine the environmental factors that are the best predictors of occurrence. In statistical models built to determine predictor variables of presence or absence of human typical cutaneous leishmaniasis, the occurrence of at least 1 reported case within a municipality was used to identify that municipality as having typical CL present. The predictor variables used in this study were the total number of square kilometers within a municipality of the various classes of: elevation, mean annual precipitation, mean annual temperature, soil type, and land

statistical analysis because no environmental data were available.

Additionally, since >97% of all cases of human typical CL were reported from just 28 of 152 municipalities, binary logistic regression analysis also was performed in an attempt to identify environmental variables that are predictors of municipalities with less than 10 reported cases and municipalities with 10 or more reported cases. The municipality of Corn Island (approximately 9 $km^2$) also was omitted from this data analysis because no environmental data were available.

# RESULTS

## Rodent collections

Three thousand six hundred-thirty eight trap nights resulted in the capture of 395 rodents representing 17 species between August 2001 and March 2006 at 13 localities in Nicaragua (Table 6). At the Bella Vista trapping site, 29 total rodents representing 6 species were captured and screened for *Leishmania* species. The numbers of rodents trapped by species were: 9 (31%) *Liomys salvini*, 6 (21%) *Mus musculus*, 1 (3%) *Nyctomys sumichrasti*, 2 (7%) *Ototylomys phyllotis*, 6 (21%) *Peromyscus mexicanus* and 5 (17%) *Rattus rattus*.

At the El Balsamo site, 33 total rodents representing 7 species were captured. The numbers of rodents trapped by species were: 15 (46%) *Melanomys caliginosus*, 1 (3%) *Nyctomys sumichrasti*, 2 (6%) *Oligoryzomys fulvescens*, 2 (6%) *Ototylomys phyllotis*, 4 (12%) *Proechimys semispinosus*, 4 (12%) *Scotinomys tequina* and 5 (15%) *Sigmodon hirsutus*.

At the El Cua site, 14 rodents representing 6 species were captured. The numbers of rodents trapped per species were: 2 (14%) *Melanomys caliginosus*, 3 (21%) *Mus musculus*, 1 (7%) *Oryzomys cousei*, 3 (21%) *Oryzomys alfaroi*, 3 (21%) *Peromyscus nudipes*, and 2 (14%) *Sigmodon hirsutus*.

At the El Paraisito site, 52 rodents representing 5 species were captured including 1 (2%) *Mus musculus*, 4 (8%) *Oligoryzomys fulvescens*, 4 (8%) *Oryzomys cousei*, 35 (67%) *Proechimys semispinosus*, and 8 (15%) *Sigmodon hirsutus*. At the El Tigre site, 9 rodents were captured representing 3 species including 1 (11%) *Oryzomys alfaroi*, 5

Table 6. Occurrence of *Leishmania* in rodents collected in Nicaragua, 2001–2006.

| | | Number Positive/Number Screened* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aug | Jan | May/Jun | May | Jul | May/June | Dec | Mar | May | Mar |
| Location | Species | 2001 | 2002 | 2002 | 2003 | 2003 | 2004 | 2004 | 2005 | 2005 | 2006 |
| Bella Vista | | | | | | | | | | | |
| | *Liomys salvini* | 0/1 | - | 0/8 | - | - | - | - | - | - | - |
| | *Mus musculus* | 0/6 | - | - | - | - | - | - | - | - | - |
| | *Nyctomys sumichrasti* | - | - | 0/1 | - | - | - | - | - | - | - |
| | *Ototylomys phyllotis* | 0/1 | 0/1 | - | - | - | - | - | - | - | - |
| | *Peromyscus mexicanus* | - | - | 0/6 | - | - | - | - | - | - | - |
| | *Rattus rattus* | 0/5 | - | - | - | - | - | - | - | - | - |
| El Balsamo | | | | | | | | | | | |
| | *Melanomys caliginosus* | - | - | - | 0/1 | 0/7 | 0/7 | - | - | - | - |
| | *Nyctomys sumichrasti* | - | - | - | - | 0/1 | - | - | - | - | - |
| | *Ototylomys phyllotis* | - | - | - | - | 0/2 | - | - | - | - | - |
| | *Proechimys semispinosus* | - | - | - | 0/2 | - | 0/2 | - | - | - | - |
| | *Oligoryzomys fulvescens* | - | - | - | - | - | 0/2 | - | - | - | - |
| | *Scotinomys tequina* | - | - | - | - | - | 0/4 | - | - | - | - |
| | *Sigmodon hirsutus* | - | - | -- | - | - | 0/5 | - | - | - | - |
| El Tigre | | | | | | | | | | | |
| | *Oryzomys alfaroi* | - | - | 0/1 | - | - | - | - | - | - | - |
| | *Peromyscus mexicanus* | - | - | 0/5 | - | - | - | - | - | - | - |
| | *Sigmodon hirsutus* | - | - | 0/3 | - | - | - | - | - | - | - |
| Las Marias | | | | | | | | | | | |
| | *Liomys salvini* | - | 0/1 | - | - | - | - | - | -- | - | - |

Table 6 continued.

| | | Number Positive/Number Screened* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Species | Aug 2001 | Jan 2002 | May/Jun 2002 | May 2003 | Jul 2003 | May/Jun 2004 | Dec 2004 | Mar 2005 | May 2005 | Mar 2006 |
| Los Mangos | | | | | | | | | | | |
| | *Liomys salvini* | - | 0/1 | - | - | - | - | - | - | - | - |
| | *Peromyscus mexicanus* | - | 0/2 | - | - | - | - | - | - | - | - |
| Rosa Grande | | | | | | | | | | | |
| | *Melanomys caliginosus* | - | - | - | - | 0/12 | - | - | - | - | - |
| | *Ototylomys phyllotis* | - | - | - | - | 0/4 | - | - | - | - | - |
| | *Peromyscus mexicanus* | - | - | - | - | 0/1 | - | - | - | - | - |
| | *Proechimys semispinosus* | - | - | - | - | 0/4 | - | - | - | - | - |
| San Cristobal | | | | | | | | | | | |
| | *Liomys salvini* | - | - | 0/1 | - | - | - | - | - | - | - |
| | *Nyctomys sumichrasti* | - | - | 0/1 | - | - | - | - | - | - | - |
| | *Ototylomys phyllotis* | - | - | 0/4 | - | - | - | - | - | - | - |
| | *Peromyscus mexicanus* | - | - | 0/2 | - | - | - | - | - | - | - |
| | *Peromyscus oaxacensis* | - | - | 0/20 | - | - | - | - | - | - | - |
| San Jacinto | | | | | | | | | | | |
| | *Peromyscus mexicanus* | - | - | 0/3 | - | - | - | - | - | - | - |
| | *Mus musculus* | - | - | 0/2 | - | - | - | - | - | - | - |

Table 6 continued.

| | | Number Positive/Number Screened* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Species | Aug 2001 | Jan 2002 | May/Jun 2002 | May 2003 | Jul 2003 | May/Jun 2004 | Dec 2004 | Mar 2005 | May 2005 | Mar 2006 |
| Selva Negra | | | | | | | | | | | |
| | *Heteromys desmarestianus* | - | 0/1 | 1/2 | 0/1 | - | - | - | - | - | - |
| | *Mus musculus* | 0/2 | - | - | - | - | - | - | - | - | - |
| | *Nyctomys sumichrasti* | 0/1 | - | - | - | - | - | - | - | - | - |
| | *Oryzomys alfaroi* | - | 0/3 | 0/3 | | - | - | - | - | - | - |
| | *Ototylomys phyllotis* | - | 0/2 | 0/2 | | - | - | - | - | - | - |
| | *Peromyscus mexicanus* | 0/2 | 1/43 | 0/48 | 0/59 | - | - | - | - | - | 0/11 |
| | *Rattus rattus* | 0/7 | 0/1 | - | - | - | - | - | - | - | - |
| | *Scotinomys tequina* | - | 0/2 | 0/2 | - | - | - | - | - | - | - |
| | *Tylomys nudicaudus* | - | - | - | 0/1 | - | - | - | - | - | - |
| El Paraisito | | | | | | | | | | | |
| | *Oligoryzomys fulvescens* | - | - | - | - | - | 0/4 | - | - | - | - |
| | *Oryzomys cousei* | - | - | - | - | - | 0/4 | - | - | - | - |
| | *Proechimys semispinosus* | - | - | - | - | - | 0/26 | 0/5 | 0/2 | 0/2 | - |
| | *Sigmodon hirsutus* | - | - | - | - | - | 0/8 | - | - | - | - |
| | *Mus musculus* | - | - | - | - | - | - | - | 0/1 | - | - |
| La Esperanzita 2 | | | | | | | | | | | |
| | *Proechimys semispinosus* | - | - | - | - | - | - | 0/2 | - | - | - |
| La Fonseca | | | | | | | | | | | |
| | *Proechimys semispinosus* | - | - | - | - | - | - | 0/5 | - | - | - |
| El Cua | | | | | | | | | | | |
| | *Melanomys caliginosus* | - | - | - | - | - | - | - | - | - | 0/2 |
| | *Mus musculus* | - | - | - | - | - | - | - | - | - | 0/3 |
| | *Oryzomys alfaroi* | - | - | - | - | - | - | - | - | - | 0/3 |
| | *Oryzomys cousei* | - | - | - | - | - | - | - | - | - | 0/1 |
| | *Peromyscus mexicanus* | - | - | - | - | - | - | - | - | - | 0/3 |
| | *Sigmodon hirsutus* | - | - | - | - | - | - | - | - | - | 0/2 |

* Screening accomplished by PCR using genus specific primers

(56%) *Peromyscus mexicanus* and 3 (33%) *Sigmodon hirsutus*. One *Proechimys semispinosus* was captured at the La Esperanzita #2 site. At the La Fonseca site, 5 rodents were captured, all were *Proechimys semispinosus*. One *Liomys salvini* was captured at the Las Marias site. At the Los Mongos site, 3 rodents representing 3 species were captured including 1 *Liomys salvini*, 1 *Peromyscus mexicanus* and 1 *Peromyscus oaxacensis*. The Rosa Grande site produced 21 rodents representing 4 species and included 12 *Melanomys caliginosus*, 4 *Ototylomys phyllotis*, 1 *Peromyscus nudipes* and 4 *Proechimys semispinosus*. The San Cristobal site produced 28 rodents representing 5 species including 1 *Liomys salvini*, 1 *Nyctomys sumichrasti,* 4 *Ototylomys phyllotis,* 2 *Peromyscus mexicanus* and 20 *Peromyscus oaxacensis*. The San Jacinto site produced 5 rodents representing 2 species including 2 *Mus musculus* and 3 *Peromyscus mexicanus*. At the Selva Negra site, 193 rodents were captured representing 9 species including 4 *Heteromys desmarestianus*, 2 *Mus musculus*, 1 *Nyctomys sumichrasti*, 6 *Oryzomys alfaroi,* 4 *Ototylomys phyllotis*, 163 *Peromyscus mexicanus*, 8 *Rattus rattus*, 4 *Scotinomys tequina*, and 1 *Tylomys nudicaudus*.

**Sand fly collections**

Between August 2001 and March 2006, 556 sand flies were collected at 8 localities in 6 districts within Nicaragua. Five hundred sixteen sand flies were identified to species, 2 were identified to subgenus, 31 were identified only as *Lutzomyia* species, 1 was identified to the genus *Brumptomyia* and 7 were identified to group (Table 7).

A total of twenty sand flies were captured at the El Balsamo study site, including

Table 7. Sand fly species collected in Nicaragua, 2001–2006.

| Location | Date | Identification | | ♀ | ♂ |
|---|---|---|---|---|---|
| El Balsamo | May 2003 | Cruciata Group | | 6 | 0 |
| | | *Lutzomyia sp.* | | 1 | 0 |
| | | *Lu. cruciata* | | 1 | 4 |
| | July 2003 | *Lu. cruciata* | | 1 | 0 |
| | | *Lu. shannoni* | | 0 | 1 |
| | | Vespertilionsis Group | | 1 | 0 |
| | | *Lutzomyia sp.* | | 2 | 0 |
| | May 2004 | *Lu. cruciata* | | 2 | 0 |
| | | *Lu. panamensis* | | 1 | 0 |
| | | | Subtotal | 15 | 5 |
| El Cua | March 2006 | *Lu. cruciata* | | 0 | 1 |
| | | *Lu. panamensis* | | 0 | 1 |
| | | | Subtotal | 0 | 2 |
| El Paraisito | May 2004 | *Lu. barrettoi majuscula* | | 2 | 1 |
| | | *Lu.* species | | 2 | 0 |
| | June 2004 | *Lu. hartmanni* | | 0 | 1 |
| | | *Lu. barrettoi majuscula* | | 1 | 0 |
| | | *Lu.* species | | 3 | 1 |
| | Mar 2005 | *Lu. barrettoi majuscula* | | 1 | 2 |
| | | *Lu.* species | | 1 | 2 |
| | May 2005 | *Lu. barrettoi majuscula* | | 3 | 0 |
| | | Subgenus Psathyromyia | | 1 | 0 |
| | | *Lu.* species | | 1 | 0 |
| | June 2005 | *Lu. barrettoi majuscula* | | 0 | 1 |
| | | | Subtotal | 15 | 8 |
| El Tigre | May 2002 | *Lu. cruciata* | | 4 | 20 |
| | | *Lu. longipalpis* | | 0 | 3 |
| | | | Subtotal | 4 | 23 |
| Los Mangos | January 2002 | *Brumptomyia* sp. | | 0 | 1 |
| | | *Lu. cruciata* | | 23 | 19 |
| | | *Lu. longipalpis* | | 1 | 0 |
| | | | Subtotal | 24 | 20 |
| Rosa Grande | July 2003 | *Lu. panamensis* | | 0 | 2 |
| | | Subgenus Psychodopygus | | 1 | 0 |
| | | | Subtotal | 1 | 2 |

Table 7 continued.

| Location | Date | Identification | ♀ | ♂ |
|---|---|---|---|---|
| San Jacinto | July 2001 | *Lutzomyia* sp. | 11 | 2 |
| | | *Lu. cayennensis* | 0 | 1 |
| | | *Lu. chiapanensis* | 14 | 5 |
| | | Subgenus Micropygomyia | 2 | 1 |
| | | *Lu. evansi* | 8 | 38 |
| | | *Lu. gomezi* | 0 | 1 |
| | | *Lu. longipalpis* | 26 | 60 |
| | | *Lu. vesicifera* | 6 | 0 |
| | August 2001 | *Lu. chiapanensis* | 5 | 2 |
| | | *Lu. cruciata* | 9 | 0 |
| | | *Lu. evansi* | 8 | 11 |
| | | *Lu. longipalpis* | 11 | 42 |
| | | *Lu. vesicifera* | 1 | 0 |
| | | *Lutzomyia* sp. | 0 | 1 |
| | January 2002 | *Lu. cruciata* | 1 | 3 |
| | | *Lu. evansi* | 17 | 13 |
| | | *Lu. longipalpis* | 22 | 109 |
| | | *Lu. gomezi* | 1 | 1 |
| | | *Lu. zeledoni* | 2 | 0 |
| | | Subtotal | 144 | 290 |
| Selva Negra | May 2003 | *Lutzomyia* sp. | 3 | 0 |
| | | Subtotal | 3 | 0 |
| | | Total | 206 | 350 |

8 *Lutzomyia cruciata* (4 female, 4 male), 1 *Lu. panamensis* female, and 1 *Lu. shannoni* male. Six specimens were identified as being *Lutzomyia* species within the Cruciata Group (6 female, 0 male), 1 female *Lutzomyia* species within the Vespertilionsis Group and 3 males were identified only to the Genus *Lutzomyia*.

A total of 2 sand flies were captured at the El Cua study site, including 1 *Lu. panamensis* male and 1 *Lu. cruciata* male. Twenty three specimens were captured at the

El Paraisito study site, including 11 *Lu. barrettoi majuscula* (7 female, 4 male) and 1 male *Lu. hartmanni* Fairchild and Hertig, 1957. This is a new record for *Lu. hartmanni* in Nicaragua. One female specimen was identified as belonging to the *Lutzomyia* subgenus *Psathryomyia* and 10 specimens (7 female and 3 male) were identified to the Genus *Lutzomyia* level.

A total of 27 sand flies were captured at the El Tigre site, with 24 identified as *Lu. cruciata* (4 female, 20 male) and 3 males as *Lu. longipalpis*. Traps at the Los Mangos study site collected a total of 44 sand flies. One male was identified as belonging to the Genus *Brumptomyia*, 42 (23 female, 19 male) as *Lu. cruciata* and 1 female as *Lu. longipalpis*. Traps at the Rosa Grande test site collected 3 specimens; 2 male *Lu. panamensis* and 1 female identified to the Subgenus *Psychodopygus*.

At San Jacinto, 434 sand flies were captured, including 1 *Lu. cayennensis* male, 26 (19 female, 7 male) *Lu. chiapanensis*, 13 (10 female, 3 male) *Lu. cruciata*, 95 (33 female, 62 male) *Lu. evansi*, 3 (1 female, 2 male) *Lu. gomezi*, 270 (59 female, 211 male) *Lu. longipalpis*, 7 female *Lu. vesicifera* and 2 female *Lu. zeledoni*. Three (2 female, 1 male) specimens were identified to the Subgenus *Micropygomyia* and 14 (11 female, 3 male) were identified only to the Genus *Lutzomyia*. The Selva Negra study site produced 3 specimens. The 3 males were identified to the Genus *Lutzomyia*.

**General distribution of sand fly species collected in Nicaragua**

Sand flies species collected during this study generally showed a distinct geographic distribution. Most species that were captured in the more arid Pacific plains region were not captured in the central highlands or Atlantic lowland plains regions and those species captured in the central highlands or Atlantic lowland plains regions were not collected during trapping within the Pacific plains regions (Table 8).

Table 8. General distribution of sand fly species collected in Nicaragua, 2001–2006.

| Species | Pacific Plains Region | Central Highlands and Atlantic Regions |
|---|---|---|
| *Lu. barrettoi majuscula* | 0 | 11 |
| *Lu. cayennensis* | 1 | 0 |
| *Lu. chiapanensis* | 26 | 0 |
| *Lu. cruciata* | 55 | 33 |
| *Lu. evansi* | 95 | 0 |
| *Lu. gomezi* | 3 | 0 |
| *Lu. hartmanni* | 0 | 1 |
| *Lu. longipalpis* | 271 | 3 |
| *Lu. panamensis* | 0 | 4 |
| *Lu. shannoni* | 0 | 1 |
| *Lu. vesicifera* | 7 | 0 |
| *Lu. zeledoni* | 2 | 0 |

## In vitro culture

Tissue samples from all 395 collected rodents were screened for the presence of *Leishmania* species using supplemented M199 culture medium. All cultures were negative.

## PCR

Of the 395 rodents screened by PCR for the presence of *Leishmania*, 2 tested positive using genus-specific primers. The positive rodents represented 2 different species. One was a *Peromyscus mexicanus* captured on 2 January 2002 and the other was a *Heteromys desmarestianus* captured on 20 May 2002. Both of the positive rodents were collected at the Selva Negra research site located in the District of Matagalpa and both are the first records of rodents infected with *Leishmania* in Nicaragua.

## Distribution of human cases of typical CL in Nicaragua

During the period from 2003 through 2005, there were a total of 5,765 reported cases of human typical cutaneous leishmaniasis throughout the entire country of Nicaragua. The bulk of the reported cases were distributed in municipalities located in the eastern half of the country (Fig. 14). Of the 152 municipalities, 78 (51.3%) reported no human cases of typical CL and 74 municipalities reported at least 1 case. The greatest number of reported cases (1,828) occurred in the municipality of El Cua-Bocay, located in the north-central region of the country within the district of Jinotega.

Twenty eight (18.3%) municipalities reported ≥ 10 cases for the entire 3 year period (Fig. 15). These 28 municipalities represented 5,622 (97.5%) of the total number of human cases of typical CL reported. Additionally, of the 5,765 total cases, 5,547 (96.2%) were reported from 19 municipalities and 5,243 (90.9%) were from just 11 municipalities (Figs. 16 and 17).

Since population and size of municipalities vary greatly, maps also were produced to illustrate the geographic distribution of human typical CL based on the ratio

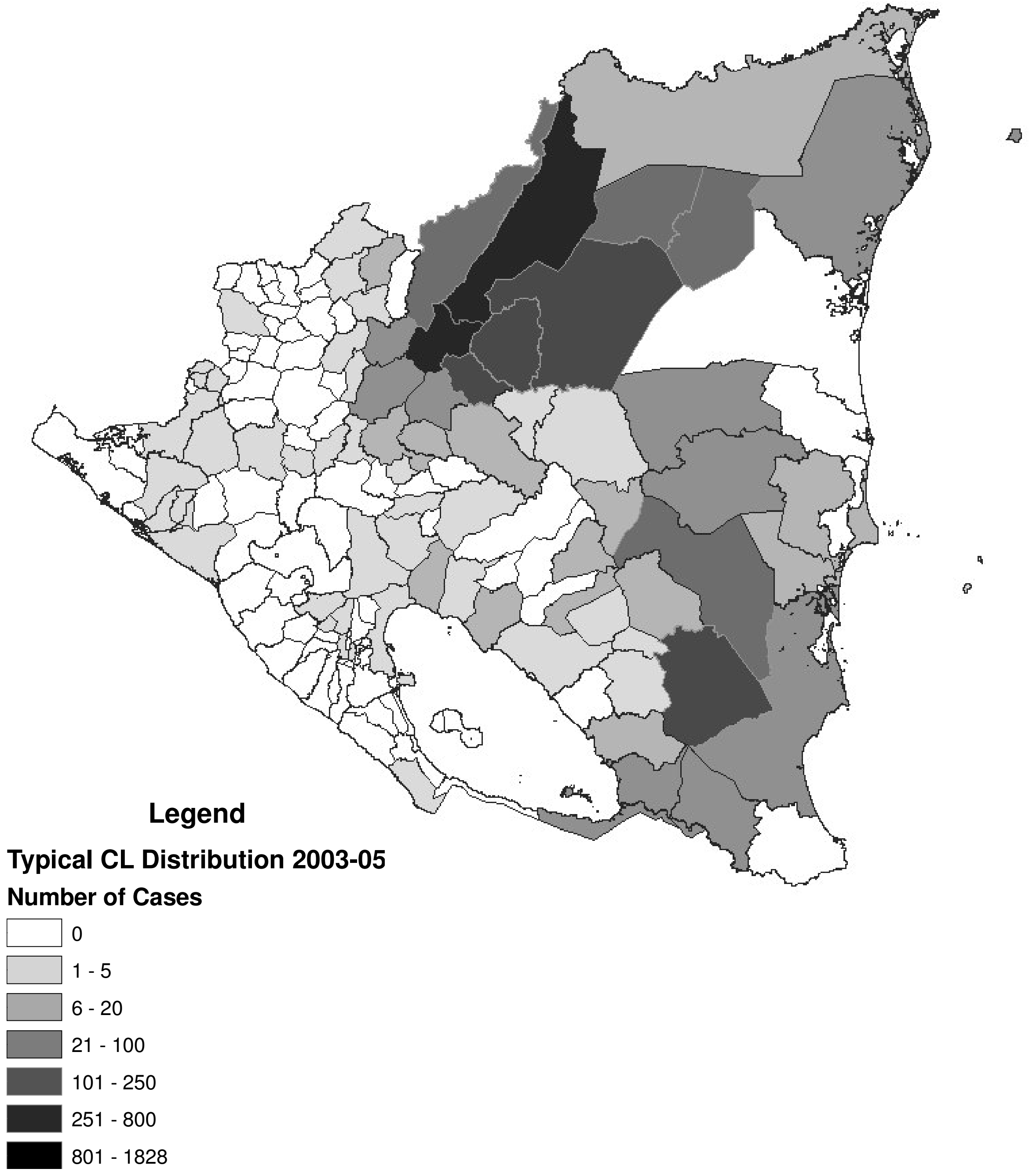


Figure 14. Map of the geographic distribution of all reported cases of human typical CL in Nicaragua, 2003–2005.

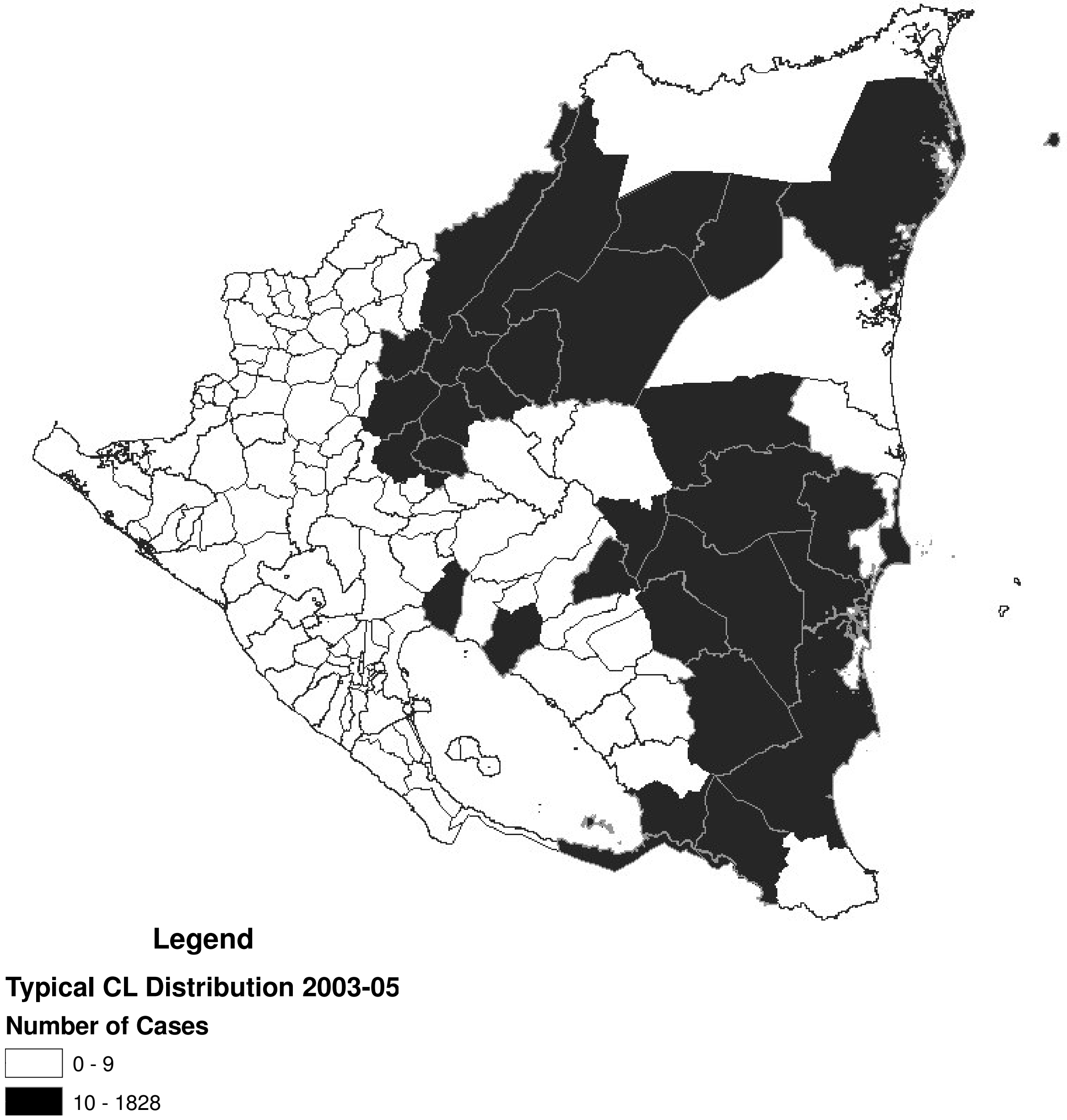


Figure 15. Map of the geographic distribution of 28 municipalities in Nicaragua with at least 10 reported cases of human typical CL representing 97.5% of all reported cases from 2003–2005.

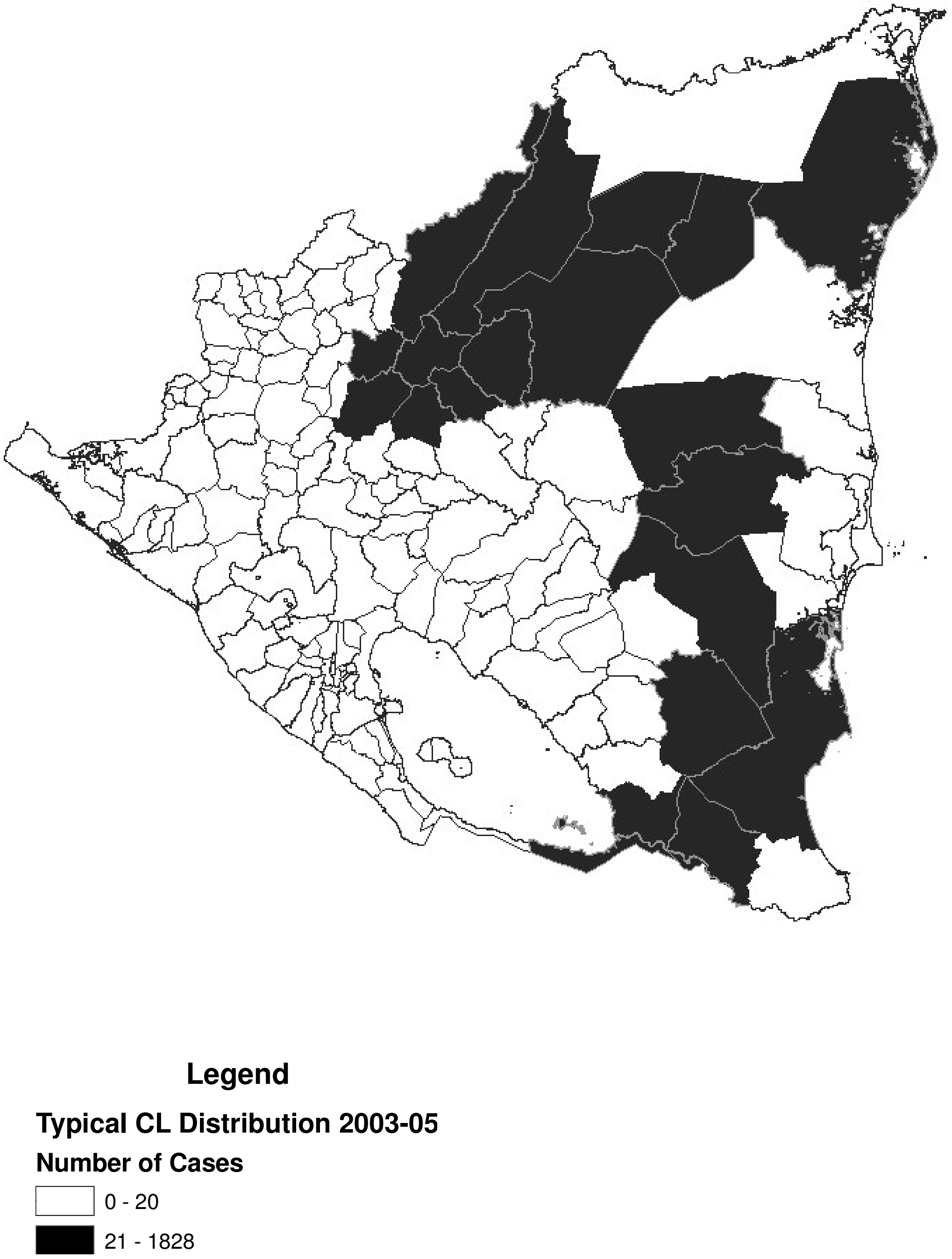


Figure 16. Map of the geographic distribution of 19 municipalities in Nicaragua with at least 20 reported cases of human typical CL representing 96.2% of all reported cases, 2003–2005.

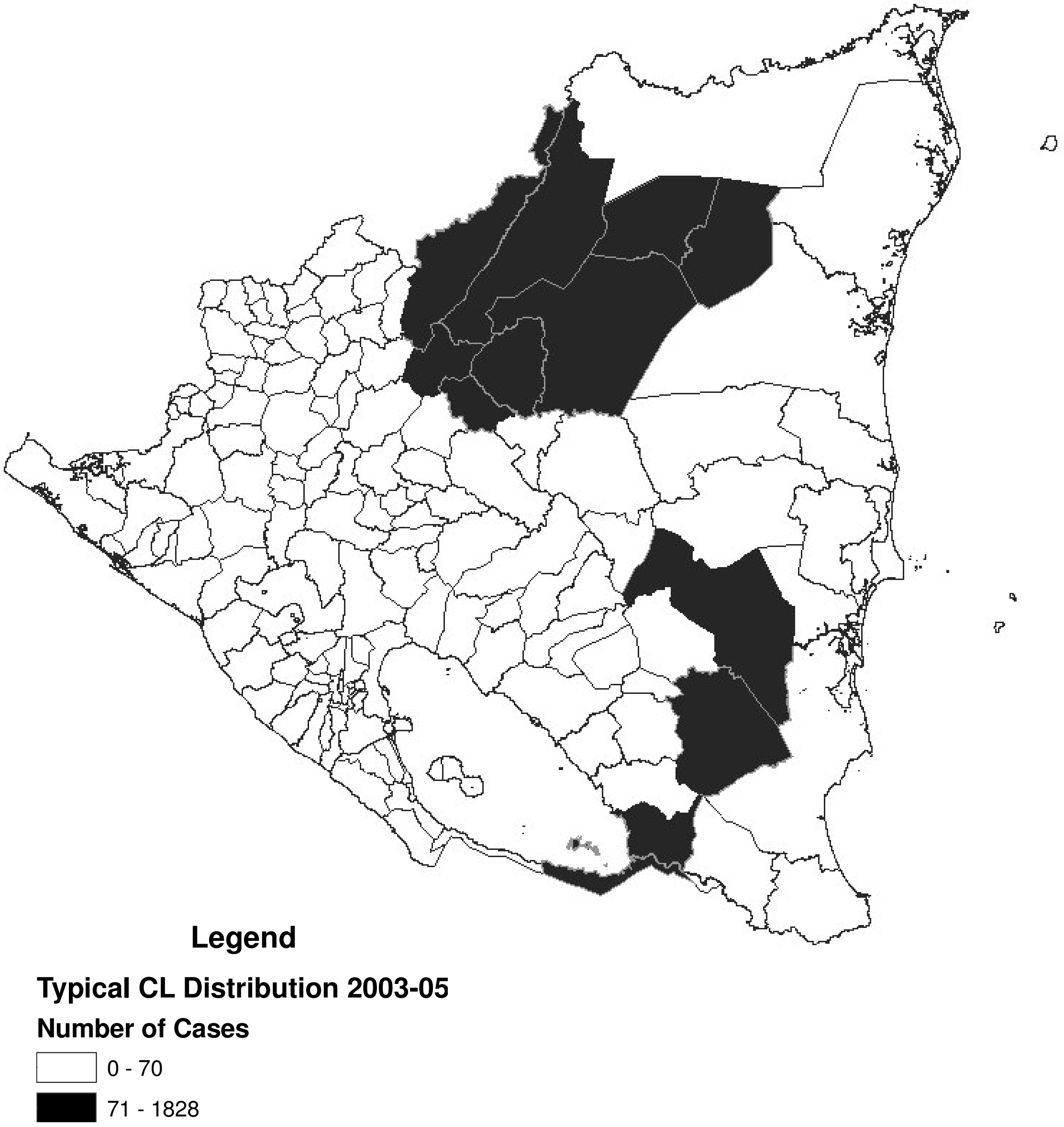


Figure 17. Map of the geographic distribution of 11 municipalities in Nicaragua that contained 90.9% of all reported cases of human typical CL, 2003–2005.

of reported cases to municipality population and the ratio of reported cases to the area of each municipality in square kilometers.

The number of human cases of typical CL reported per municipality population varied from 0.000 to 0.024 (Fig. 18). One hundred thirty six municipalities had a ratio of human cases to total municipality population between 0.000 and 0.001. All of the municipalities located within the Pacific plains region of the country fell within this first category. Six municipalities had a ratio of human cases to total municipality population between 0.001 and 0.003. Four municipalities had a human case per municipality population ratio between 0.003 and 0.005. Three municipalities had a ratio between 0.005 and 0.015. Three municipalities had a ratio of human cases per municipality population between 0.015 and 0.024. All of the municipalities with a ratio higher than 0.001 were located within either the central highlands or Atlantic lowland plains regions. Additionally, the 3 municipalities with the highest number of reported cases per municipality population were located in the northern section of the country within the central highlands region.

The number of human cases of typical CL reported per square kilometer of municipality area varied from 0.00 to 2.20 (Fig. 19). One hundred thirty three of 152 municipalities reported fewer than 0.028 cases of typical CL per km$^2$. Fourteen municipalities reported between 0.028 and 0.127 cases per km$^2$. Three municipalities reported between 0.127 and 0.318 cases per km$^2$. One municipality reported 0.688 cases per km$^2$ and 1 municipality reported 2.280 cases per km$^2$. The 2 municipalities with the highest number of cases per km$^2$ were located within the central highlands region in

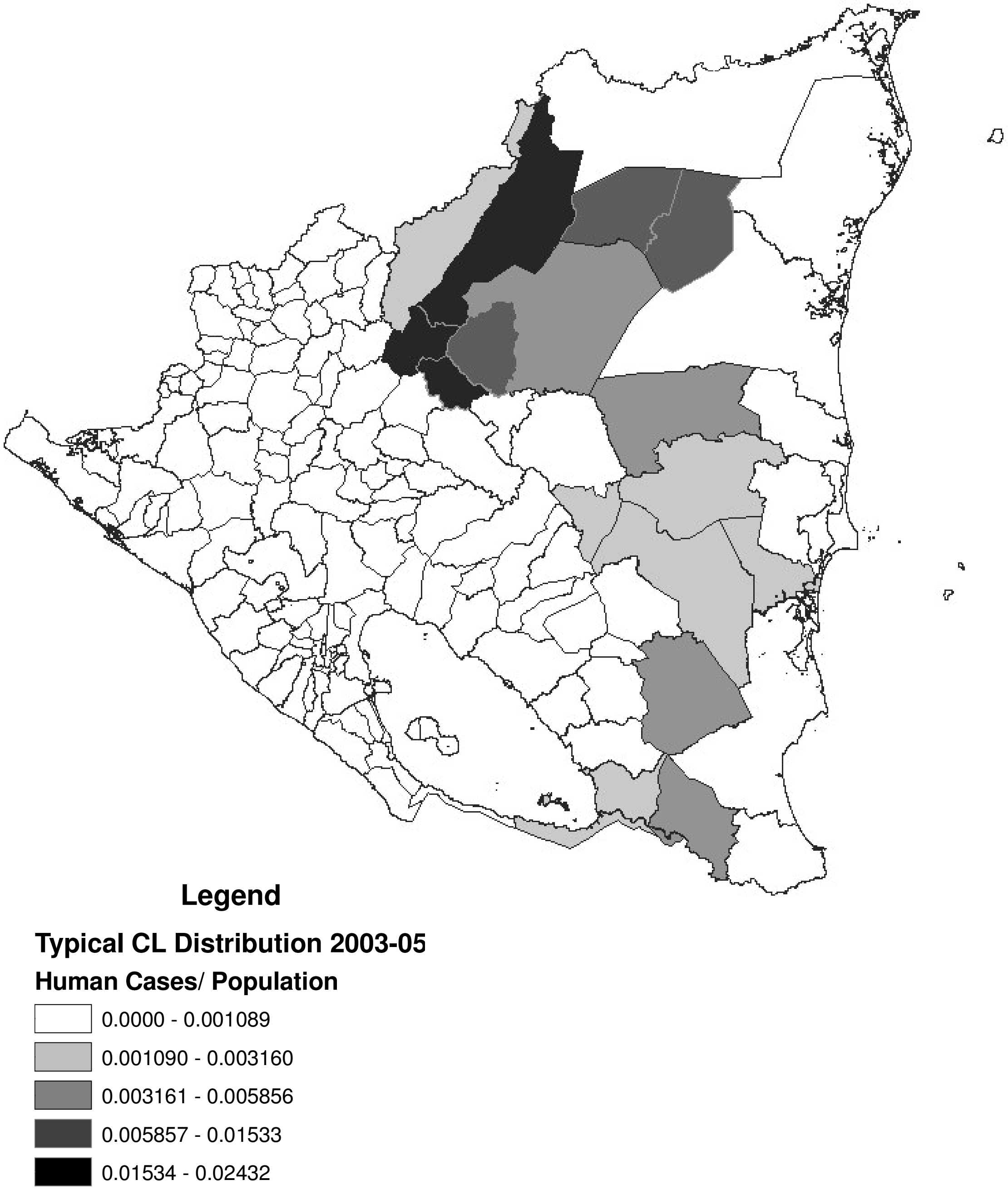


Figure 18. Map of the geographic distribution of human typical CL in Nicaragua based on the ratio of reported cases per municipality population, 2003–2005.

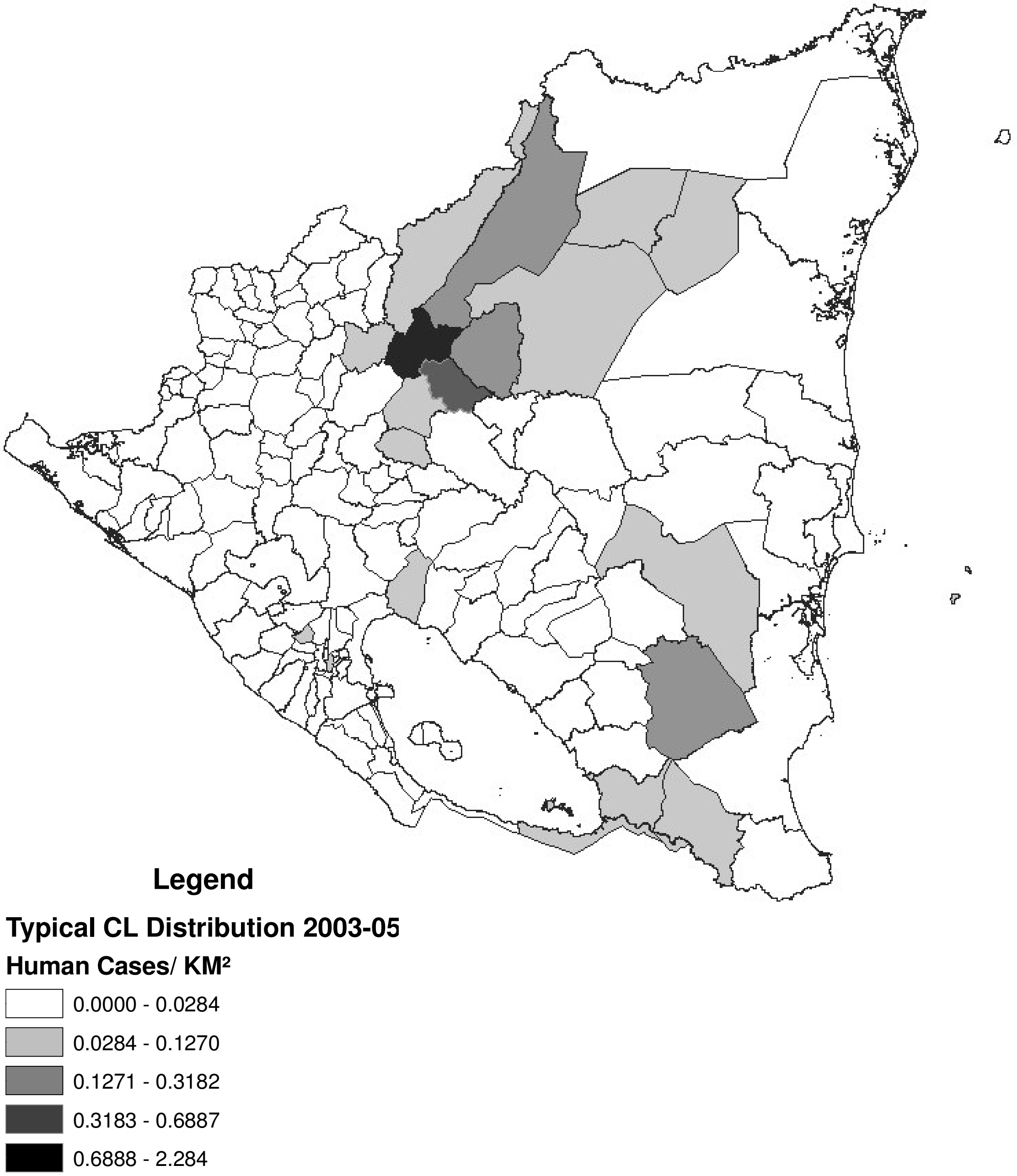


Figure 19. Map of the geographic distribution of typical human CL in Nicaragua based on the ratio of reported cases per municipality area (km$^2$), 2003–2005.

northern Nicaragua.

### GIS analysis of environmental and ecosystem coverage of municipalities

GIS analysis results for number of $km^2$ contained within each of the 151 municipalities analyzed for categories of elevation, mean annual precipitation, mean annual temperature, soil type and land usage/cover are found in Appendices C-F.

### Statistical analysis, logistic regression models

The results of elevation, mean annual precipitation, mean annual temperature, soil types and land use/coverage predictor variables on the dependent variable of human typical CL presence or absence within a municipality initially were evaluated in separate models. The empty model (-2 Log Likelihood (LL) = 209.17, N=151), which contains only the constant in the model, identified those categories of predictor variables that may be significant in the full model (Tables 9–13). The overall statistic tests the null hypothesis ($H_0$) that variable coefficients included in the model are zero versus the alternative hypothesis ($H_A$) that model coefficients are not zero. An overall p-value $\leq$ 0.050 indicated that the $H_0$ can be rejected and that 1 or more variables used in the model will improve the model's predictive power over the empty model that includes only the constant. The overall statistic for each of the full models indicated that they would be better at predicting human typical CL presence or absence within a municipality than the empty model.

Table 9. Elevation variables for Nicaragua not in the equation for the empty model. Variables with a p-value < 0.050 might be significant in the full model.

| Elevation Variables* | Score | df | Sig |
|---|---|---|---|
| 0–100 | 2.965 | 1 | 0.085 |
| 100–200 | 15.029 | 1 | 0.000 |
| 200–400 | 15.641 | 1 | 0.000 |
| 400–600 | 8.404 | 1 | 0.004 |
| 600–800 | 4.686 | 1 | 0.030 |
| 800–1,000 | 1.399 | 1 | 0.237 |
| 1,000–1,200 | 0.608 | 1 | 0.436 |
| 1,200–1,400 | 0.065 | 1 | 0.799 |
| 1,400–2,000 | 0.082 | 1 | 0.774 |
| Overall Statistics | 27.084 | 9 | 0.001 |

*Elevation in meters above mean sea level

Table 10. Precipitation variables for Nicaragua not in the equation for the empty model. Variables with a p-value < 0.050 might be significant in the full model.

| Precipitation Variables* | Score | df | Sig |
|---|---|---|---|
| <800 | 3.965 | 1 | 0.046 |
| 800–900 | 0.912 | 1 | 0.340 |
| 900–1,000 | 0.589 | 1 | 0.443 |
| 1,000–1,200 | 0.064 | 1 | 0.800 |
| 1,200–1,400 | 1.823 | 1 | 0.177 |
| 1,400–1,600 | 2.974 | 1 | 0.085 |
| 1,600–1,800 | 11.436 | 1 | 0.001 |
| 1,800–2,000 | 3.398 | 1 | 0.065 |
| 2,000–2,400 | 10.365 | 1 | 0.001 |
| 2,400–2,800 | 7.401 | 1 | 0.007 |
| 2,800–3,200 | 0.439 | 1 | 0.508 |
| 3,200–4,000 | 3.089 | 1 | 0.079 |
| >4,000 | 0.073 | 1 | 0.787 |
| Overall Statistics | 38.290 | 13 | 0.000 |

*Mean annual precipitation in millimeters

Table 11. Temperature variables for Nicaragua not in the equation for the empty model. Variables with a p-value < 0.050 might be significant in the full model.

| Temperature Variables* | Score | df | Sig |
|---|---|---|---|
| 18.5 | 0.236 | 1 | 0.627 |
| 19.5 | 0.001 | 1 | 0.976 |
| 20.5 | 1.548 | 1 | 0.213 |
| 21.5 | 0.020 | 1 | 0.888 |
| 22.5 | 1.180 | 1 | 0.277 |
| 23.5 | 5.746 | 1 | 0.017 |
| 24.5 | 9.577 | 1 | 0.002 |
| 25.5 | 12.304 | 1 | 0.000 |
| 26.5 | 8.914 | 1 | 0.003 |
| 27.5 | 0.853 | 1 | 0.356 |
| Overall Statistics | 27.281 | 10 | 0.002 |

*Mean annual temperatures in degrees Celsius

Table 12. Soil type variables for Nicaragua not in the equation for the empty model. Variables with a p-value < 0.050 might be significant in the full model.

| Soil type variables | Score | df | Sig |
|---|---|---|---|
| Heavy Clay | 0.705 | 1 | 0.401 |
| Clay | 14.275 | 1 | 0.000 |
| Loamy Sand | 1.087 | 1 | 0.297 |
| Sandy | 0.596 | 1 | 0.440 |
| Loam | 0.320 | 1 | 0.572 |
| Clay Loam | 0.546 | 1 | 0.460 |
| Sandy Loam | 0.080 | 1 | 0.777 |
| Overall Statistics | 18.288 | 7 | 0.011 |

Table 13. Land use/cover variables for Nicaragua not in the equation for the empty model. Variables with a p-value < 0.050 might be significant in the full model.

| Variable | Score | df | Sig |
|---|---|---|---|
| Urban Areas | 1.794 | 1 | 0.180 |
| Cropland | 0.431 | 1 | 0.511 |
| Agricultural 10–25% Natural Vegetation | 16.166 | 1 | 0.000 |
| Agricultural 25–50% Natural Vegetation | 11.882 | 1 | 0.001 |
| Mangroves | 0.168 | 1 | 0.682 |
| Coastal Transition Vegetation | 0.127 | 1 | 0.721 |
| Savannah | 1.525 | 1 | 0.217 |
| Grassland with Deciduous Shrubs | 0.023 | 1 | 0.878 |
| Shrubland | 4.013 | 1 | 0.045 |
| TDBSF&TEMSF | 0.671 | 1 | 0.413 |
| TEPSF | 0.278 | 1 | 0.598 |
| TESF | 0.774 | 1 | 0.379 |
| TEBSF | 6.826 | 1 | 0.009 |
| Overall Statistics | 38.797 | 13 | 0.000 |

TDBSF= Tropical deciduous broadleaf seasonal forest; TEMSF=Tropical evergreen mixed seasonal forest, TEPSF=Tropical evergreen pine seasonal forest; TESF=Tropical evergreen swamp forest; TEBSF=Tropical evergreen broadleaf seasonal forest.

The classification of results of observed response categories and predicted response categories for the empty model including only the constant are shown in Table 14.

Table 14. Percentage of correct model predictions of presence and absence of human typical CL in Nicaragua by the empty model including only the constant.

|  | Predicted Human Typical CL |  |  |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 78 | 0 | 100.0 |
| Present | 73 | 0 | 0.0 |
| Overall | – | – | 51.7 |

**Logistic regression analysis of elevation and human typical CL presence or absence**

Results of the omnibus test of model coefficients indicate the model that includes all elevation variables is significantly better at predicting the presence or absence of human typical CL than the empty model that includes only the constant (-2 LL = 160.38, $\chi^2 = 48.79$, df = 9, P = 0.000). The Hosmer-Lemeshow test ($\chi^2 = 12.509$, df = 8, $P$ = 0.130) indicates that the model adequately fits the data. The $R^2$ values for Cox and Snell and Nagelkerke were 0.276 and 0.368 respectively.

The model's ability to predict membership of municipalities within either of the 2 groups of the outcome variable (presence or absence) is summarized in Table 15. The model was correctly able to classify 65 of 78 (83.3%) municipalities as not having human typical CL and correctly able to classify 41 of 73 (56.2%) municipalities as having presence of human typical CL. Overall, with the inclusion of elevation predictor variables, the model correctly identified 70.2% of municipalities with regard to presence or absence of typical CL. This was an improvement over the 51.7% correctly predicted in the empty model.

Table 15. Percentages of correct model predictions of presence and absence of human typical CL within municipalities in Nicaragua; with only the constant and elevation variables included in the model.

|  | Predicted Human Typical CL |  |  |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 65 | 13 | 83.3 |
| Present | 32 | 41 | 56.2 |
| Overall | – | – | 70.2 |

Statistical analysis results of the full model including all elevation predictor variables are summarized in Table 16. The only elevation predictor variable that was significant at the 95% CI was the range of 100–200 m above MSL. However, the elevation range from 600–800 m above MSL with a p-value of 0.054 is just outside the significant range.

Table 16. Coefficients and goodness of fit of logistic binary model predicting presence and absence of human typical CL in Nicaragua based on all elevation predictor variables.

| Elevation Variable* | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| 0–100 | 0.000 | 0.000 | 0.613 | 1 | 0.433 | 1.000 |
| 100–200 | 0.007 | 0.002 | 8.922 | 1 | 0.003 | 1.007 |
| 200–400 | 0.002 | 0.002 | 1.469 | 1 | 0.226 | 1.002 |
| 400–600 | 0.002 | 0.003 | 0.628 | 1 | 0.432 | 1.002 |
| 600–800 | 0.010 | 0.005 | 3.710 | 1 | 0.054 | 1.010 |
| 800–1,000 | -0.005 | 0.011 | 0.228 | 1 | 0.633 | 0.995 |
| 1,000–1,200 | 0.018 | 0.014 | 1.616 | 1 | 0.204 | 1.019 |
| 1,200–1,400 | -0.026 | 0.027 | 0.916 | 1 | 0.339 | 0.974 |
| 1,400–2,000 | -0.014 | 0.054 | 0.071 | 1 | 0.789 | 0.986 |
| Constant | -1.453 | 0.323 | 20.207 | 1 | 0.000 | 0.234 |

* Elevation ranges in meters above mean sea level

## Logistic regression analysis of precipitation and human typical CL presence or absence

Results of the omnibus test of model coefficients indicate the model that includes all precipitation variables is significantly better at predicting the presence or absence of human typical CL than the empty model that includes only the constant (-2 LL = 150.59 $\chi^2 = 58.58$, df = 13, P = 0.000). The Hosmer-Lemeshow test ($\chi^2 = 8.27$, df = 8, $P$ = 0.407) indicates that the model adequately fits the data. Values for $R^2$ for Cox and Snell and Nagelkerke tests were 0.322 and 0.429 respectively.

The model’s ability to predict membership of municipalities within the 2 groups of the outcome variable (presence or absence) is summarized in Table 17. The model was correctly able to classify 68 of 78 (87.2%) municipalities as not having typical CL and correctly able to classify 49 of 73 (67.1%) municipalities as having presence of typical CL. Overall, with the inclusion of all precipitation predictor variables, the model correctly identified 77.5% of municipalities with regard to presence or absence of human typical CL. This was an improvement over the 51.7% correctly predicted in the empty model and the 70.2% correctly predicted by the model including all elevation predictor variables.

Table 17. Percentages of correct model predictions of presence and absence of human typical CL within municipalities in Nicaragua; with the constant and precipitation variables included in the model.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 68 | 10 | 87.2 |
| Present | 24 | 49 | 67.1 |
| Overall | – | – | 77.5 |

Statistical analysis results of the full model including all precipitation predictor variables are summarized in Table 18. Three mean annual precipitation predictor variables were significant at the 95% CI: 900–1,000 mm, 1,200–1,400 mm, 1,600–1,800 mm and 2,000–2,400 mm.

Table 18. Coefficients and goodness of fit of logistic binary model predicting presence and absence of human typical CL in Nicaragua based on precipitation variables.

| Variable | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| <800 | -0.050 | 0.090 | 0.303 | 1 | 0.582 | 0.951 |
| 800–900 | -0.001 | 0.003 | 0.060 | 1 | 0.806 | 0.999 |
| 900–1,000 | 0.007 | 0.003 | 5.009 | 1 | 0.025 | 1.007 |
| 1,000–1,200 | 0.000 | 0.002 | 0.021 | 1 | 0.885 | 1.000 |
| 1,200–1,400 | 0.006 | 0.002 | 5.674 | 1 | 0.017 | 1.006 |
| 1,400–1,600 | 0.009 | 0.001 | 0.106 | 1 | 0.745 | 1.000 |
| 1,600–1,800 | 0.007 | 0.004 | 4.988 | 1 | 0.026 | 1.009 |
| 1,800–2,000 | -0.001 | 0.004 | 0.103 | 1 | 0.748 | 0.999 |
| 2,000–2,400 | 0.007 | 0.004 | 4.128 | 1 | 0.042 | 1.007 |
| 2,400–2,800 | 0.002 | 0.001 | 1.852 | 1 | 0.174 | 1.002 |
| 2,800–3,200 | 0.000 | 0.000 | 0.130 | 1 | 0.718 | 1.000 |
| 3,200–4,000 | 0.005 | 0.003 | 3.475 | 1 | 0.062 | 1.005 |
| >4,000 | -0.004 | 0.000 | 1.107 | 1 | 0.293 | 0.996 |
| Constant | -1.387 | 0.346 | 16.059 | 1 | 0.000 | 0.250 |

**Logistic regression analysis of temperature and human typical CL presence or absence**

Results of the omnibus test of model coefficients indicate the model that includes all temperature variables is significantly better at predicting the presence or absence of human typical CL than the empty model that includes only the constant (-2 LL = 169.81, $\chi^2 = 39.35$, df = 10, $P = 0.000$). The Hosmer-Lemeshow test ($\chi^2 = 9.76$, df = 8, P = 0.283) indicated that the model adequately fits the data. Values for $R^2$ for Cox and Snell and Nagelkerke tests were 0.299 and 0.306, respectively.

The model's ability to predict membership of municipalities within the 2 groups of the outcome variable (presence or absence) is summarized in Table 19. The model was correctly able to classify 66 of 78 (84.6%) municipalities as not having typical CL and correctly able to classify 46 of 73 (63.0%) municipalities as having presence of typical CL. Overall, with the inclusion of all mean annual temperature predictor variables, the model correctly identified 74.2% of municipalities with regard to presence or absence of human typical CL.

Table 19. Percentages of correct model predictions of presence and absence of human typical CL within municipalities in Nicaragua, with the constant and temperature variables included in the model.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 66 | 12 | 84.6 |
| Present | 27 | 46 | 63. 0 |
| Overall | – | – | 74.2 |

Statistical analysis results of the full model including all temperature predictor variables are summarized in Table 20. Only the mean annual temperature variable of 26.5° C was significant at the 95% CI.

Table 20. Coefficients and goodness of fit of logistic binary model predicting presence and absence of human typical CL in Nicaragua based on mean annual temperature variables in degrees Celsius.

| Variable | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| Temperature 18.5 | -2.562 | 2.252 | 1.294 | 1 | 0.255 | 0.077 |
| Temperature 19.5 | 0.005 | 0.280 | 0.000 | 1 | 0.985 | 1.005 |
| Temperature 20.5 | 0.089 | 0.080 | 1.221 | 1 | 0.269 | 1.093 |
| Temperature 21.5 | -0.058 | 0.033 | 3.129 | 1 | 0.077 | 0.944 |
| Temperature 22.5 | 0.026 | 0.016 | 2.476 | 1 | 0.116 | 1.026 |
| Temperature 23.5 | 0.002 | 0.006 | 0.125 | 1 | 0.723 | 1.002 |
| Temperature 24.5 | 0.001 | 0.005 | 0.081 | 1 | 0.775 | 1.001 |
| Temperature 25.5 | 0.003 | 0.002 | 3.348 | 1 | 0.067 | 1.003 |
| Temperature 26.5 | 0.001 | 0.000 | 6.174 | 1 | 0.013 | 1.001 |
| Temperature 27.5 | -0.001 | 0.002 | 0.262 | 1 | 0.608 | 0.999 |
| Constant | -0.984 | 0.287 | 11.745 | 1 | 0.001 | 0.374 |

### Logistic regression analysis of soil types and human typical CL presence or absence

Results of the omnibus test of model coefficients indicate the model that includes all soil type variables is significantly better at predicting the presence or absence of human typical CL than the empty model that includes only the constant (-2 LL = 184.15, $\chi^2 = 25.016$, df = 7, P = 0.001). The Hosmer-Lemeshow test ($\chi^2 = 13.02$, df = 8, $P$ = 0.111) indicated that the model adequately fits the data. Values for $R^2$ for Cox and Snell and Nagelkerke tests were 0.153 and 0.204, respectively.

The model's ability to predict membership of municipalities within the 2 groups of the outcome variable (presence or absence) is summarized in Table 21. The model was correctly able to classify 65 of 78 (83.3%) municipalities as not having typical CL and correctly able to classify only 34 of 73 (46.6%) municipalities as having presence of typical CL. Overall, with the inclusion of all soil type predictor variables, the model correctly identified just 65.6% of municipalities with regard to presence or absence of human typical CL.

Table 21. Percentages of correct model predictions of presence and absence of human typical CL within municipalities in Nicaragua; with the constant and soil type variables included in the model.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 65 | 13 | 83.3 |
| Present | 39 | 34 | 46.6 |
| Overall | – | – | 65.6 |

Statistical analysis results of the full model including all soil type predictor variables are summarized in Table 22. Only the predictor variable of clay soils was significant at the 95% CI.

Table 22. Coefficients and goodness of fit of logistic binary model predicting presence and absence of human typical CL in Nicaragua based on soil type variables used in the model.

| Variable | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| Heavy Clay | 0.002 | 0.002 | 1.496 | 1 | 0.221 | 1.002 |
| Clay | 0.001 | 0.000 | 10.352 | 1 | 0.001 | 1.001 |
| Loamy Sand | -0.011 | 0.012 | 0.830 | 1 | 0.362 | 0.989 |
| Sandy | 0.142 | 0.237 | 0.358 | 1 | 0.549 | 1.152 |
| Loam | 0.001 | 0.004 | 0.110 | 1 | 0.740 | 1.001 |
| Clay Loam | 0.001 | 0.001 | 0.174 | 1 | 0.676 | 1.001 |
| Sandy Loam | 0.003 | 0.005 | 0.411 | 1 | 0.521 | 1.003 |
| Constant | -0.751 | 0.298 | 6.336 | 1 | 0.012 | 0.472 |

### Logistic regression analysis of land cover/use types and human typical CL presence or absence

Results of the omnibus test of model coefficients indicate the model that includes all land use/coverage predictor variables is significantly better at predicting the presence or absence of human typical CL than the empty model that includes only the constant (-2 LL = 155.27, $\chi^2 = 53.891$, df = 13, *P* = 0.000). The results of the Hosmer-Lemeshow test ($\chi^2 = 6.88$, df = 8, *P* = 0.549) indicated that the model adequately fits the data. Values for $R^2$ for Cox and Snell and Nagelkerke tests were 0.300 and 0.400 respectively.

The model's ability to predict membership of municipalities within the 2 groups of the outcome variable (presence or absence) is summarized in Table 23. The model was correctly able to classify 67 of 78 (85.9%) municipalities as not having typical CL and correctly able to classify only 49 of 73 (67.1%) municipalities as having presence of typical CL. Overall, with the inclusion of all land use/coverage predictor variables, the model correctly identified 76.8% of municipalities with regard to presence or absence of human typical CL.

Table 23. Percentages of correct model predictions of presence and absence of human typical CL within municipalities in Nicaragua; with the constant and land use/coverage variables included in the model.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | Absence | Presence | Percentage correct (%) |
| Absence | 67 | 11 | 85.9 |
| Presence | 24 | 49 | 67.1 |
| Overall | – | – | 76.8 |

Statistical analysis results of the full model including all land use/coverage predictor variables are summarized in Table 24. Three predictor variables of land use/coverage were significant at the 95% CI: Agricultural systems with 10–25% natural vegetation, and tropical evergreen broadleaf seasonal forests showed a significant positive correlation while tropical evergreen seasonal pine forests showed a significant negative correlation.

The sensitivity and specificity comparison analysis of all predictive models for the presence or absence of human typical CL are summarized in Table 25.

Table 24. Coefficients and goodness of fit of logistic binary model predicting presence and absence of human typical CL in Nicaragua based on land use/coverage predictor variables.

| Variable | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| Urban Areas | 0.067 | 0.078 | 0.735 | 1 | 0.391 | 1.069 |
| Cropland | 0.005 | 0.003 | 2.568 | 1 | 0.109 | 1.005 |
| Agricultural 10–25% Natural Vegetation | 0.004 | 0.001 | 7.724 | 1 | 0.005 | 1.004 |
| Agricultural 25–50% Natural Vegetation | 0.002 | 0.001 | 3.060 | 1 | 0.080 | 1.002 |
| Mangroves | -0.009 | 0.011 | 0.658 | 1 | 0.417 | 0.991 |
| Coastal Transition Vegetation | -0.005 | 0.006 | 0.659 | 1 | 0.417 | 0.995 |
| Savannah | 0.008 | 0.006 | 1.829 | 1 | 0.176 | 1.008 |
| Grassland with Deciduous Shrubs | -0.005 | 0.005 | 0.961 | 1 | 0.327 | 0.995 |
| Shrubland | 0.021 | 0.011 | 3.497 | 1 | 0.061 | 1.021 |
| Tropical Deciduous Broadleaf Forest | 0.001 | 0.003 | 0.199 | 1 | 0.655 | 1.001 |
| Tropical Evergreen Pine Forest | -0.003 | 0.001 | 3.888 | 1 | 0.049 | 0.997 |
| Tropical Evergreen Swamp Forest | -0.001 | 0.002 | 0.186 | 1 | 0.666 | 0.999 |
| Tropical Evergreen Broadleaf Forest | 0.002 | 0.001 | 4.005 | 1 | 0.045 | 1.002 |
| Constant | -1.404 | 0.322 | 19.049 | 1 | 0.000 | 0.246 |

Table 25. Sensitivity and specificity of all models for presence or absence of human typical CL in Nicaragua.

| Predictor Variable Used | Sensitivity (%) | Specificity (%) | Overall (%) |
|---|---|---|---|
| Elevation | 56.2 | 83.3 | 70.2 |
| Precipitation | 67.1 | 87.2 | 77.5 |
| Temperature | 63.0 | 84.6 | 74.2 |
| Soil Type | 46.6 | 83.3 | 65.6 |
| Land Use/Cover | 67.1 | 85.9 | 76.8 |

### Logistic regression analysis of combinations of predictive environmental variables

Logistic regression analysis also was performed pair wise among all possible combinations of the predictor variables: elevation, precipitation, temperature, soil types and land use/cover. The models containing the combined variables of elevation-temperature, elevation-soil type, elevation-land cover/use, temperature-soil type were less efficient at identifying the presence or absence of typical cutaneous leishmaniasis within a municipality than the model containing precipitation data alone. The 2 models containing the combined variables of precipitation-land cover and temperature-land cover were rejected due to Hosmer-Lemeshow test statistics of $P=0.000$ and $P=0.024$, respectively that indicated that the models do not adequately fit the data. The remaining 4 pair wise combinations of environmental predictor variables, elevation-precipitation, precipitation-temperature, precipitation-soil type and soil type-land cover/use did slightly improve upon the 77.5% percent of municipalities identified as having presence or absence of typical CL in the model containing mean annual precipitation variables alone. However, these four models were rejected based on evaluation of Peterson correlation coefficients which indicated severe co-linearity between the predictor variables.

### Logistic regression analysis of the environmental predictor variables and occurrence of < 10 or ≥ 10 cases of human typical CL

Overall 97.5% of all reported cases of human typical CL occurred within 28 municipalities that reported 10 or more cases from 2003–2005. Logistic regression results of the predictor variables of elevation, mean annual precipitation, mean annual temperature, soil types and land use/coverage on the dichotomous dependent variable of <10 reported cases and ≥10 reported cases were evaluated.

The empty model (N = 151, -2 Log Likelihood (LL) = 144.82), containing only the constant, identified those predictor variables that may be significant in the full model. Again, the null hypothesis ($H_O$) that predictor variable coefficients used in the model are zero versus the alternative hypothesis ($H_A$) that 1 or more predictor variables used in the model will improve the model's predictive power over the constant coefficient alone is tested. The $H_O$ is rejected at an overall statistic p-value of ≤ 0.050. The overall test statistics for variables not in the empty model were: elevation variable coefficents $P = 0.000$, mean annual precipitation coefficients $P = 0.000$, mean annual temperature coefficients $P = 0.000$, soil type coefficients $P = 0.000$ and land use/cover coefficients $P = 0.000$. This indicated that all models using environments predictor variables had 1 or more predictor variables that significantly improved the model's predictive power over the empty model including only the constant. However, only the model including land use/cover predictor variables displayed an ability to correctly predict the occurrence of ≥10 reported cases of typical CL within a municipality greater than 50% of the time. Only the results of the full model including land use/cover classes of predictor variables are presented. The empty model classification of municipalities as having < 10 or ≥ 10 cases typical human CL is summarized in Table 26.

Table 26. Percentage of correct predictions of < 10 or ≥ 10 cases of human typical CL in Nicaragua by the empty model including only the constant.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | < 10 | ≥ 10 | Percentage correct (%) |
| < 10 | 123 | 0 | 100.0 |
| ≥ 10 | 28 | 0 | 0.0 |
| Overall | – | – | 81.5 |

Results of the omnibus test of model coefficients indicate the full model that includes all land use/cover variables is significantly better at predicting the occurrence of < 10 or ≥ 10 cases of human typical CL than the empty model that includes only the constant (-2 LL = 57.04, $\chi^2 = 87.78$, df = 13, $P = 0.000$). The Hosmer-Lemeshow test ($\chi^2 = 3.13$, df = 8, $P = 0.926$) indicates that the model adequately fits the data. The $R^2$ values for Cox and Snell and Nagelkerke were 0.441 and 0.715 respectively.

The model's ability to predict membership of municipalities within the 2 groups of the outcome variable (< 10 or ≥ 10 cases of typical CL) is summarized in Table 27. The model was correctly able to predict 119 of 123 municipalities as having less than 10 cases of typical CL and correctly able to identify 20 of 28 municipalities as having 10 or more reported cases of typical CL. The overall percentage correct predicted by the model of 92.1% is an improvement over the 81.5% correctly predicted by the empty model. Four land use/cover predictor variable classes were significant at the 95% CI: cropland, agricultural use with 10–25% natural vegetation, agricultural use with 25–50% natural vegetation, and tropical evergreen broadleaf forest (Table 28).

Table 27. Percentages of correct model predictions of < 10 or ≥ 10 cases of typical CL within municipalities in Nicaragua; with the constant and land use/coverage variables included in the model.

| | Predicted Human Typical CL | | |
|---|---|---|---|
| Observed | < 10 Cases | ≥ 10 Cases | Percentage correct (%) |
| < 10 Cases | 119 | 4 | 96.7 |
| ≥ 10 Cases | 8 | 20 | 71.4 |
| Overall | - | - | 92.1 |

Table 28. Coefficients and goodness of fit of logistic model predicting < 10 or ≥ 10 cases of typical CL in Nicaragua based on land cover/use predictor variables.

| Variable | B | SE | Wald | df | Sig | Exp(B) |
|---|---|---|---|---|---|---|
| Urban Areas | -0.188 | 0.360 | 0.272 | 1 | 0.602 | 0.829 |
| Cropland | 0.019 | 0.006 | 9.155 | 1 | 0.002 | 1.019 |
| Agricultural 10–25% Natural Vegetation | 0.002 | 0.001 | 3.940 | 1 | 0.047 | 1.002 |
| Agricultural 25−50% Natural Vegetation | 0.006 | 0.002 | 10.482 | 1 | 0.001 | 1.006 |
| Mangroves | -0.340 | 0.325 | 1.095 | 1 | 0.295 | 0.377 |
| Coastal Transition Vegetation | -0.257 | 0.146 | 1.149 | 1 | 0.284 | 0.642 |
| Savannah | 0.339 | 0.351 | 0.930 | 1 | 0.335 | 1.403 |
| Grassland with Deciduous Shrubs | -0.044 | 0.027 | 2.627 | 1 | 0.102 | 0.957 |
| Shrubland | 0.008 | 0.006 | 1.631 | 1 | 0.202 | 1.008 |
| Tropical Deciduous Broadleaf Forest | 0.002 | 0.006 | 0.158 | 1 | 0.691 | 1.002 |
| Tropical Evergreen Pine Forest | -0.114 | 0.131 | 0.758 | 1 | 0.758 | 0.892 |
| Tropical Evergreen Swamp Forest | 0.002 | 0.006 | .0082 | 1 | 0.082 | 1.002 |
| Tropical Evergreen Broadleaf Forest | 0.003 | 0.001 | 8.216 | 1 | 0.004 | 1.003 |
| Constant | -4.382 | 0.861 | 25.915 | 1 | 0.000 | 0.012 |

### Forest degradation from 1983 to 2000 and the distribution of human cases of typical CL

Most of the 28 municipalities that included 97.5% of the reported cases of human typical CL during the period 2003–2005 also showed a large loss of forest cover between the years 1983 and 2000. Maps of these 28 municipalities, when overlaid with maps of the extent of forest degradation, clearly demonstrate that the vast majority of reported cases of human typical CL were from municipalities where significant forest degradation has occurred over the past 2 decades (Fig. 20).

### Temporal distribution of reported cases of human typical CL for the period 2003–2005

Analysis of the temporal distribution of typical human CL for the period from 2003 to 2005 showed that there was an increase in the average number of the weekly reported cases by municipality from approximately late May through early July. The peak average occurred in the twenty-third week of the year with 134 reported cases. This period roughly corresponds with the beginning of the rainy season throughout Nicaragua. The lowest average number of reported cases for the data period available occurred from approximately October through January and falls within the yearly dry season (Fig. 21).

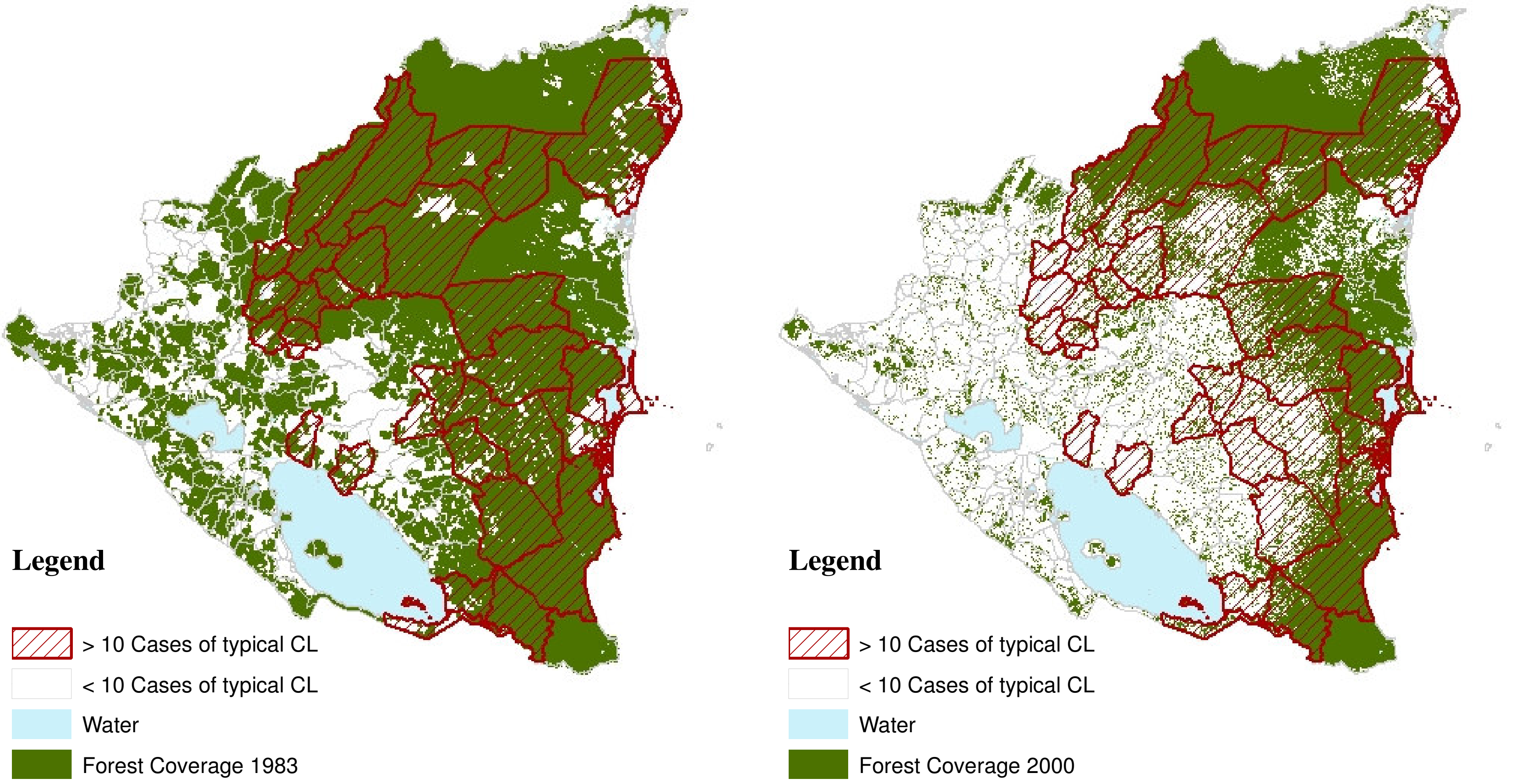


Figure 20. Twenty-eight municipalities representing 97.5% of reported cases of typical human CL for the years 2003–2005; overlaid onto forest coverage maps from the years 1983 and 2000.

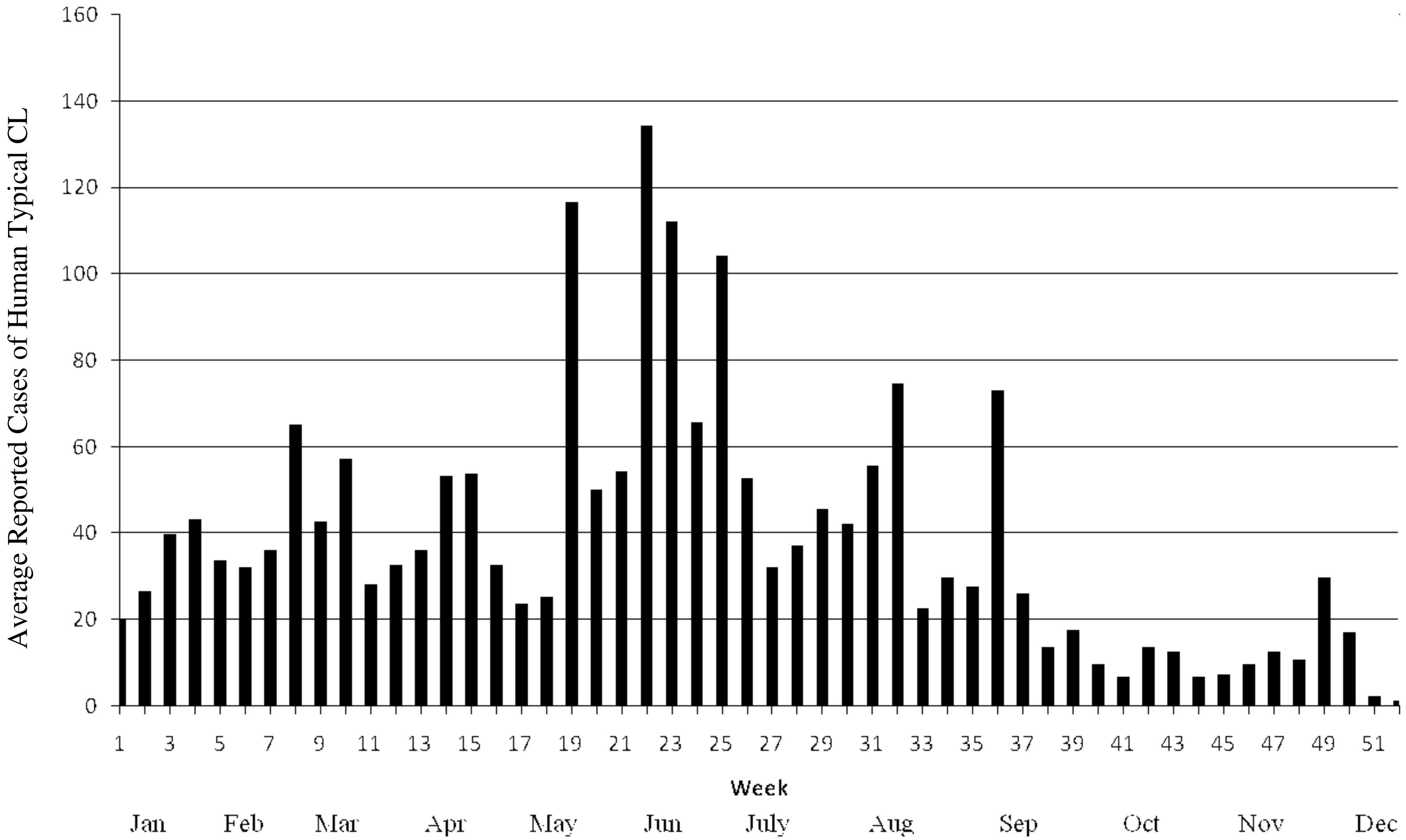


Figure 21. Chart of the average number of reported cases of human typical CL in Nicaragua by week for the 3 year period from 2003–2005.

# DISCUSSION

## Overview

Human leishmaniasis in Nicaragua occurs in 3 primary clinical forms: visceral, mucocutaneous, and cutaneous leishmaniasis. Cutaneous leishmaniasis in Nicaragua is manifested by 2 distinct disease syndromes, ulcerative typical CL and non-ulcerative atypical CL. The vast majority of human cases within the more arid regions of the Pacific plains are atypical cutaneous leishmaniasis caused by *L.* (*L.*) *chagasi* (Belli et al., 1999). In the wetter, rural regions of the central highlands and the Atlantic lowland plains, the predominant form of the disease is typical cutaneous leishmaniasis and it is most likely a result of infections with *L.* (*L.*) *panamensis* and to a lesser extent *L.* (*V.*) *braziliensis* (Belli et al. 1994).

Typical CL, as well as other clinical manifestations associated with human infection with *Leishmania*, tends to display a focal geographic distribution. Within these foci, conditions must exist that allow for the coming together of principle players in the transmission cycle. *Leishmania* species are obligate heteroxenous parasites; requiring that suitable reservoirs and vectors coexist within an ecological habitat. Humans typically enter the equation as incidental hosts (Ashford, 1996).

Sand flies are notorious poor fliers, so human infections result when individuals live, work or travel within areas where natural transmission cycles exist. Little is known of the natural reservoir vector systems responsible for the maintenance and transmission of *Leishmania* species that cause human typical cutaneous leishmaniasis in Nicaragua. No proven reservoirs or sand fly vectors have been described. In fact, little is known about the distribution of rodent species within the various geographic areas of the country. This study attempted to explore the potential reservoir hosts and sand fly vectors within natural systems at 13 study localities in diverse environmental regions of Nicaragua, as well as the environmental factors that correlate with the distribution of reported human cases of typical CL.

Additionally, since the distribution of *Leishmania* species and the various clinical diseases that they cause clearly occur in different and distinct geographical regions of Nicaragua, this study attempted to identify various environmental factors that correlate with areas where human typical CL is predominant.

### Rodent collections within the central highlands region, Selva Negra

This study identified 2 rodents that tested positive by PCR for infection with *Leishmania*. These are the first records of *Leishmania* identified in rodents captured in Nicaragua. The positive rodents included 1 *Peromyscus mexicanus* captured in January 2002 and 1 *Heteromys desmarestianus* captured in May 2002; both positive specimens were collected at the Selva Negra study site. *Heteromys desmarestianus* previously has been implicated as a potential reservoir host of *Leishmania* (*L.*) *mexicana* in Belize where it is considered to be a very common species (Lainson and Strangways-Dixon, 1964; Disney, 1964; Zeledon et al., 1977). *Heteromys desmarestianus* also has been shown to be a host of *Leishmania* (V.) *panamensis* in Costa Rica (Zeledon et al., 1977). A related species of *Peromyscus mexicanus*, *P. yucatanicus,* has been found to be a host and potential reservoir of *Leishmania* (*L.*) *mexicana* in Mexico (Chable-Santos et al., 1995; Canto Lara et al., 1999).

Although the percentage (25%) of infected *H. desmarestianus* individuals captured during this study at Selva Negra fulfilled the WHO criteria of infected population proportion for establishing a species as reservoir host, the total number of *H. desmarestianus* collected was quite small. Of the 193 total rodents representing 10 species which were captured at Selva Negra, just 4 *H. desmarestianus* were captured. Assuming that *H. desmarestianus* has equal affinity for entering the traps as other captured species, it is unlikely that *H. desmarestianus* is sufficiently abundant at the Selva Negra locality to serve as the primary reservoir for *Leishmania*. Also of note, the Selva Negra site was the only locality in which *H. desmarestianus* were captured. This would indicate that *H. desmarestianus* is insufficiently dispersed and abundant to serve as a widespread host of *Leishmania* in Nicaragua.

*Peromyscus mexicanus* appear to be abundantly common within the Selva Negra study site, and were by far the most frequently captured. Of 193 rodents collected, 163 (84.5%) were identified as *P. mexicanus*. This satisfies an additional criterian established by the World Health Organization for incrimination of a reservoirs host in that this species is sufficiently abundant to serve as a common source of blood meals for sand flies (WHO, 1990). However, the proportion of infected individuals captured was low 1/162 (0.6%). The low rate of infection among captured individuals may be indicative of a low infection rate within the entire population, which would suggest that *P. mexicanus* is unlikely to be a significant reservoir of *Leishmania*. Alternatively, given that infections of reservoir hosts with *Leishmania* species tend to be highly focal in nature and that *Leishmania* infection was found to be present within the population, it is quite possible that *P. mexicanus* does contribute some role as a reservoir host and the primary focus of infections is, as of yet, undiscovered. *Peromyscus mexicanus* also is widespread throughout much of Nicaragua; this species was captured in 8 of the 13 study sites.

Although *P. mexicanus* and *H. desmarestianus* were the only species identified as having *Leishmania* infections at the Selva Negra study site, 3 other captured species or genera have been shown to be suitable hosts of *Leishmania* species that cause typical human cutaneous leishmaniasis elsewhere. These include *Nyctomys sumichrasti*, *Oryzomys* sp., and *Ototylomys phyllotis. Nyctomys sumichrasti* has been demonstrated to be a host of *Leishmania* (*L.) mexicana* in Mexico and Belize (Disney, 1964; Lainson and Strangways-Dixon, 1964; Chable-Santos et al., 1995). A number of species within the genus *Oryzomys* have been found to be hosts of parasites within the *Leishmania mexicana* Complex at localities in Mexico, Central and South America (Herrer et al., 1973; Lainson and Shaw, 1992; Chable-Santos et al., 1995; Kerr et al., 2006; Rotureau, 2006). Additionally, *Leishmania* (*V.*) *braziliensis* has been identified or isolated from at least 3 species of *Oryzomys* in Brazil (Lainson and Shaw, 1969; Forattini et al., 1972; Forattini et al., 1973; Oliveira et al., 2005). *Ototylomys phyllotis* is a host of *Leishmania* (*L.*) *mexicana* in Belize, Guatemala, and Mexico (Chable-Santos et al., 1995; Lainson and Strangways-Dixon, 1964; Disney, 1968; Zeledon, 1985).

The relatively low numbers of individuals within these 3 genera that were captured at the Selva Negra study site are perhaps an indicator that they may not be a reservoir host of *Leishmania* here. However, all have been incriminated as suitable hosts for parasites within the *Leishmania* (*L.*) *mexicana* Complex at other localities and cannot be ruled out as playing at least some role in the host-vector transmission cycle. Although the only rodents that were identified as being infected with *Leishmania* parasites during this study were captured at the Selva Negra research site, useful information about the occurrence and distribution of rodent species that may serve as potential hosts and reservoirs was obtained.

One additional species, *Rattus rattus*, was captured at the Selva Negra research site and it has been shown to be a potential host of *Leishmania* in South America. Parasites within the *L.* (*V.*) *donovani* Complex were identified in 1 *R. rattus* in a focus of visceral leishmaniasis in Venezuela (Zulueta et al., 1999). *Leishmania* (*V.*) *braziliensis* and *L.* (*L.*) *mexicana* also have been identified in *Rattus rattus* in Brazil, Columbia, and Venezuela (Alexander et al., 1998; De Lima et al., 2002; Brandao-Filho et al., 2003; Oliveira et al., 2005). Although it is possible that *Rattus rattus* serve some role in the transmission cycle involving humans, the only specimens captured in Nicaragua were from within residences. No specimens were captured in the wild and it is doubtful that this species plays a major role in the natural sylvatic transmission cycle in Nicaragua.

### Rodent collections within the central highlands region; El Cua and El Tigre

Although the only positive rodents captured during this study were from the Selva Negra site, rodent collections at other sites identified species that were present and perhaps provide insight into species that are most likely to serve as natural reservoirs of *Leishmania*. A number of these species have been identified as suitable hosts of *Leishmania* elsewhere. The 3 species captured at El Tigre, *Oryzomys alfaroi*, *Peromyscus mexicanus*, and *Sigmodon hirsutus* also were captured at El Cua. Three additional species were collected at El Cua; *Melanomys caliginosus*, *Mus musculus*, and *Oryzomys cousei*. *Oryzomys* and *Peromyscus* also were captured at Selva Negra and have been implicated as reservoirs of *Leishmania* as mentioned previously. *Melanomys caliginosus* were captured at El Cua and it has been found to be a potential host of *L.* (*V.*) *braziliensis* in Columbia (Alexander et al., 1998). *Sigmodon hirsutus* were collected from both localities and this genus has been shown to be a suitable host for *L.* (*L.*) *mexicana* in Belize and Mexico (Disney, 1968; Chable-Santos et al., 1995). De Lima et al. (2002) identified *L.* (*V.*) *braziliensis* within *Sigmodon* in Venezuela.

### Rodent collections within the Atlantic lowland plains (north); El Balsamo and Rosa Grande

The 4 species of captured rodents at the Rosa Grande site, *Melanomys caliginosus*, *Ototylomys phyllotis*, *Peromyscus mexicanus* and *Proechimys semispinosus*, also were captured the El Balsamo site. The El Balsamo site also produced *Nyctomys sumichrasti*, *Oligoryzomys fulvescens*, *Scotinomys tequina*, and *Sigmodon hirsutus*. *Leishmania* infections have been reported previously in all but *Scotinomys tequina*. In addition to those species previously discussed, *Oligoryzomys* spp. and *Proechimys* spp. also have been identified has suitable hosts of *Leishmania*. *Oligoryzomys* spp. was reported to be a putative reservoir of *L.* (*L.*) *amazonensis* in Bolivia (Telleria et al., 1999). *Proechimys* spp. has been implicated as a host in a number of studies in Brazil, Columbia, French Guiana, and Panama (Lainson and Shaw, 1968; Herrer et al., 1973, Dedet et al., 1989; Travi et al., 1998).

*Melanomys caliginosus* was the most commonly captured species at both the El Balsamo and Rosa Grande study sites and it has been implicated as a possible host of *L.* (*V.*) *braziliensis* in Columbia (Alexander et al., 1998). This species represented 12 of 21 individuals captured at Rosa Grande and 15 of 33 individuals captured at El Balsamo. Its relative abundance at both areas makes it a good candidate for a suitable reservoir.

### Rodent collections within the Atlantic lowland plains (south); El Paraisito, La Esperanzito 2, and La Fonseca

Five species of rodents were captured at the El Paraisito study site. These included *Oligoryzomys fulvescens*, *Oryzomys cousei*, *Proechimys semispinosus*, *Sigmodon hirsutus*, and *Mus musculus*. As previously mentioned, all of these have been implicated as hosts of *Leishmania*, with the exception of *Mus musculus*. *Proechimys semispinosus* was by far the most commonly-captured species at these localities. This species represented 35 of 52 rodents captured at El Paraisito. Two rodents were captured at La Esperanzita 2 and 5 at La Fonseca; all were *Proechimys semispinosus*. This species appears to be widespread and abundant in this region of Nicaragua. Giving its relative abundance and its implication as a reservoir host of *Leishmania* in several countries in Central and South America, *Proechimys* is a likely reservoir candidate in this region of Nicaragua and deserves further study.

### Rodent collections in areas where atypical cutaneous leishmaniasis is predominant

Very few cases of typical CL (< 3%) have been reported from the Pacific plains region of western Nicaragua. Most municipalities within this region reported 0 cases for the 2003–2005 time period of this study, and no municipality reported more than 3 cases of typical human CL. The predominant form of leishmaniasis in this region is reported to be atypical CL; and it is associated with *Leishmania* (*L.*) *chagasi*, the same species that is known to cause visceral leishmaniasis (Belli et al., 1999). *Leishmania* (*L.*) *chagasi* and its primary sand fly vectors (*Lu. longipalpis* and *Lu. evansi*) have been demonstrated to exhibit a peridomestic transmission cycle with canids serving as the major reservoir hosts (Morrison et al., 1993). This differs from the primarily sylvatic vector-reservoir systems associated with the causative agents of typical cutaneous leishmaniasis. To my knowledge, no studies in Nicaragua have isolated *L.* (*L.*) *chagasi* from domestic or wild canids; but, it is likely dogs serve as a primary reservoir host here.

Because the reported numbers of human cases of typical CL are low within the Pacific plains region of Nicaragua and data received from the Ministry of Health only included human cases seen within local clinics, it is impossible to discern if the disease was contracted within the reporting municipality or acquired elsewhere. It is likely that some of these infections were acquired at foci within the central highlands or Atlantic lowlands plains and then treated and reported in another municipality. However, typical cutaneous leishmaniasis transmission cycles endemic to the Pacific plains regions cannot be ruled out and potential reservoirs and vectors within this region were explored.

### Rodent collections within the Pacific plains region; Bella Vista

Of the 29 rodents representing 6 species collected at the Bella Vista study site, 4 species have been identified as suitable hosts of *Leishmania*. These included the previously mentioned *Nyctomys sumichrasti*, *Ototylomys phyllotis*, *Peromyscus mexicanus*, and *Rattus rattus*. Only 1 *Nyctomys sumichrasti* and 2 *Ototylomys phyllotis* were captured, indicating that they may not be of sufficient abundance to serve as a suitable reservoir. Again, all of the *Rattus rattus* (5) were captured in human dwellings and are probably not a significant contributor to maintenance of the parasite within its natural system. *Peromyscus mexicanus* appears to be fairly common in this area as they represented 6 of the 29 individuals captured. Its relatively high abundance and the fact that *Peromyscus* has been identified as a reservoir of *Leishmania* in Mexico (Chable-Santos et al., 1995; Canto Lara et al., 1999) make it the most likely candidate for a potential reservoir host here. The most frequently captured species was *Liomys salvini* (9 of 29), and it has not previously been implicated as a host of *Leishmania.*

### Rodent collections within the Pacific plains region; San Cristobal

Of the 28 rodents representing 5 species captured at the San Cristobal study site, 2 species and 1 genus have been identified as suitable hosts of *Leishmania*. *Nyctomys sumichrasti* and *Ototylomys phyllotis* represented 1 and 4 individuals captured, respectively. Although they have been implicated as hosts elsewhere, as at the Bella Vista study site they appear to be insufficiently abundant to serve as a primary reservoir. The genus *Peromyscus* represented 22 of the 28 captured individuals, with 2 *Peromyscus mexicanus* and 20 *Peromyscus oaxacensis*. Their relatively high abundance and known suitability as reservoirs again make them the prime candidate for reservoirs within this area as well.

### Rodent collections within the Pacific plains region; Las Marias, Los Mangos, and San Jacinto.

Insufficient individuals were captured at these 3 sites to gain insight into the numbers and relative abundance of species. However, 2 *Peromyscus mexicanus* were captured at Las Mangos and 3 were captured at San Jacinto. Two *Liomys salvini* were captured at Los Mangos and 1 at Las Marias. This species has not been shown to be a host of *Leishmania* elsewhere.

### Sand fly collections

All but 1 species of sand flies collected in Nicaragua during this study appear to have a distinct geographic distribution between the Pacific plains region in the western half of the country where atypical cutaneous leishmaniasis is the dominant form of human disease and the central highlands and Atlantic regions in the eastern half of the country where typical cutaneous leishmaniasis is the dominant form. *Lutzomyia cruciata* was captured with some success throughout the country, with 55 individuals captured in areas with the Pacific plains and 33 individuals captured in the central highlands and Atlantic regions. These results are in contrast to the results of Valle and Rivera (1995), who captured over 2,000 *Lu. cruciata* from areas within the central highlands and Atlantic regions while capturing only 4 individuals from within the Pacific plains region. They reported that *Lu. cruciata* was the dominate species captured within the central highlands and Atlantic regions. Although total numbers of sand flies captured during this study were considerably lower, *Lu. cruciata* also was captured more frequently within the central highlands and Atlantic plains regions of the country than any other species. Although *Lu. cruciata* was the species that was captured more than any other in this region, in the eastern half of the country its distribution appears to be primarily limited to the central highlands and the northern sections of the Atlantic lowland plains. This supports the results of Valle and Rivera (1995), who captured the vast majority of *Lu. cruciata* specimens near Matagalpa in the central highlands.

*Lutzomyia cayennensis*, *Lu. chiapanensis*, *Lu. evansi*, *Lu. gomezi*, *Lu. vesiscifera*, and *Lu. zeledoni* only were captured in the Pacific plains region. These results generally support the results of previous researchers in Nicaragua. Valle and Rivera (1995) found *Lu. evansi* to be the most abundant species captured within the Pacific plains region and Fairchild and Hertig (1959) only captured this species near the Pacific coast. *Lutzomyia cayennensis* and all but 1 *Lu. chiapanensis* also were captured by Valle and Rivera (1959) only in the Pacific plains region. *Lutzomyia vesiscifera* appears to be fairly widespread, although in low numbers, throughout the country; this study identified it only at San Jacinto. It previously has been captured from all regions, but is most prevalent in the Pacific plains (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995). *Lutzomyia gomezi* also previously has been identified in low numbers throughout much of the country (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995). This study identified *Lu. gomezi* only at San Jacinto.

*Lutzomyia barrettoi majuscula*, *Lu. hartmanni*, *Lu. panamensis*, and *Lu.shannoni*, only were captured within the central highlands or Atlantic lowland plains regions. Fairchild and Hertig (1959) captured *Lu. barrettoi majuscula* near Managua in the Pacific plains and Valle and Rivera (1995) captured it in only the central highlands and the northern region of the Atlantic plains. This study identified *Lu. barrettoi majuscula* at El Paraisito. This extends its known range within the country into the southern part of the Atlantic plains. One *Lu. hartmanni* was captured at El Paraisito. This represents a new record for this species in Nicaragua. *Lutzomyia panamensis* was collected from El Cua, located in the central highlands, and from El Balsamo and Rosa Grande in the northern region of the Atlantic lowland plains. Previous studies have identified its range within Nicaragua extending throughout most of the central highlands and Atlantic lowland plains, but not into the Pacific plains (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995). Although this study identified *Lu. shannnoni* only at El Balsamo, within the northern region of the Atlantic lowland plains, it previously has been reported from all regions (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995).

*Lutzomyia longipalpis* was the most abundantly-captured species. Of the 274 individuals captured, 270 were collected from San Jacinto located in the Pacific plains. This supports the results obtained by previous investigators, who found this species only within the Pacific plains (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995). Three *Lu. longipalpis* were captured in El Tigre, which extends the known range of this species into the central highlands.

## Distribution of sand fly species in relationship to clinical forms of leishmaniasis

*Lutzomyia longipalpis* and *Lu. evansi* were by far the most commonly captured species within the Pacific plains region during the course of this study. These results support the findings of others in Nicaragua (Fairchild and Hertig, 1959; Zeledon and Murillo, 1983; Valle and Rivera, 1995). This is significant, because *Lu. longipalpis* and *Lu. evansi* are known vectors of *L.* (*L.*) *chagasi* (Killick-Kendrick, 1999). *Leishmania* (*L.*) *chagasi* is the primary etiological agent of human VL leishmaniasis in the New World and this species also has been isolated from patients suffering from VL and atypical CL in Nicaragua. The geographic distribution of VL and atypical CL in Nicaragua corresponds with the geographic distribution of *Lu. longipalpis* and *Lu. evansi*. Although *L. chagasi* has not been isolated from either *Lu. longipalpis* or *Lu. evansi* collected in Nicaragua, the large relative abundance of *Lu. longipalpis* and *Lu. evansi* along with the overlapping geographic distributions of the sand flies and the occurrence of human VL and atypical CL hint that they are the most-likely vectors of *L.*(*L.*) *chagasi* in Nicaragua.

Additionally, the Pacific plains region of Nicaragua is the driest region of the country and *Lu. longipalpis* previously has been reported to inhabit arid to semi-arid habitats (Morrison et al., 1993). *Lutzomyia evansi* previously has been reported to be a sympatric species of *Lu. longipalpis* (Travi et al., 2002). The capture of *Lu. longipalpis* and *Lu. evansi* primarily within the relatively dry Pacific plains region during this study supports this.

In the central highlands and Atlantic lowland plains regions of Nicaragua, the predominant form of human leishmaniasis is typical CL. The most prevalent species of sand fly captured during this study were *Lu. cruciata* and *Lu. barrettoi majuscula*. Both species are anthropophilic (Young and Duncan, 1994; Rebollar-Téllez et al., 1996a).

Although *Lu. cruciata* has not been proven to be a vector of *Leishmania*, it is anthropophilic; and it has been found to be associated with areas of human typical CL in the New World and it has been found to be naturally infected with leishmanial parasites (Young and Duncan, 1984; Rebollar-Téllez et al., 1996a; Rebollar-Téllez et al., 1996b). *Lutzomyia cruciata* also has been found to be capable of transmitting *Leishmania* (*L.*) *mexicana* under experimental conditions (Williams, 1966).

As in previous studies of the sand fly fauna of Nicaragua, *Lu. panamensis* only was captured in the central highlands and Atlantic lowland plains regions. This is significant in that it has been reported to be a suspected vector of *L.* (*L.*) *mexicana* and *L.* (*V.*) *braziliensis;* both known to be etiology agents of human typical CL (Killick-Kendrick, 1999). The distribution of *Lu. panamensis* corresponds with the distribution of human cases of typical CL in Nicaragua. Given that *Lu. cruciata* and *Lu. panamensis* are abundant in the central highlands and Atlantic lowland plains regions of Nicaragua, and that they are capable of serving as hosts for *L.* (*L.*) *mexicana* and *L.* (*V.*) *braziliensis*, I believe that these 2 species play an important role as the vectors of the causative agents of human typical CL in these regions of Nicaragua.

### Spatial distribution of human typical CL

Human cutaneous leishmaniasis in Nicaragua occurs in 2 primary clinical forms, typical CL and atypical CL. When reviewing the geographic distribution of human cases of typical CL and atypical CL, it becomes apparent that these forms of the disease are spatially segregated. The vast majority of cases of typical CL occur in the central highlands and Atlantic lowland plains regions in the eastern half of the country. In fact, greater than 96% of the human cases of typical CL reported by the Nicaraguan Ministry of Health were from just 19 municipalities. All of these 19 municipalities are located within the central highlands or Atlantic lowland plains regions. Within the Pacific plains region of western Nicaragua, most municipalities reported no cases of typical CL and no municipality reported greater than 3 cases. Conversely, human cases of atypical CL primarily are reported from municipalities within the Pacific plains region. These 2 forms of CL are the result of infections within different species of *Leishmania*.

Parasites isolated from typical cutaneous lesions in patients from Nicaragua previously have been reported to be *Leishmania* (*V.*) *braziliensis* and *Leishmania* (*V.*) *panamensis*, while those isolated from atypical cutaneous lesion have been characterized as *Leishmania* (*L.*) *chagasi* (Belli et al., 1994; 1998; 1999). The isolation of different species of *Leishmania* from human patients with different clinical forms of the disease is significant in that these species are known to have different vector-reservoir systems as well as different primary ecological habitats (Jimenez et al., 2000).

### Logistic regression models of environmental factors associated with the presence or absence of reported cases of human typical CL

Reported human cases of typical CL are likely to be concentrated within geographic areas where the parasite, suitable reservoir hosts, suitable vectors and a susceptible human population intersect. This study used GIS, remotely-sensed satellite data, and logistic regression analysis to investigate the environmental relationships between geographic areas where human typical CL was reported and areas where no human cases were reported from 2003–2005.

Logistic regression models demonstrated that mean annual precipitation and land use/cover classes were the best predictors for the dichotomous dependent variable of human typical CL presence or absence within a municipality between the years 2003–2005. Both models displayed equal sensitivity (67.1%); however, using precipitation as the predictor variable increased the specificity to 87.2% over 85.9% in the model using land use/cover as the predictor variable. Using temperature as the predictor variable resulted in a less efficient model than either precipitation or land use/cover with a sensitivity of 63.0% and a specificity of 84.6%. The individual models using elevation and soil type as the predictor variables were poor predictors of the occurrence of typical CL with model sensitivities of 56.2% and 46.6%, respectively. The results of this study provide partial support to the conclusions of the study conducted in Columbia, as described by King et al. (2004), which found that land cover classes were better predictors of the occurrence of typical cutaneous leishmaniasis than elevation. Although King et al. (2004) did not look at precipitation as a predictor variable, this study showed than its inclusion in a predictive model for the presence or absence of CL may improve the model's capabilities.

One potential drawback that may be associated with the predictive models for the presence or absence of typical CL is that the model does not distinguish municipalities that had 1 reported case from those that had hundreds of cases during the period 2003–2005. It was assumed that transmission of the parasite occurred in the municipalities in which the cases were reported. Travel is difficult within Nicaragua and it is possibly that all cases of human typical CL were acquired within municipality where they are reported. However, it is possible, and even somewhat likely, that not all reported cases of infection with typical CL causing parasites were acquired within the reporting municipality, but rather may have been acquired during working or visiting endemic areas. Greater than 97% of all reported cases of human typical CL were reported from 28 of 151 municipalities that documented 10 or more cases during the 3 year period analyzed during this study. When logistic regression analysis was applied to environmental predictor variables for these 28 municipalities, land use/cover was by far the best predictor for identifying municipalities with 10 or more cases.

A number of rodent species, which previously have been identified as suspected or proven reservoir hosts for species of *Leishmania* that are known to cause typical CL, are widely-distributed throughout Nicaragua and appear to be better suited to adapt to a variety of ecosystems than species of *Lutzomyia*. Sand fly vectors of *Leishmania* species known to cause human typical CL within Nicaragua are geographically-distinct in their distribution, as this study and others have shown. Therefore, environmental factors are more likely to play a role in the spatial distribution patterns of sand fly species than potential reservoir hosts.

Land use/cover was a good predictor variable in both the model for presence versus absence and for the model predicting the occurrence of 10 and more cases versus less than 10 cases of typical CL. In the model predicting presence or absence, agricultural systems with 10–25% natural vegetation and tropical evergreen broadleaf forests were the 2 variables that were significant positive predictors of human typical CL. In the model predicting the occurance of $\geq$10 cases versus <10 cases of human typical CL, cropland, agricultural systems with 10–25% natural vegetation, agricultural systems with 25–50%, and tropical evergreen broadleaf forest were significant positive predictors. This appears to provide evidence that typical CL is associated with rural activities and primarily is found in areas where human activities such as farming and ranching begin to encroach into sylvatic areas where the natural transmission cycle occurs.

Maps of the 28 municipalities in which greater than 97% of the cases of human typical CL were reported in the years 2003−2005, overlaid with maps of the extent of forest coverage in Nicaragua in 1983 and in 2000, clearly show that the vast majority of reported cases have been from municipalities where significant forest degradation has occurred (Fig. 20). The natural system in which the transmission of *Leishmania* parasites among suitable sylvatic rodent hosts , or other forest dwelling mammals, by sand flies probably has existed in Nicaragua since time immemorial. As increasing numbers of a naive and susceptible human population clear native forested lands and establish agricultural systems within geographic areas where the natural transmission cycle of *Leishmania* occurs, the numbers of reported human cases of typical CL likely will continue to remain high. There also is a distinct possibility that the sand fly vectors and rodent reservoir hosts will adapt from a strictly sylvatic lifecycle to a peridomestic lifecycle, further increasing the exposure risk of humans to the parasite.

### Temporal distribution of reported cases of human typical CL for the period 2003–2005

Data provided by the Nicaragua Ministry of Health showed that the average weekly number of reported cases of human typical CL increased during May through July and this time frame corresponds with the early part of the rainy season throughout the country. The period of time from inoculation to demonstration of typical cutaneous lesions can take weeks and it is likely that in many cases patients delay seeking of treatment until lesions are serious. This would indicate that the most likely period of transmission from the sand fly vector to human hosts occurs during the drier periods of the year from approximately November through April. One possible explanation for the occurance of peak transmission during the dry season is the poor flying capabilities of the sand fly vectors. Movement of sand flies is likely impeded by periods of heavy rain. Further research is required to demonstrate the periods of peak sand fly activity.

# SUMMARY AND CONCLUSIONS

In this dissertation, new data have been provided on the occurrence of leishmaniasis in rodent species, the geographic distribution of sand fly species and potential rodent reservoir hosts, and environmental factors associated with geographic distribution of human cases of typical cutaneous leishmaniasis in Nicaragua. This is the first study to investigate potential rodent reservoirs of *Leishmania* in Nicaragua and is the first study to investigate the abiotic and biotic environmental components that are associated with the geographic distribution of human cases of typical CL in Nicaragua. During field studies, 395 rodents representing 17 species were collected from 13 localities from August 2001–March 2006 and screened for *Leishmania* infections. One *Heteromys desmarestianus* and 1 *Peromyscus mexicanus* were found to be positive for leishmanial infections by PCR. These positives represent the first report of *Leishmania* infections in rodents in Nicaragua.

*Heteromys desmarestianus* does not appear to be sufficiently abundant or adequately widespread geographically to play an important role as a reservoir host of *Leishmania* in Nicaragua. *Peromyscus mexicanus* occur frequently throughout the Central Highland and the northern regions of the Atlantic lowland plains and this species probably does serve as a reservoir of *Leishmania* here. *Proechimys semispinosus* occur throughout the Atlantic lowland plains and it is likely that this species serves as a primary reservoir for *Leishmania* species.

Five hundred fifty six sand flies representing 12 species were collected from 8 localities, including *Lutzomyia hartmanni*, a new record for this species in Nicaragua.

The predominant species captured in western Nicaragua were *Lutzomyia longipalpis* and *Lutzomyia evansi*. These 2 species are mostly likely the vectors of *L. chagasi*, the etiolological agent of visceral and atypical cutaneous leishmaniasis in Nicaragua. The predominant species captured in central and eastern Nicaragua was *Lutzomyia cruciata*. This species is likely to be a primary or amplifying vector of *Leishmania* species causing typical CL in this region. Although this study yielded but 4 specimens of *Lu. panamensis*, previous studies have shown it to be abundant throught the central highlands and Pacific plains and is a known vector of *L. braziliensis*, a known causative agent of typical CL and MCL. The geographic distribution of sand flies in this study provides additional support to previously-published reports of suspected vectors of *Leishmania* species causing typical and atypical forms of cutaneous leishmaniasis in Nicaragua.

Distribution data of human cases of typical cutaneous leishmaniasis obtained from the Nicaraguan Ministry of Health, along with GIS and remotely-sensed data of elevation, precipitation, temperature, soil types and land use/cover classes, were used to developed predictive logistic regression models for the presence or absence of human cases within 151 municipalities. Mean annual precipitation and land use/cover were determined to be the best environmental variable predictors for the occurrence of typical cutaneous leishmaniasis. In models using mean annual precipitation as the predictor variable for the dichotomous dependent variable of presence or absence, precipitation ranges of 900–1,000, 1,200–1,400, 1,600–1,800, and 2,000–2,400 mm per year showed a significant positive correlation at the 95% CI; while, precipitation ranges below 900 and above 2,400 millimeters per year did not show a significant positive or negative correlation.

Logistic regression models using land use and cover as the predictor variable for the presence or absence of typical cutaneous leishmaniasis demonstrated that the disease is primarily associated with rural activities, with the land use/cover classes of agricultural systems with 10–25% natural vegetation, agricultural systems with 25–50% natural vegetation, cropland, and tropical evergreen deciduous forest showing significant positive correlation at the 95% CI.

The research presented in this study will serve to increase the awareness among parasitologists, medical entomologists and public health workers of the geographic segregation of typical and atypical cutaneous leishmaniasis, the geographic distribution of potential sand fly vectors and potential rodent reservoir hosts, and the biotic and abiotic environmental factors that may influence the distribution of the disease in Nicaragua. Further research is needed to incriminate vectors and reservoirs hosts within distinct regions of the country in order to better understand local transmission cycles. Research in the following areas would be of use: screening of additional rodent species and of non-rodent species to clarify sylvatic and peridomestic transmission risk; screening of sand fly species to clarify vectors; refinement of statistical models using GIS and remotely-sensed data to determine if models developed for Nicaragua might be useful in predicting the occurrence of leishmaniasis at additional localities.

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# APPENDIX A

Table A–1. Rodents collected in Nicaragua; raw data, 2001–2006.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 8-Aug-01 | Nica-001 | 72915 | *Mus musculus* | F | 11 | Bella Vista |
| 8-Aug-01 | Nica-002 | 72909 | *Mus musculus* | M | 9 | Bella Vista |
| 9-Aug-01 | Nica-003 | 72907 | *Rattus rattus* | M | 111 | Bella Vista |
| 9-Aug-01 | Nica-004 | 72908 | *Rattus rattus* | F | 89 | Bella Vista |
| 9-Aug-01 | Nica-005 | 72910 | *Rattus rattus* | F | 90 | Bella Vista |
| 9-Aug-01 | Nica-006 | 72911 | *Rattus rattus* | F | 110 | Bella Vista |
| 9-Aug-01 | Nica-007 | 72912 | *Liomys salvini* | M | 41 | Bella Vista |
| 9-Aug-01 | Nica-008 | 72913 | *Rattus rattus* | M | 40 | Bella Vista |
| 9-Aug-01 | Nica-009 | 72914 | *Mus musculus* | F | 19 | Bella Vista |
| 9-Aug-01 | Nica-010 | 72916 | *Mus musculus* | F | 20 | Bella Vista |
| 9-Aug-01 | Nica-011 | 72918 | *Mus musculus* | F | 19 | Bella Vista |
| 9-Aug-01 | Nica-012 | 72917 | *Mus musculus* | M | 9 | Bella Vista |
| 9-Aug-01 | Nica-013 | 72923 | *Ototylomys phyllotis* | F | 88 | Bella Vista |
| 14-Aug-01 | Nica-014 | 72924 | *Rattus rattus* | F | 135 | Selva Negra |
| 14-Aug-01 | Nica-015 | 72921 | *Rattus rattus* | M | 30 | Selva Negra |
| 14-Aug-01 | Nica-016 | 72922 | *Nyctomys sumichrasti* | M | 50 | Selva Negra |
| 14-Aug-01 | Nica-017 | 72919 | *Rattus rattus* | M | 105 | Selva Negra |
| 14-Aug-01 | Nica-018 | 72920 | *Mus musculus* | M | 15 | Selva Negra |
| 14-Aug-01 | Nica-019 | 72925 | *Mus musculus* | F | 20 | Selva Negra |
| 14-Aug-01 | Nica-020 | 72926 | *Rattus rattus* | M | 65 | Selva Negra |
| 14-Aug-01 | Nica-021 | 72927 | *Rattus rattus* | M | 115 | Selva Negra |
| 14-Aug-01 | Nica-022 | 72931 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 14-Aug-01 | Nica-023 | 72932 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 15-Aug-01 | Nica-024 | 72933 | *Rattus rattus* | M | 35 | Selva Negra |
| 15-Aug-01 | Nica-025 | 72934 | *Rattus rattus* | F | 125 | Selva Negra |
| 16-Aug-01 | Nica-026 | 72936 | *Mus musculus* | F | 15 | San Jancito |
| 5-Jan-02 | Nica-027 | 93598 | *Liomys salvini* | F | 25 | Las Marias |
| 8-Jan-02 | Nica-029 | 93683 | *Peromyscus mexicanus* | F | 65 | San Jacinto |
| 9-Jan-02 | Nica-030 | 93701 | *Rattus rattus* | F | 100 | Selva Negra |
| 9-Jan-02 | Nica-031 | 93647 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 9-Jan-02 | Nica-032 | 93648 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 9-Jan-02 | Nica-033 | 93649 | *Peromyscus mexicanus* | F | 50 | Selva Negra |
| 9-Jan-02 | Nica-034 | 93651 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 9-Jan-02 | Nica-035 | 93652 | *Peromyscus mexicanus* | M | 30 | Selva Negra |
| 9-Jan-02 | Nica-036 | 93596 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 9-Jan-02 | Nica-037 | 93653 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 9-Jan-02 | Nica-038 | 93620 | *Peromyscus mexicanus* | M | 30 | Selva Negra |
| 9-Jan-02 | Nica-039 | 93650 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 9-Jan-02 | Nica-040 | 93656 | *Peromyscus mexicanus* | M | 52 | Selva Negra |
| 9-Jan-02 | Nica-041 | 93658 | *Peromyscus mexicanus* | M | 35 | Selva Negra |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 9-Jan-02 | Nica-042 | 93657 | *Peromyscus mexicanus* | M | 55 | Selva Negra |
| 10-Jan-02 | Nica-043 | 93621 | *Peromyscus mexicanus* | M | 60 | Selva Negra |
| 10-Jan-02 | Nica-044 | 93655 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 10-Jan-02 | Nica-045 | 93645 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 10-Jan-02 | Nica-046 | 93659 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 10-Jan-02 | Nica-047 | 93654 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 10-Jan-02 | Nica-048 | 93646 | *Oryzomys alfaroi* | M | 30 | Selva Negra |
| 10-Jan-02 | Nica-049 | 93622 | *Peromyscus mexicanus* | M | 37 | Selva Negra |
| 10-Jan-02 | Nica-050 | 93682 | *Heteromys desmarsestianus* | M | 80 | Selva Negra |
| 10-Jan-02 | Nica-051 | 93684 | *Ototylomys phyllotis* | M | 50 | Selva Negra |
| 10-Jan-02 | Nica-052 | 93686 | *Peromyscus mexicanus* | F | 30 | Selva Negra |
| 10-Jan-02 | Nica-053 | 93687 | *Peromyscus mexicanus* | F | 50 | Selva Negra |
| 10-Jan-02 | Nica-054 | 93681 | *Peromyscus mexicanus* | M | 30 | Selva Negra |
| 10-Jan-02 | Nica-055 | 93694 | *Peromyscus mexicanus* | M | 55 | Selva Negra |
| 11-Jan-02 | Nica-056 | 93688 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 11-Jan-02 | Nica-057 | 93675 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 11-Jan-02 | Nica-058 | 93689 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 11-Jan-02 | Nica-059 | 93601 | *Ototylomys phyllotis* | F | 90 | Selva Negra |
| 11-Jan-02 | Nica-060 | 93696 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 11-Jan-02 | Nica-061 | 93644 | *Scotinomys teguina* | M | 10 | Selva Negra |
| 11-Jan-02 | Nica-062 | 93695 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 11-Jan-02 | Nica-063 | 93575 | *Scotinomys teguina* | M | ? | Selva Negra |
| 11-Jan-02 | Nica-064 | 93700 | *Oryzomys alfaroi* | M | 20 | Selva Negra |
| 11-Jan-02 | Nica-065 | 93697 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 11-Jan-02 | Nica-066 | 93698 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 11-Jan-02 | Nica-067 | 93699 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 13-Jan-02 | Nica-068 | 93685 | *Didelphis marsupialis* | F | 145 | San Lucas |
| 15-Jan-02 | Nica-069 | 93577 | *Liomys salvini* | F | 40 | Los Mangos |
| 15-Jan-02 | Nica-070 | 93600 | *Peromyscus nudipes* | F | 61 | Los Mangos |
| 15-Jan-02 | Nica-071 | 93678 | *Peromyscus mexicanus* | M | 50 | Los Mangos |
| 16-Jan-02 | Nica-072 |  | *Rattus rattus* | M | 80 | San Jacinto |
| 17-Jan-02 | Nica-073 | 93679 | *Mus musculus* | F | 15 | San Jacinto |
| 24-Jan-02 | Nica-080 | 93690 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 24-Jan-02 | Nica-081 | 93691 | *Peromyscus mexicanus* | M | 30 | Selva Negra |
| 24-Jan-02 | Nica-082 | 93676 | *Peromyscus mexicanus* | M | 35 | Selva Negra |
| 24-Jan-02 | Nica-083 | 93677 | *Peromyscus mexicanus* | M | 30 | Selva Negra |
| 24-Jan-02 | Nica-084 | 93692 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 24-Jan-02 | Nica-085 | 93693 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 24-Jan-02 | Nica-086 | 93680 | *Peromyscus mexicanus* | F | 30 | Selva Negra |
| 24-Jan-02 | Nica-087 | 93599 | *Peromyscus mexicanus* | M | 35 | Selva Negra |
| 24-Jan-02 | Nica-088 | 93576 | *Oryzomys sp.* | M | 45 | Selva Negra |
| 24-Jan-02 | Nica-089 | 93702 | *Peromyscus mexicanus* | M | 55 | Selva Negra |
| 24-Jan-02 | Nica-090 | 93703 | *Peromyscus mexicanus* | M | 35 | Selva Negra |
| 24-Jan-02 | Nica-091 | 93704 | *Peromyscus mexicanus* | M | 35 | Selva Negra |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 24-Jan-02 | Nica-092 | 93705 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 24-Jan-02 | Nica-093 | 93706 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 18-May-02 | Nica-094 | 113501 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 18-May-02 | Nica-095 | 113502 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 18-May-02 | Nica-096 | 113503 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 18-May-02 | Nica-097 | 113508 | *Scotinomys teguina* | F | 12 | Selva Negra |
| 18-May-02 | Nica-098 | 113507 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 18-May-02 | Nica-099 | 113504 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 18-May-02 | Nica-100 | 113506 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 18-May-02 | Nica-101 | 113505 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 18-May-02 | Nica-102 | 113509 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 18-May-02 | Nica-103 | 113510 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 18-May-02 | Nica-104 | 113511 | *Peromyscus oaxacensis* | M | 50 | Selva Negra |
| 19-May-02 | Nica-105 | 113512 | *Ototylomys phyllotis* | F | 120 | Selva Negra |
| 19-May-02 | Nica-106 | 113525 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 19-May-02 | Nica-107 | 113526 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 19-May-02 | Nica-108 | 113527 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 19-May-02 | Nica-109 | 113528 | *Peromyscus mexicanus* | F | 35 | Selva Negra |
| 19-May-02 | Nica-110 | 113529 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 20-May-02 | Nica-111 | 113530 | *Peromyscus mexicanus* | F | 48 | Selva Negra |
| 20-May-02 | Nica-112 | 113516 | *Oryzomys sp.* | M | 25 | Selva Negra |
| 20-May-02 | Nica-113 | 113531 | *Heteromys desmarsianus* | M | 45 | Selva Negra |
| 20-May-02 | Nica-114 | 113532 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 20-May-02 | Nica-115 | 113534 | *Ototylomys phyllotis* | F | 105 | Selva Negra |
| 20-May-02 | Nica-116 | 113533 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 20-May-02 | Nica-117 | 113535 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 20-May-02 | Nica-118 | 113536 | *Peromyscus mexicanus* | M | 55 | Selva Negra |
| 20-May-02 | Nica-119 | 113542 | *Peromyscus mexicanus* | F | 50 | Selva Negra |
| 20-May-02 | Nica-120 | 113541 | *Peromyscus mexicanus* | M | 46 | Selva Negra |
| 20-May-02 | Nica-121 | 113537 | *Peromyscus mexicanus* | M | 38 | Selva Negra |
| 20-May-02 | Nica-122 | 113538 | *Peromyscus mexicanus* | F | 38 | Selva Negra |
| 20-May-02 | Nica-123 | 113539 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 20-May-02 | Nica-124 | 113540 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 20-May-02 | Nica-125 | 113543 | *Peromyscus mexicanus* | M | 55 | Selva Negra |
| 20-May-02 | Nica-126 | 113515 | *Heteromys desmarsianus* | F | 50 | Selva Negra |
| 21-May-02 | Nica-127 | 113513 | *Oryzomys sp.* | M | 35 | Selva Negra |
| 21-May-02 | Nica-128 | 113544 | *Peromyscus mexicanus* | M | 35 | Selva Negra |
| 21-May-02 | Nica-129 | 113514 | *Oryzomys sp.* | M | 25 | Selva Negra |
| 21-May-02 | Nica-130 | 113545 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 21-May-02 | Nica-131 | 113548 | *Peromyscus mexicanus* | F | 50 | Selva Negra |
| 22-May-02 | Nica-132 | 113518 | *Sigmodon hirsutus* | M | 60 | El Tigre |
| 22-May-02 | Nica-133 | 113546 | *Sigmodon hirsutus* | M | 105 | El Tigre |
| 22-May-02 | Nica-134 | 113547 | *Peromyscus mexicanus* | M | 50 | El Tigre |
| 22-May-02 | Nica-135 | 113549 | *Peromyscus mexicanus* | M | 45 | El Tigre |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 22-May-02 | Nica-136 | 113550 | *Peromyscus mexicanus* | M | 35 | El Tigre |
| 22-May-02 | Nica-137 | 113555 | *Peromyscus mexicanus* | F | 30 | El Tigre |
| 23-May-02 | Nica-138 | 113519 | *Sigmodon hirsutus* | M | 80 | El Tigre |
| 23-May-02 | Nica-139 | 113552 | *Peromyscus mexicanus* | M | 50 | El Tigre |
| 23-May-02 | Nica-140 | 113553 | *Oryzomys sp.* | M | 40 | El Tigre |
| 26-May-02 | Nica-141 | 113554 | *Peromyscus mexicanus* | M | 60 | Selva Negra |
| 26-May-02 | Nica-142 | 113551 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 26-May-02 | Nica-143 | 113556 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 26-May-02 | Nica-144 | 113558 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 26-May-02 | Nica-145 | 113559 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 26-May-02 | Nica-146 | 113560 | *Peromyscus mexicanus* | F | 50 | Selva Negra |
| 26-May-02 | Nica-147 | 113561 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 26-May-02 | Nica-148 | 113524 | *Scotinomys teguina* | M | 15 | Selva Negra |
| 26-May-02 | Nica-149 | 113562 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 26-May-02 | Nica-150 | 113563 | *Peromyscus mexicanus* | M | 40 | Selva Negra |
| 26-May-02 | Nica-151 | 113564 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 26-May-02 | Nica-152 | 113565 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 26-May-02 | Nica-153 | 113566 | *Peromyscus mexicanus* | M | 50 | Selva Negra |
| 26-May-02 | Nica-154 | 113573 | *Peromyscus mexicanus* | F | 40 | Selva Negra |
| 26-May-02 | Nica-155 | 113581 | *Peromyscus mexicanus* | F | 55 | Selva Negra |
| 26-May-02 | Nica-156 | 113571 | *Peromyscus mexicanus* | F | 45 | Selva Negra |
| 26-May-02 | Nica-157 | 113570 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 26-May-02 | Nica-158 | 113572 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 26-May-02 | Nica-159 | 113567 | *Peromyscus mexicanus* | M | 45 | Selva Negra |
| 31-May-02 | Nica-160 | 113517 | *Nyctomys sumichrasti* | F | 50 | Bella Vista |
| 31-May-02 | Nica-161 | 113575 | *Peromyscus mexicanus* | M | 40 | Bella Vista |
| 31-May-02 | Nica-162 | 113576 | *Peromyscus mexicanus* | F | 45 | Bella Vista |
| 31-May-02 | Nica-163 | 113577 | *Peromyscus mexicanus* | F | 40 | Bella Vista |
| 31-May-02 | Nica-164 | 113574 | *Peromyscus mexicanus* | M | 40 | Bella Vista |
| 1-Jun-02 | Nica-165 | 113580 | *Liomys salvini* | F | 35 | Bella Vista |
| 1-Jun-02 | Nica-166 | 113578 | *Liomys salvini* | M | 40 | Bella Vista |
| 1-Jun-02 | Nica-167 | 113579 | *Liomys salvini* | M | 35 | Bella Vista |
| 1-Jun-02 | Nica-168 | 113568 | *Ototylomys phyllotis* | F | 80 | Bella Vista |
| 1-Jun-02 | Nica-169 | 113522 | *Liomys salvini* | F | 25 | Bella Vista |
| 1-Jun-02 | Nica-170 | 113582 | *Peromyscus mexicanus* | F | 45 | Bella Vista |
| 1-Jun-02 | Nica-171 | 113583 | *Liomys salvini* | M | 40 | Bella Vista |
| 1-Jun-02 | Nica-172 | 113586 | *Peromyscus mexicanus* | F | 45 | Bella Vista |
| 2-Jun-02 | Nica-173 | 113584 | *Liomys salvini* | M | 25 | Bella Vista |
| 2-Jun-02 | Nica-174 | 113569 | *Liomys salvini* | F | 40 | Bella Vista |
| 2-Jun-02 | Nica-175 | 113587 | *Liomys salvini* | M | 35 | Bella Vista |
| 6-Jun-02 | Nica-176 | 113585 | *Peromyscus mexicanus* | F | 40 | San Cristobal |
| 6-Jun-02 | Nica-177 | 113588 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 6-Jun-02 | Nica-178 | 113589 | *Peromyscus oaxacensis* | F | 25 | San Cristobal |
| 6-Jun-02 | Nica-179 | 113592 | *Peromyscus oaxacensis* | F | 45 | San Cristobal |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 6-Jun-02 | Nica-180 | 113590 | *Peromyscus oaxacensis* | M | 40 | San Cristobal |
| 6-Jun-02 | Nica-181 | 113520 | *Ototylomys phyllotis* | F | 95 | San Cristobal |
| 6-Jun-02 | Nica-182 | 113591 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 6-Jun-02 | Nica-183 | 113593 | *Peromyscus oaxacensis* | M | 35 | San Cristobal |
| 6-Jun-02 | Nica-184 | 113594 | *Peromyscus oaxacensis* | F | 45 | San Cristobal |
| 6-Jun-02 | Nica-185 | 113595 | *Nyctomys sumichrasti* | M | 50 | San Cristobal |
| 6-Jun-02 | Nica-186 | 113596 | *Peromyscus oaxacensis* | F | 45 | San Cristobal |
| 6-Jun-02 | Nica-187 | 113597 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 6-Jun-02 | Nica-188 | 113599 | *Peromyscus oaxacensis* | M | 40 | San Cristobal |
| 10-Jun-02 | Nica-189 | 113598 | *Ototylomys phyllotis* | F | 80 | San Cristobal |
| 10-Jun-02 | Nica-190 | 113600 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 10-Jun-02 | Nica-191 | 113601 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 10-Jun-02 | Nica-192 | 113602 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 10-Jun-02 | Nica-193 | 113521 | *Ototylomys phyllotis* | F | 90 | San Cristobal |
| 10-Jun-02 | Nica-194 | 113605 | *Peromyscus oaxacensis* | M | 40 | San Cristobal |
| 10-Jun-02 | Nica-195 | 113606 | *Peromyscus oaxacensis* | F | 40 | San Cristobal |
| 10-Jun-02 | Nica-196 | 113607 | *Liomys salvini* | F | 20 | San Cristobal |
| 10-Jun-02 | Nica-197 | 113608 | *Peromyscus oaxacensis* | F | 35 | San Cristobal |
| 10-Jun-02 | Nica-198 | 113609 | *Peromyscus oaxacensis* | M | 30 | San Cristobal |
| 11-Jun-02 | Nica-199 | 113610 | *Peromyscus oaxacensis* | M | 45 | San Cristobal |
| 11-Jun-02 | Nica-200 | 113611 | *Peromyscus oaxacensis* | F | 35 | San Cristobal |
| 11-Jun-02 | Nica-201 | 113603 | *Ototylomys phyllotis* | M | 60 | San Cristobal |
| 11-Jun-02 | Nica-202 | 113523 | *Peromyscus mexicanus* | M | 25 | San Cristobal |
| 11-Jun-02 | Nica-203 | 113604 | *Peromyscus oaxacensis* | M | 35 | San Cristobal |
| 11-May-03 | Nica-204 | 115175 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 11-May-03 | Nica-205 | 115176 | *Peromyscus nudipes* | F | 40 | Selva Negra |
| 11-May-03 | Nica-206 | 115177 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 11-May-03 | Nica-207 | 115178 | *Peromyscus nudipes* | F | 40 | Selva Negra |
| 11-May-03 | Nica-208 | 115179 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 11-May-03 | Nica-209 | 115180 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 11-May-03 | Nica-210 | 115181 | *Peromyscus nudipes* | F | 35 | Selva Negra |
| 11-May-03 | Nica-211 | 115182 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 11-May-03 | Nica-212 | 115199 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 11-May-03 | Nica-213 | 115183 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 11-May-03 | Nica-214 | 115184 | *Peromyscus nudipes* | F | 40 | Selva Negra |
| 12-May-03 | Nica-215 | 115185 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 12-May-03 | Nica-216 | 115186 | *Peromyscus nudipes* | M | 48 | Selva Negra |
| 12-May-03 | Nica-217 | 115187 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 12-May-03 | Nica-218 | 115188 | *Peromyscus nudipes* | M | 52 | Selva Negra |
| 12-May-03 | Nica-219 | 115189 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-May-03 | Nica-220 | 115190 | *Peromyscus nudipes* | M | 39 | Selva Negra |
| 12-May-03 | Nica-221 | 115191 | *Peromyscus nudipes* | F | 35 | Selva Negra |
| 12-May-03 | Nica-222 | 115192 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-May-03 | Nica-223 | 115195 | *Peromyscus nudipes* | M | 45 | Selva Negra |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 12-May-03 | Nica-224 | 115196 | *Heteromys desmarsestianus* | F | 68 | Selva Negra |
| 12-May-03 | Nica-225 | 115197 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 12-May-03 | Nica-226 | 115198 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-May-03 | Nica-227 | 115193 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-May-03 | Nica-228 | 115194 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 12-May-03 | Nica-229 | 115200 | *Peromyscus nudipes* | M | 49 | Selva Negra |
| 13-May-03 | Nica-230 | 115201 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 13-May-03 | Nica-231 | 115202 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 13-May-03 | Nica-232 | 115203 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 13-May-03 | Nica-233 | 115204 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 13-May-03 | Nica-234 | 115205 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 13-May-03 | Nica-235 | 115206 | *Peromyscus nudipes* | F | 35 | Selva Negra |
| 13-May-03 | Nica-236 | 115207 | *Peromyscus nudipes* | M | 55 | Selva Negra |
| 13-May-03 | Nica-237 | 115208 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 13-May-03 | Nica-238 | 115209 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 13-May-03 | Nica-239 | 115210 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 14-May-03 | Nica-240 | 115219 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 14-May-03 | Nica-241 | 115220 | *Peromyscus nudipes* | F | 42 | Selva Negra |
| 14-May-03 | Nica-242 | 115221 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 14-May-03 | Nica-243 | 115222 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 14-May-03 | Nica-244 | 115223 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 14-May-03 | Nica-245 | 115224 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 14-May-03 | Nica-246 | 115225 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 18-May-03 | Nica-247 | 115226 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 18-May-03 | Nica-248 | 115227 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 18-May-03 | Nica-249 | 115228 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 18-May-03 | Nica-250 | 115211 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 18-May-03 | Nica-251 | 115212 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 18-May-03 | Nica-252 | 115213 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 18-May-03 | Nica-253 | 115214 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 18-May-03 | Nica-254 | 115215 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 19-May-03 | Nica-255 | 115216 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 19-May-03 | Nica-256 | 115217 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 19-May-03 | Nica-257 | 115218 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 19-May-03 | Nica-258 | 115229 | *Peromyscus nudipes* | F | 40 | Selva Negra |
| 19-May-03 | Nica-259 | 115230 | *Peromyscus nudipes* | M | 48 | Selva Negra |
| 19-May-03 | Nica-260 | 115235 | *Tylomys nudicaudus* | M | 90 | Selva Negra |
| 19-May-03 | Nica-261 | 115236 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 19-May-03 | Nica-262 | 115231 | *Peromyscus nudipes* | F | 35 | Selva Negra |
| 19-May-03 | Nica-263 | 115232 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 19-May-03 | Nica-264 | 115239 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 21-May-03 | Nica-266 | 115237 | *Proechimys semispinosus* | M | 245 | El Balsamo |
| 22-May-03 | Nica-267 | 115238 | *Proechimys semispinosus* | M | 90 | El Balsamo |
| 22-May-03 | Nica-268 | 115233 | *Sigmodon hirsutus* | M | 20 | El Balsamo |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 22-May-03 | Nica-269 | 115234 | *Melanomys caliginosus* | M | 40 | El Balsamo |
| 19-Jul-03 | Nica-270 | 121424 | *Nyctomys sumichrasti* | M | 70 | El Balsamo |
| 19-Jul-03 | Nica-271 | 121412 | *Melanomys caliginosus* | M | 47 | El Balsamo |
| 21-Jul-03 | Nica-272 | 121413 | *Ototylomys phyllotis* | M | 80 | El Balsamo |
| 21-Jul-03 | Nica-273 | 121414 | *Melanomys caliginosus* | M | 30 | El Balsamo |
| 21-Jul-03 | Nica-274 | 121415 | *Melanomys caliginosus* | F | 30 | El Balsamo |
| 21-Jul-03 | Nica-275 | 121416 | *Melanomys caliginosus* | M | 40 | El Balsamo |
| 21-Jul-03 | Nica-276 | 121417 | *Melanomys caliginosus* | M | 37 | El Balsamo |
| 21-Jul-03 | Nica-277 | 121418 | *Melanomys caliginosus* | F | 40 | El Balsamo |
| 22-Jul-03 | Nica-278 | 121419 | *Ototylomys phyllotis* | F | 120 | El Balsamo |
| 22-Jul-03 | Nica-279 | 121420 | *Melanomys caliginosus* | F | 20 | El Balsamo |
| 24-Jul-03 | Nica-280 | 121421 | *Ototylomys phyllotis* | M | 118 | Rosa Grande |
| 24-Jul-03 | Nica-281 | 121422 | *Ototylomys phyllotis* | F | 50 | Rosa Grande |
| 25-Jul-03 | Nica-282 | 121423 | *Melanomys caliginosus* | M | 55 | Rosa Grande |
| 25-Jul-03 | Nica-283 | 121427 | *Melanomys caliginosus* | F | 50 | Rosa Grande |
| 25-Jul-03 | Nica-284 | 121428 | *Melanomys caliginosus* | M | 58 | Rosa Grande |
| 25-Jul-03 | Nica-285 | 121425 | *Proechimys semispinosus* | F | 325 | Rosa Grande |
| 26-Jul-03 | Nica-286 | 121426 | *Ototylomys phyllotis* | F | 80 | Rosa Grande |
| 26-Jul-03 | Nica-287 | 121429 | *Melanomys caliginosus* | F | 389 | Rosa Grande |
| 26-Jul-03 | Nica-288 | 121430 | *Melanomys caliginosus* | F | 346 | Rosa Grande |
| 26-Jul-03 | Nica-289 | 121431 | *Melanomys caliginosus* | M | 57 | Rosa Grande |
| 26-Jul-03 | Nica-290 | 121480 | *Proechimys semispinosus* | F | 453 | Rosa Grande |
| 26-Jul-03 | Nica-291 | 123014 | *Proechimys semispinosus* | F | 365 | Rosa Grande |
| 26-Jul-03 | Nica-292 | 121457 | *Melanomys caliginosus* | M | 41 | Rosa Grande |
| 26-Jul-03 | Nica-293 | 121439 | *Melanomys caliginosus* | M | 396 | Rosa Grande |
| 26-Jul-03 | Nica-294 | 121438 | *Melanomys caliginosus* | F | 37 | Rosa Grande |
| 26-Jul-03 | Nica-295 | 121432 | *Melanomys caliginosus* | F | 27 | Rosa Grande |
| 27-Jul-03 | Nica-296 | 121433 | *Peromyscus nudipes* | M | 42 | Rosa Grande |
| 27-Jul-03 | Nica-297 | 121434 | *Ototylomys phyllotis* | M | 92 | Rosa Grande |
| 27-Jul-03 | Nica-298 | 121435 | *Melanomys caliginosus* | F | 40 | Rosa Grande |
| 27-Jul-03 | Nica-299 | 121436 | *Proechimys semispinosus* | M | 462 | Rosa Grande |
| 27-Jul-03 | Nica-300 | 121437 | *Melanomys caliginosus* | M | 45 | Rosa Grande |
| 19-May-04 | Nica-301 | 119158 | *Scotinomys teguina* | F | 15 | El Balsamo |
| 19-May-04 | Nica-302 | 119161 | *Scotinomys teguina* | F | 15 | El Balsamo |
| 19-May-04 | Nica-303 | 119122 | *Proechimys semispinosus* | F | 300 | El Balsamo |
| 19-May-04 | Nica-304 | 119146 | *Sigmodon hirsutus* | F | 110 | El Balsamo |
| 19-May-04 | Nica-305 | 119159 | *Scotinomys teguina* | M | 15 | El Balsamo |
| 19-May-04 | Nica-306 | 119160 | *Scotinomys teguina* | M | 15 | El Balsamo |
| 20-May-04 | Nica-307 | 119165 | *Sigmodon hirsutus* | M | 55 | El Balsamo |
| 20-May-04 | Nica-308 | 119163 | *Melanomys caliginosus* | M | 45 | El Balsamo |
| 20-May-04 | Nica-309 | 119166 | *Melanomys caliginosus* | F | 35 | El Balsamo |
| 20-May-04 | Nica-310 | 119162 | *Melanomys caliginosus* | M | 40 | El Balsamo |
| 20-May-04 | Nica-311 | 119164 | *Melanomys caliginosus* | M | 40 | El Balsamo |
| 20-May-04 | Nica-312 | 119123 | *Proechimys semispinosus* | M | 400 | El Balsamo |

Table A−1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 21-May-04 | Nica-313 | 119167 | *Oligoryzomys fulvescens* | M | 35 | El Balsamo |
| 21-May-04 | Nica-314 | 119168 | *Melanomys caliginosus* | M | 35 | El Balsamo |
| 21-May-04 | Nica-315 | 119147 | *Sigmodon hirsutus* | M | 150 | El Balsamo |
| 21-May-04 | Nica-316 | 119169 | *Melanomys caliginosus* | M | 30 | El Balsamo |
| 21-May-04 | Nica-317 | 119148 | *Sigmodon hirsutus* | F | 120 | El Balsamo |
| 21-May-04 | Nica-318 | 119170 | *Oligoryzomys fulvescens* | M | 50 | El Balsamo |
| 21-May-04 | Nica-319 | 119171 | *Melanomys caliginosus* | F | 40 | El Balsamo |
| 25-May-04 | Nica-320 | 119124 | *Proechimys semispinosus* | M | 490 | El Paraisito |
| 25-May-04 | Nica-321 | 119149 | *Proechimys semispinosus* | M | 480 | El Paraisito |
| 25-May-04 | Nica-322 | 119138 | *Proechimys semispinosus* | M | 55 | El Paraisito |
| 26-May-04 | Nica-323 | 119150 | *Proechimys semispinosus* | M | 555 | El Paraisito |
| 26-May-04 | Nica-324 | 119151 | *Proechimys semispinosus* | M | 520 | El Paraisito |
| 26-May-04 | Nica-325 | 119154 | *Proechimys semispinosus* | M | 175 | El Paraisito |
| 27-May-04 | Nica-326 | 119173 | *Oryzomys cousei* | M | 90 | El Paraisito |
| 27-May-04 | Nica-327 | 119174 | *Sigmodon hirsutus* | M | 75 | El Paraisito |
| 27-May-04 | Nica-328 | 119157 | *Proechimys semispinosus* | F | 500 | El Paraisito |
| 27-May-04 | Nica-329 | 119152 | *Proechimys semispinosus* | F | 450 | El Paraisito |
| 27-May-04 | Nica-330 | 119153 | *Proechimys semispinosus* | F | 325 | El Paraisito |
| 27-May-04 | Nica-331 | 119155 | *Proechimys semispinosus* | M | 190 | El Paraisito |
| 27-May-04 | Nica-332 | 119172 | *Oryzomys cousei* | M | 485 | El Paraisito |
| 28-May-04 | Nica-333 | 119176 | *Sigmodon hirsutus* | M | 100 | El Paraisito |
| 28-May-04 | Nica-334 | 119125 | *Proechimys semispinosus* | F | 405 | El Paraisito |
| 28-May-04 | Nica-335 | 119144 | *Proechimys semispinosus* | M | 545 | El Paraisito |
| 28-May-04 | Nica-336 | 119175 | *Sigmodon hirsutus* | M | 45 | El Paraisito |
| 28-May-04 | Nica-337 | 119156 | *Proechimys semispinosus* | M | 65 | El Paraisito |
| 29-May-04 | Nica-338 | 119135 | *Proechimys semispinosus* | M | >600 | El Paraisito |
| 30-May-04 | Nica-339 | 119178 | *Sigmodon hirsutus* | M | 80 | El Paraisito |
| 30-May-04 | Nica-340 | 119179 | *Sigmodon hirsutus* | M | 145 | El Paraisito |
| 30-May-04 | Nica-341 | 119181 | *Oligoryzomys fulvescens* | F | 285 | El Paraisito |
| 31-May-04 | Nica-342 | 119134 | *Proechimys semispinosus* | F | 415 | El Paraisito |
| 31-May-04 | Nica-343 | 119180 | *Oligoryzomys fulvescens* | F | 35 | El Paraisito |
| 1-Jun-04 | Nica-344 | 119130 | *Proechimys semispinosus* | F | 440 | El Paraisito |
| 2-Jun-04 | Nica-345 | 119139 | *Proechimys semispinosus* | M | 425 | El Paraisito |
| 3-Jun-04 | Nica-346 | 119143 | *Proechimys semispinosus* | M | 425 | El Paraisito |
| 3-Jun-04 | Nica-347 | 119131 | *Proechimys semispinosus* | M | 45 | El Paraisito |
| 3-Jun-04 | Nica-348 | 119182 | *Oryzomys cousei* | M | 80 | El Paraisito |
| 7-Jun-04 | Nica-349 | 119132 | *Proechimys semispinosus* | M | 275 | El Paraisito |
| 7-Jun-04 | Nica-350 | 119183 | *Oryzomys cousei* | M | 70 | El Paraisito |
| 7-Jun-04 | Nica-351 | 119145 | *Sigmodon hirsutus* | F | 325 | El Paraisito |
| 7-Jun-04 | Nica-352 | 119184 | *Sigmodon hirsutus* | M | 115 | El Paraisito |
| 7-Jun-04 | Nica-353 | 119188 | *Proechimys semispinosus* | M | 65 | El Paraisito |
| 7-Jun-04 | Nica-354 | 119185 | *Oligoryzomys fulvescens* | M | 50 | El Paraisito |
| 8-Jun-04 | Nica-355 | 119177 | *Proechimys semispinosus* | M | >600 | El Paraisito |
| 8-Jun-04 | Nica-356 | 119142 | *Proechimys semispinosus* | F | 355 | El Paraisito |

Table A–1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 8-Jun-04 | Nica-357 | 119133 | *Proechimys semispinosus* | M | 110 | El Paraisito |
| 8-Jun-04 | Nica-358 | 119187 | *Sigmodon hirsutus* | M | 125 | El Paraisito |
| 8-Jun-04 | Nica-359 | 119186 | *Oligoryzomys fulvescens* | M | 45 | El Paraisito |
| 9-Jun-04 | Nica-360 | 119136 | *Proechimys semispinosus* | F | 520 | El Paraisito |
| 9-Jun-04 | Nica-361 | 119137 | *Proechimys semispinosus* | F | 390 | El Paraisito |
| 16-Dec-04 | Nica-368 | 137043 | *Proechimys semispinosus* | F | 408 | La Sardina |
| 17-Dec-04 | Nica-369 | 137054 | *Proechimys semispinosus* | M | 175 | El Paraisito |
| 17-Dec-04 | Nica-370 | 137035 | *Proechimys semispinosus* | M | 253 | El Paraisito |
| 18-Dec-04 | Nica-371 | 137053 | *Proechimys semispinosus* | F | 145 | El Paraisito |
| 18-Dec-04 | Nica-372 | 137042 | *Proechimys semispinosus* | M | 250 | El Paraisito |
| 19-Dec-04 | Nica-373 | 137038 | *Proechimys semispinosus* | F | 295 | El Paraisito |
| 21-Dec-04 | Nica-374 | 137037 | *Proechimys semispinosus* | F | 290 | Esperanzita #2 |
| 22-Dec-04 | Nica-375 | 137042 | *Proechimys semispinosus* | F | 315 | La Fonseca |
| 22-Dec-04 | Nica-376 | 137-57 | *Proechimys semispinosus* | F | 395 | La Fonseca |
| 23-Dec-04 | Nica-377 | 137039 | *Proechimys semispinosus* | M | 233 | La Fonseca |
| 23-Dec-04 | Nica-378 | 137051 | *Proechimys semispinosus* | M | 130 | La Fonseca |
| 23-Dec-04 | Nica-379 | 137050 | *Proechimys semispinosus* | M | 397 | La Fonseca |
| 16-Mar-05 | Nica-380 | 137040 | *Proechimys semispinosus* | M | 360 | El Paraisito |
| 17-Mar-05 | Nica-381 | 137069 | *Mus musculus* | F | 10 | El Paraisito |
| 18-Mar-05 | Nica-382 | 137036 | *Proechimys semispinosus* | F | 385 | El Paraisito |
| 12-May-05 | Nica-382B | 137034 | *Proechimys semispinosus* | F | 325 | El Paraisito |
| 13-May-05 | Nica-383 | 137041 | *Proechimys semispinosus* | M | 510 | Nueva Guinea |
| 12-Mar-06 | Nica-384 | 137046 | *Peromyscus nudipes* | M | 34 | Selva Negra |
| 12-Mar-06 | Nica-385 | 137044 | *Peromyscus nudipes* | M | 44 | Selva Negra |
| 12-Mar-06 | Nica-386 | 137047 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 12-Mar-06 | Nica-387 | 133252 | *Peromyscus nudipes* | M | 40 | Selva Negra |
| 12-Mar-06 | Nica-388 | 137048 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-Mar-06 | Nica-389 | 137045 | *Peromyscus nudipes* | M | 38 | Selva Negra |
| 12-Mar-06 | Nica-390 | 137049 | *Peromyscus nudipes* | F | 50 | Selva Negra |
| 12-Mar-06 | Nica-391 | 137058 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 12-Mar-06 | Nica-392 | 137055 | *Peromyscus nudipes* | M | 45 | Selva Negra |
| 12-Mar-06 | Nica-393 | 137059 | *Peromyscus nudipes* | F | 45 | Selva Negra |
| 12-Mar-06 | Nica-394 | 137052 | *Peromyscus nudipes* | M | 50 | Selva Negra |
| 14-Mar-06 | Nica-395 | 137061 | *Sigmodon hirsutus* | M | 70 | El Cua |
| 14-Mar-06 | Nica-396 | 137073 | *Sigmodon hirsutus* | F | 70 | El Cua |
| 14-Mar-06 | Nica-397 | 137066 | *Peromyscus nudipes* | M | 40 | El Cua |
| 14-Mar-06 | Nica-398 | 137063 | *Oryzomys cousei* | M | 50 | El Cua |
| 14-Mar-06 | Nica-399 | 137071 | *Melanomys caliginosus* | M | 30 | El Cua |
| 14-Mar-06 | Nica-400 | 137070 | *Mus musculus* | M | 7 | El Cua |
| 14-Mar-06 | Nica-401 | 137064 | *Melanomys caliginosus* | M | 45 | El Cua |
| 14-Mar-06 | Nica-402 | 137072 | *Mus musculus* | F | 10 | El Cua |
| 14-Mar-06 | Nica-403 | 137062 | *Mus musculus* | M | 5 | El Cua |
| 15-Mar-06 | Nica-404 | 137068 | *Oryzomys alfaroi* | M | 26 | El Cua |

Table A–1 continued.

| Date | UIW Number | Texas Tech TK # | Species ID | Sex | Weight grams | Locality |
|---|---|---|---|---|---|---|
| 15-Mar-06 | Nica-405 | 137060 | *Peromyscus nudipes* | M | 50 | El Cua |
| 15-Mar-06 | Nica-406 | 137065 | *Oryzomys alfaroi* | M | 35 | El Cua |
| 15-Mar-06 | Nica-407 | 133251 | *Peromyscus nudipes* | F | 45 | El Cua |
| 15-Mar-06 | Nica-408 | 137067 | *Oryzomys alfaroi* | M | 35 | El Cua |

# APPENDIX B

Table B−2. Human typical CL cases reported per municipality, 2003-2005.

| Municipality | Cases | Municipality | Cases |
|---|---|---|---|
| Achuapa | 0 | El Rosario | 0 |
| Acoyapa | 1 | El Sauce | 3 |
| Altagracia | 0 | El Tortuguero | 31 |
| Belen | 0 | El Tuma - La Dalia | 21 |
| Bluefields | 40 | El Viejo | 0 |
| Boaco | 2 | Esquipulas | 0 |
| Bonanza | 241 | Esteli | 0 |
| Buenos Aires | 0 | Granada | 1 |
| Camoapa | 0 | Jalapa | 3 |
| Cardenas | 0 | Jinotega | 25 |
| Catarina | 0 | Jinotepe | 0 |
| Chichigalpa | 2 | Juigalpa | 11 |
| Chinandega | 2 | Kukrahill | 10 |
| Cinco Pinos | 1 | La Concepcion | 0 |
| Ciudad Antigua | 0 | La Concordia | 0 |
| Ciudad Dario | 0 | La Conquista | 0 |
| Ciudad Sandino | 0 | La Cruz de Rio Grande | 64 |
| Comalapa | 1 | La Libertad | 0 |
| Condega | 0 | La Paz Centro | 0 |
| Corinto | 0 | La Paz de Carazo | 0 |
| Cua-Bocay | 1828 | La Trinidad | 0 |
| Desembocadura | 0 | Laguna de Perlas | 10 |
| Dipilto | 0 | Larreynaga | 0 |
| Diria | 0 | Las Sabanas | 0 |
| Diriamba | 0 | Leon | 1 |
| Diriomo | 0 | Macuelizo | 0 |
| Dolores | 0 | Managua | 3 |
| El Almendro | 4 | Masatepe | 1 |
| El Ayote | 18 | Masaya | 0 |
| El Castillo | 57 | Matagalpa | 10 |
| El Coral | 3 | Mateare | 0 |
| El Crucero | 0 | Matiguas | 7 |
| El Jicaral | 0 | Morrito | 0 |
| El Jicaro | 1 | Mosonte | 0 |
| El Rama | 146 | Moyogalpa | 0 |
| El Realejo | 0 | Mulle de los Bueyos | 11 |
| Murra | 6 | San Juan del Norte | 0 |
| Muy Muy | 0 | San Juan del Rio Coco | 0 |
| Nagarote | 0 | San Juan del Sur | 1 |
| Nandaime | 0 | San Lorenzo | 19 |
| Nandasmo | 0 | San Lucas | 0 |
| Nindiri | 1 | San Marcos | 0 |
| Niquinohomo | 1 | San Miguelito | 8 |

Table B−2 continued.

| Municipality | Cases | Municipality | Cases |
|---|---|---|---|
| Nueva Guinea | 494 | San Nicolas | 0 |
| Ocotal | 0 | San Pedro de Lovago | 1 |
| Paiwas | 1 | San Pedro Del Norte | 1 |
| Palacaguina | 0 | San Rafael del Norte | 5 |
| Posoltega | 1 | Santa Maria | 0 |
| Potosi | 0 | Santa Maria de Pantasma | 21 |
| Prinzapolka | 0 | Santa Rosa del Penon | 1 |
| Pueblo Nuevo | 0 | Santa Teresa | 0 |
| Puerto Cabezas | 45 | Santo Domingo | 10 |
| Puerto Morazan | 0 | Santo Tomas | 8 |
| Quezalguaque | 1 | Santo Tomas del Norte | 0 |
| Quilali | 2 | Sebaco | 1 |
| Rancho Grande | 412 | Siuna | 466 |
| Rio Blanco | 3 | Somotillo | 1 |
| Rivas | 0 | Somoto | 1 |
| Rosita | 207 | Telica | 0 |
| San Carlos | 95 | Telpaneca | 0 |
| San Dionisio | 1 | Terrabona | 0 |
| San Fernando | 0 | Teustepe | 1 |
| San Francisco de Cuapa | 0 | Ticuantepe | 2 |
| San Francisco del Norte | 1 | Tipitapa | 3 |
| San Francisco Libre | 0 | Tisma | 0 |
| San Isidro | 2 | Tola | 0 |
| San Jorge | 0 | Totogalpa | 0 |
| San Jose de Bocay | 818 | Villa Carlos Fonseca | 0 |
| San Jose de Cusmapa | 0 | Villa Sandino | 2 |
| San Jose de Los Remates | 1 | Villanueva | 1 |
| San Juan de Limay | 0 | Waslala | 423 |
| San Juan de Oriente | 0 | Waspan | 7 |
| San Rafael del Sur | 0 | Wiwili de Jinotega | 113 |
| San Ramon | 16 | Wiwili de Nueva Segovia | 0 |
| San Sebastian de Yali | 1 | Yalaguina | 0 |
| Santa Lucia | 0 | | |

# APPENDIX C

Table C−1. Area in square kilometers of temperature zones within municipalities.

| | Temperature Zones (Degrees Celsius) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 18.5 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.5 | 27.5 |
| Achuapa | 0 | 0 | 0 | 3 | 14 | 35 | 90 | 170 | 78 | 0 |
| Acoyapa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 1329 | 0 |
| Altagracia | 0 | 0 | 1 | 5 | 10 | 9 | 20 | 41 | 108 | 0 |
| Belen | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 22 | 0 |
| Bluefields | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 433 | 3650 | 311 |
| Boaco | 0 | 0 | 0 | 0 | 5 | 96 | 387 | 562 | 34 | 0 |
| Bonanza | 0 | 0 | 0 | 0 | 10 | 71 | 225 | 860 | 751 | 0 |
| Buenos Aires | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 0 |
| Camoapa | 0 | 0 | 0 | 0 | 3 | 9 | 266 | 1005 | 201 | 0 |
| Cardenas | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 20 | 169 | 3 |
| Catarina | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 3 | 0 | 0 |
| Chichigalpa | 0 | 0 | 0 | 0 | 2 | 4 | 4 | 18 | 85 | 108 |
| Chinandega | 0 | 1 | 1 | 9 | 12 | 19 | 40 | 59 | 508 | 8 |
| Cinco Pinos | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 47 | 0 | 0 |
| Ciudad Antigua | 0 | 0 | 0 | 0 | 0 | 60 | 66 | 0 | 0 | 0 |
| Ciudad Dario | 0 | 0 | 0 | 0 | 3 | 16 | 45 | 575 | 80 | 6 |
| Ciudad Sandino | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 42 | 0 |
| Comalapa | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 274 | 303 | 0 |
| Condega | 0 | 0 | 4 | 78 | 149 | 171 | 0 | 0 | 0 | 0 |
| Corinto | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 |
| Cua-Bocay | 0 | 6 | 16 | 29 | 93 | 261 | 353 | 41 | 3 | 0 |
| Desembocadura | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1716 | 0 |
| Dipilto | 0 | 3 | 3 | 51 | 40 | 7 | 0 | 0 | 0 | 0 |
| Diria | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 23 | 0 | 0 |
| Diriamba | 0 | 0 | 0 | 0 | 0 | 14 | 50 | 52 | 219 | 12 |
| Diriomo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 |
| Dolores | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| El Almendro | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 41 | 962 | 0 |
| El Ayote | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 138 | 687 | 0 |
| El Castillo | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 176 | 1460 | 0 |
| El Coral | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 303 | 0 |
| El Crucero | 0 | 0 | 0 | 0 | 7 | 67 | 80 | 66 | 3 | 0 |
| El Jicaral | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76 | 117 | 232 |
| El Jicaro | 0 | 0 | 0 | 0 | 26 | 164 | 240 | 0 | 0 | 0 |
| El Rama | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 54 | 3680 | 0 |

Table C−1 continued.

| | Temperature Zones (Degrees Celsius) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 18.5 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.5 | 27.5 |
| El Realejo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 89 |
| El Rosario | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| El Sauce | 0 | 0 | 0 | 0 | 0 | 41 | 74 | 144 | 434 | 0 |
| El Tortuguero | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3082 | 0 |
| El Tuma - La Dalia | 0 | 1 | 8 | 20 | 84 | 163 | 245 | 125 | 0 | 0 |
| El Viejo | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 53 | 640 | 467 |
| Esquipulas | 0 | 0 | 0 | 3 | 26 | 40 | 96 | 54 | 0 | 0 |
| Esteli | 0 | 0 | 13 | 233 | 428 | 99 | 17 | 0 | 0 | 0 |
| Granada | 0 | 0 | 0 | 2 | 5 | 10 | 19 | 57 | 388 | 33 |
| Jalapa | 0 | 0 | 16 | 46 | 110 | 383 | 90 | 0 | 0 | 0 |
| Jinotega | 0 | 12 | 88 | 197 | 403 | 92 | 24 | 0 | 0 | 0 |
| Jinotepe | 0 | 0 | 0 | 0 | 0 | 6 | 49 | 33 | 188 | 6 |
| Juigalpa | 0 | 0 | 0 | 0 | 0 | 10 | 45 | 107 | 554 | 0 |
| Kukrahill | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1116 | 67 |
| La Concepcion | 0 | 0 | 0 | 0 | 0 | 28 | 34 | 5 | 0 | 0 |
| La Concordia | 0 | 1 | 1 | 54 | 61 | 33 | 0 | 0 | 0 | 0 |
| La Conquista | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 59 | 0 |
| La Cruz de Rio Grande | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3315 | 0 |
| La Libertad | 0 | 0 | 0 | 0 | 0 | 17 | 262 | 400 | 96 | 0 |
| La Paz Centro | 0 | 0 | 0 | 0 | 0 | 11 | 5 | 26 | 273 | 347 |
| La Paz de Carazo | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 15 | 0 | 0 |
| La Trinidad | 0 | 0 | 0 | 13 | 40 | 111 | 113 | 0 | 0 | 0 |
| Laguna de Perlas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1810 | 128 |
| Larreynaga | 0 | 0 | 0 | 0 | 0 | 2 | 7 | 16 | 585 | 135 |
| Las Sabanas | 0 | 7 | 17 | 32 | 10 | 0 | 0 | 0 | 0 | 0 |
| Leon | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 30 | 329 | 417 |
| Macuelizo | 0 | 0 | 0 | 24 | 91 | 96 | 52 | 0 | 0 | 0 |
| Managua | 0 | 0 | 0 | 0 | 0 | 4 | 23 | 81 | 158 | 0 |
| Masatepe | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 13 | 0 | 0 |
| Masaya | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 27 | 114 | 0 |
| Matagalpa | 0 | 0 | 35 | 80 | 120 | 198 | 191 | 22 | 0 | 0 |
| Mateare | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76 | 209 | 3 |
| Matiguas | 0 | 0 | 0 | 16 | 34 | 74 | 334 | 886 | 184 | 0 |
| Morrito | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 665 | 0 |
| Mosonte | 3 | 22 | 18 | 20 | 23 | 45 | 81 | 0 | 0 | 0 |
| Moyogalpa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 53 | 0 |
| Mulle de los Bueyos | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 371 | 1025 | 0 |

Table C−1 continued.

| | Temperature Zones (Degrees Celsius) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 18.5 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.5 | 27.5 |
| Murra | 0 | 0 | 0 | 64 | 169 | 119 | 77 | 0 | 0 | 0 |
| Muy Muy | 0 | 0 | 0 | 2 | 6 | 31 | 60 | 280 | 0 | 0 |
| Nagarote | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 361 | 236 |
| Nandaime | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 347 | 0 |
| Nandasmo | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 3 | 0 | 0 |
| Nindiri | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 31 | 121 | 0 |
| Niquinohomo | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 10 | 0 | 0 |
| Nueva Guinea | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 187 | 2479 | 0 |
| Ocotal | 0 | 0 | 0 | 0 | 0 | 36 | 60 | 0 | 0 | 0 |
| Paiwas | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 533 | 1813 | 0 |
| Palacaguina | 0 | 0 | 0 | 0 | 9 | 62 | 94 | 0 | 0 | 0 |
| Posoltega | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 23 | 105 | 9 |
| Potosi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 144 | 0 |
| Prinzapolka | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 6693 | 0 |
| Pueblo Nuevo | 0 | 4 | 13 | 22 | 52 | 106 | 0 | 0 | 0 | 0 |
| Puerto Cabezas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1028 | 4714 | 0 |
| Puerto Morazan | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 360 | 87 |
| Quezalguaque | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 59 | 18 |
| Quilali | 0 | 0 | 0 | 0 | 33 | 109 | 122 | 83 | 0 | 0 |
| Rancho Grande | 0 | 0 | 0 | 10 | 32 | 174 | 137 | 136 | 102 | 0 |
| Rio Blanco | 0 | 0 | 0 | 4 | 10 | 15 | 70 | 296 | 278 | 0 |
| Rivas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 277 | 0 |
| Rosita | 0 | 0 | 0 | 0 | 0 | 2 | 7 | 101 | 2088 | 0 |
| San Carlos | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1348 | 2 |
| San Dionisio | 0 | 0 | 0 | 1 | 11 | 49 | 76 | 34 | 0 | 0 |
| San Fernando | 1 | 8 | 14 | 22 | 75 | 81 | 32 | 0 | 0 | 0 |
| San Francisco de Cuapa | 0 | 0 | 0 | 0 | 0 | 17 | 86 | 148 | 24 | 0 |
| San Francisco del Norte | 0 | 0 | 0 | 0 | 0 | 11 | 24 | 73 | 12 | 0 |
| San Francisco Libre | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 81 | 548 |
| San Isidro | 0 | 0 | 0 | 0 | 11 | 36 | 91 | 155 | 0 | 0 |
| San Jorge | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 |
| San Jose de Bocay | 0 | 3 | 3 | 8 | 38 | 465 | 786 | 2383 | 46 | 0 |
| San Jose de Cusmapa | 0 | 0 | 6 | 16 | 30 | 30 | 31 | 18 | 0 | 0 |
| San Jose de Los Remates | 0 | 0 | 0 | 1 | 49 | 42 | 146 | 43 | 0 | 0 |
| San Juan de Limay | 0 | 0 | 8 | 21 | 38 | 72 | 98 | 179 | 19 | 0 |
| San Juan de Oriente | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 1 | 0 | 0 |
| San Juan del Norte | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 12 | 1499 | 59 |

Table C−1 continued.

| | Temperature Zones (Degrees Celsius) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 18.5 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.5 | 27.5 |
| San Juan del Rio Coco | 0 | 0 | 0 | 25 | 70 | 59 | 23 | 10 | 0 | 0 |
| San Juan del Sur | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 120 | 279 | 0 |
| San Lorenzo | 0 | 0 | 0 | 0 | 0 | 10 | 55 | 136 | 339 | 0 |
| San Lucas | 0 | 0 | 3 | 33 | 57 | 58 | 0 | 0 | 0 | 0 |
| San Marcos | 0 | 0 | 0 | 0 | 0 | 53 | 45 | 18 | 3 | 0 |
| San Miguelito | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 1072 | 0 |
| San Nicolas | 0 | 0 | 5 | 26 | 39 | 56 | 34 | 14 | 0 | 0 |
| San Pedro de Lovago | 0 | 0 | 0 | 0 | 0 | 2 | 39 | 384 | 35 | 0 |
| San Pedro Del Norte | 0 | 0 | 2 | 6 | 7 | 20 | 21 | 10 | 0 | 0 |
| San Rafael del Norte | 0 | 4 | 13 | 83 | 100 | 35 | 0 | 0 | 0 | 0 |
| San Rafael del Sur | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 54 | 271 | 21 |
| San Ramon | 0 | 0 | 0 | 18 | 112 | 143 | 107 | 40 | 0 | 0 |
| San Sebastian de Yali | 0 | 3 | 12 | 40 | 159 | 141 | 38 | 11 | 0 | 0 |
| Santa Lucia | 0 | 0 | 0 | 0 | 15 | 31 | 34 | 38 | 8 | 0 |
| Santa Maria | 0 | 0 | 0 | 0 | 19 | 52 | 63 | 16 | 0 | 0 |
| Santa Maria de Pantasma | 0 | 0 | 0 | 0 | 63 | 76 | 219 | 191 | 0 | 0 |
| Santa Rosa del Penon | 0 | 0 | 0 | 0 | 0 | 9 | 39 | 107 | 71 | 0 |
| Santa Teresa | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 70 | 130 | 0 |
| Santo Domingo | 0 | 0 | 0 | 0 | 0 | 1 | 55 | 309 | 311 | 0 |
| Santo Tomas | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 358 | 74 | 0 |
| Santo Tomas del Norte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 11 | 0 |
| Sebaco | 0 | 0 | 0 | 2 | 16 | 28 | 136 | 109 | 0 | 0 |
| Siuna | 0 | 1 | 11 | 17 | 68 | 207 | 452 | 1054 | 3269 | 0 |
| Somotillo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 699 | 0 |
| Somoto | 0 | 0 | 0 | 18 | 83 | 245 | 112 | 0 | 0 | 0 |
| Telica | 0 | 0 | 0 | 0 | 0 | 11 | 28 | 58 | 293 | 0 |
| Telpaneca | 0 | 0 | 3 | 31 | 58 | 144 | 117 | 1 | 0 | 0 |
| Terrabona | 0 | 0 | 0 | 1 | 17 | 51 | 91 | 85 | 0 | 0 |
| Teustepe | 0 | 0 | 0 | 0 | 0 | 4 | 85 | 282 | 267 | 0 |
| Ticuantepe | 0 | 0 | 0 | 0 | 0 | 3 | 21 | 38 | 1 | 0 |
| Tipitapa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 637 | 208 |
| Tisma | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 109 | 5 |
| Tola | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 424 | 0 |
| Totogalpa | 0 | 0 | 0 | 0 | 15 | 81 | 51 | 0 | 0 | 0 |
| Villa Carlos Fonseca | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 47 | 403 | 97 |
| Villa Sandino | 0 | 0 | 0 | 0 | 0 | 0 | 69 | 431 | 183 | 0 |
| Villanueva | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 792 | 0 |

Table C−1 continued.

| | Temperature Zones (Degrees Celsius) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 18.5 | 19.5 | 20.5 | 21.5 | 22.5 | 23.5 | 24.5 | 25.5 | 26.5 | 27.5 |
| Waslala | 0 | 0 | 0 | 6 | 79 | 116 | 167 | 780 | 180 | 0 |
| Waspan | 0 | 0 | 0 | 0 | 2 | 24 | 92 | 1542 | 6820 | 0 |
| Wiwili de Jinotega | 2 | 12 | 24 | 43 | 134 | 562 | 672 | 851 | 146 | 1 |
| Wiwili de Nueva Segovia | 0 | 0 | 0 | 3 | 27 | 83 | 152 | 142 | 0 | 0 |
| Yalaguina | 0 | 0 | 0 | 0 | 0 | 61 | 9 | 0 | 0 | 0 |

# APPENDIX D

Table D–1. Area in square kilometers of mean annual precipitation zones within municipalities.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| Achuapa | 0 | 0 | 0 | 9 | 36 | 53 | 115 | 177 | 0 | 0 | 0 | 0 | 0 |
| Acoyapa | 0 | 0 | 0 | 0 | 84 | 1044 | 207 | 40 | 0 | 0 | 0 | 0 | 0 |
| Altagracia | 0 | 0 | 0 | 0 | 45 | 158 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Belen | 0 | 0 | 0 | 76 | 109 | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bluefields | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 186 | 818 | 2005 | 1400 |
| Boaco | 0 | 0 | 0 | 141 | 383 | 332 | 143 | 81 | 4 | 0 | 0 | 0 | 0 |
| Bonanza | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 71 | 313 | 1521 | 0 | 0 | 0 |
| Buenos Aires | 0 | 0 | 0 | 26 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Camoapa | 0 | 0 | 0 | 191 | 219 | 205 | 103 | 104 | 331 | 331 | 0 | 0 | 0 |
| Cardenas | 0 | 0 | 0 | 0 | 0 | 0 | 71 | 32 | 100 | 0 | 0 | 0 | 0 |
| Catarina | 0 | 0 | 0 | 0 | 10 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chichigalpa | 0 | 0 | 0 | 0 | 0 | 17 | 162 | 42 | 0 | 0 | 0 | 0 | 0 |
| Chinandega | 0 | 0 | 0 | 0 | 93 | 224 | 267 | 73 | 0 | 0 | 0 | 0 | 0 |
| Cinco Pinos | 0 | 0 | 0 | 0 | 40 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Antigua | 0 | 18 | 66 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Dario | 0 | 636 | 69 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Sandino | 0 | 0 | 0 | 1 | 40 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Comalapa | 0 | 0 | 0 | 600 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Condega | 198 | 158 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corinto | 0 | 0 | 0 | 0 | 0 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cua-Bocay | 0 | 0 | 0 | 0 | 0 | 802 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Desembocadura | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1219 | 512 | 0 |
| Dipilto | 0 | 24 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diria | 0 | 0 | 0 | 0 | 19 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diriamba | 0 | 0 | 0 | 0 | 0 | 351 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diriomo | 0 | 0 | 0 | 1 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolores | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Almendro | 0 | 0 | 0 | 0 | 0 | 9 | 316 | 468 | 210 | 0 | 0 | 0 | 0 |
| El Ayote | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 825 | 0 | 0 | 0 |

Table D–1 continued.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| El Castillo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 476 | 800 | 313 | 0 |
| El Coral | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 295 | 0 | 0 | 0 | 0 |
| El Crucero | 0 | 0 | 0 | 0 | 0 | 223 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Jicaral | 0 | 0 | 11 | 361 | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Jicaro | 0 | 0 | 2 | 215 | 213 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Rama | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 634 | 2562 | 546 | 0 |
| El Realejo | 0 | 0 | 0 | 0 | 0 | 69 | 27 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Rosario | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Sauce | 0 | 0 | 0 | 26 | 128 | 252 | 204 | 83 | 0 | 0 | 0 | 0 | 0 |
| El Tortuguero | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 113 | 2606 | 363 | 0 |
| El Tuma - La Dalia | 0 | 0 | 0 | 0 | 100 | 422 | 114 | 10 | 0 | 0 | 0 | 0 | 0 |
| El Viejo | 0 | 0 | 0 | 0 | 0 | 944 | 195 | 77 | 0 | 0 | 0 | 0 | 0 |
| Esquipulas | 0 | 0 | 10 | 53 | 145 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Esteli | 0 | 526 | 204 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Granada | 0 | 0 | 242 | 157 | 184 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jalapa | 0 | 0 | 0 | 0 | 168 | 475 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jinotega | 0 | 0 | 19 | 136 | 338 | 337 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jinotepe | 0 | 0 | 0 | 0 | 76 | 212 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Juigalpa | 0 | 0 | 0 | 346 | 183 | 189 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kukrahill | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 1178 | 0 |
| La Concepcion | 0 | 0 | 0 | 0 | 0 | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Concordia | 0 | 99 | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Conquista | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Cruz de Rio Grande | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 390 | 2936 | 0 | 0 |
| La Libertad | 0 | 0 | 0 | 0 | 93 | 180 | 140 | 79 | 127 | 156 | 0 | 0 | 0 |
| La Paz Centro | 0 | 0 | 0 | 357 | 159 | 168 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Paz de Carazo | 0 | 0 | 0 | 0 | 11 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Trinidad | 0 | 245 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Laguna de Perlas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 1925 | 0 |
| Larreynaga | 0 | 0 | 0 | 365 | 290 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Las Sabanas | 0 | 0 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table D–1 continued.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| Leon | 0 | 0 | 0 | 0 | 51 | 601 | 154 | 0 | 0 | 0 | 0 | 0 | 0 |
| Macuelizo | 0 | 112 | 151 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Managua | 0 | 0 | 0 | 86 | 76 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Masatepe | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Masaya | 0 | 0 | 4 | 30 | 103 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Matagalpa | 0 | 27 | 66 | 199 | 315 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mateare | 0 | 0 | 0 | 167 | 114 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Matiguas | 0 | 0 | 0 | 0 | 0 | 0 | 99 | 261 | 1144 | 24 | 0 | 0 | 0 |
| Morrito | 0 | 0 | 0 | 0 | 0 | 569 | 97 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mosonte | 27 | 90 | 66 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Moyogalpa | 0 | 0 | 0 | 0 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mulle de los Bueyos | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 159 | 1195 | 42 | 0 | 0 |
| Murra | 0 | 0 | 0 | 93 | 256 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Muy Muy | 0 | 0 | 0 | 0 | 3 | 239 | 136 | 1 | 0 | 0 | 0 | 0 | 0 |
| Nagarote | 0 | 0 | 0 | 33 | 179 | 388 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nandaime | 0 | 0 | 0 | 368 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nandasmo | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nindiri | 0 | 0 | 1 | 48 | 83 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Niquinohomo | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nueva Guinea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1070 | 1329 | 271 | 2 | 0 |
| Ocotal | 0 | 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paiwas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 134 | 2219 | 0 | 0 | 0 |
| Palacaguina | 118 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Posoltega | 0 | 0 | 0 | 0 | 0 | 0 | 136 | 14 | 0 | 0 | 0 | 0 | 0 |
| Potosi | 0 | 0 | 0 | 88 | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prinzapolka | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1491 | 5298 | 0 | 0 |
| Pueblo Nuevo | 0 | 48 | 88 | 61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Puerto Cabezas | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 57 | 1803 | 3247 | 644 | 0 | 0 |
| Puerto Morazan | 0 | 0 | 0 | 0 | 189 | 263 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| Quezalguaque | 0 | 0 | 0 | 0 | 0 | 0 | 82 | 0 | 0 | 0 | 0 | 0 | 0 |
| Quilali | 0 | 0 | 0 | 100 | 247 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table D–1 continued.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| Rancho Grande | 0 | 0 | 0 | 0 | 0 | 59 | 255 | 146 | 135 | 0 | 0 | 0 | 0 |
| Rio Blanco | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 673 | 0 | 0 | 0 | 0 |
| Rivas | 0 | 0 | 0 | 0 | 144 | 125 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rosita | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1176 | 1023 | 0 | 0 | 0 |
| San Carlos | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 617 | 650 | 90 | 0 | 0 | 0 |
| San Dionisio | 0 | 0 | 0 | 67 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Fernando | 0 | 4 | 13 | 108 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco de Cuapa | 0 | 0 | 0 | 158 | 105 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco del Norte | 0 | 0 | 0 | 0 | 64 | 54 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco Libre | 0 | 11 | 272 | 370 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Isidro | 0 | 240 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Jorge | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Jose de Bocay | 0 | 0 | 0 | 0 | 0 | 2070 | 590 | 1066 | 9 | 0 | 0 | 0 | 0 |
| San Jose de Cusmapa | 0 | 0 | 0 | 9 | 103 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Jose de Los Remates | 0 | 73 | 58 | 105 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Juan de Limay | 0 | 0 | 24 | 75 | 116 | 116 | 68 | 36 | 0 | 0 | 0 | 0 | 0 |
| San Juan de Oriente | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Juan del Norte | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 553 | 1015 |
| San Juan del Rio Coco | 0 | 0 | 93 | 94 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Juan del Sur | 0 | 0 | 0 | 0 | 1 | 291 | 127 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Lorenzo | 0 | 0 | 342 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Lucas | 0 | 0 | 76 | 74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Marcos | 0 | 0 | 0 | 0 | 0 | 119 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Miguelito | 0 | 0 | 0 | 0 | 0 | 2 | 440 | 406 | 256 | 0 | 0 | 0 | 0 |
| San Nicolas | 0 | 2 | 98 | 67 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Pedro de Lovago | 0 | 0 | 0 | 0 | 119 | 110 | 53 | 44 | 134 | 0 | 0 | 0 | 0 |
| San Pedro Del Norte | 0 | 0 | 0 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Rafael del Norte | 0 | 1 | 114 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Rafael del Sur | 0 | 0 | 0 | 0 | 0 | 360 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table D–1 continued.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| San Ramon | 0 | 0 | 0 | 0 | 178 | 206 | 36 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Sebastian de Yali | 7 | 113 | 185 | 99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Lucia | 0 | 0 | 9 | 117 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Maria | 0 | 0 | 149 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Maria de Pantasma | 0 | 0 | 0 | 189 | 297 | 63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Rosa del Penon | 0 | 0 | 28 | 142 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Teresa | 0 | 0 | 0 | 59 | 105 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Domingo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 314 | 316 | 0 | 0 | 0 |
| Santo Tomas | 0 | 0 | 0 | 0 | 0 | 110 | 99 | 57 | 182 | 44 | 0 | 0 | 0 |
| Santo Tomas del Norte | 0 | 0 | 0 | 0 | 5 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sebaco | 0 | 222 | 59 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Siuna | 0 | 0 | 0 | 0 | 0 | 1101 | 557 | 687 | 1761 | 976 | 0 | 0 | 0 |
| Somotillo | 0 | 0 | 0 | 0 | 412 | 218 | 83 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somoto | 0 | 146 | 313 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Telica | 0 | 0 | 0 | 0 | 48 | 103 | 239 | 0 | 0 | 0 | 0 | 0 | 0 |
| Telpaneca | 149 | 96 | 108 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Terrabona | 0 | 87 | 104 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Teustepe | 0 | 261 | 360 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ticuantepe | 0 | 0 | 0 | 0 | 1 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipitapa | 0 | 240 | 706 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tisma | 0 | 0 | 74 | 38 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tola | 0 | 0 | 0 | 0 | 11 | 467 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totogalpa | 95 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villa Carlos Fonseca | 0 | 0 | 0 | 0 | 5 | 556 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villa Sandino | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 189 | 407 | 8 | 0 | 0 | 0 |
| Villanueva | 0 | 0 | 0 | 0 | 40 | 215 | 539 | 2 | 0 | 0 | 0 | 0 | 0 |
| Waslala | 0 | 0 | 0 | 0 | 0 | 408 | 373 | 320 | 231 | 0 | 0 | 0 | 0 |
| Waspan | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5212 | 2840 | 455 | 0 | 0 | 0 |
| Wiwili de Jinotega | 0 | 0 | 0 | 0 | 202 | 1792 | 371 | 89 | 0 | 0 | 0 | 0 | 0 |

Table D–1 continued.

| | Mean Annual Precipitation Zones (millimeters) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | <800 | 800-900 | 900-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 | 2000-2400 | 2400-2800 | 2800-3200 | 3200-4000 | >4000 |
| Wiwili de Nueva Segovia | 0 | 0 | 0 | 0 | 379 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Yalaguina | 13 | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

# APPENDIX E

Table E–1. Area in square kilometers of elevation zones within municipalities.

| | Elevation Zones (Height Above MSL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
| Achuapa | 2 | 40 | 189 | 78 | 64 | 13 | 4 | 0 | 0 | 0 | 0 |
| Acoyapa | 898 | 402 | 73 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Altagracia | 62 | 46 | 43 | 21 | 13 | 10 | 7 | 1 | 0 | 0 | 0 |
| Belen | 46 | 169 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bluefields | 2801 | 1071 | 552 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Boaco | 0 | 0 | 499 | 420 | 151 | 14 | 0 | 0 | 0 | 0 | 0 |
| Bonanza | 30 | 775 | 773 | 295 | 42 | 3 | 0 | 0 | 0 | 0 | 0 |
| Buenos Aires | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Camoapa | 0 | 60 | 1000 | 389 | 31 | 4 | 0 | 0 | 0 | 0 | 0 |
| Cardenas | 139 | 34 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Catarina | 0 | 1 | 1 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chichigalpa | 155 | 31 | 24 | 7 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chinandega | 371 | 106 | 81 | 46 | 23 | 16 | 11 | 3 | 0 | 0 | 0 |
| Cinco Pinos | 0 | 1 | 44 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Antigua | 0 | 0 | 0 | 0 | 107 | 19 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Dario | 0 | 4 | 50 | 483 | 157 | 35 | 1 | 0 | 0 | 0 | 0 |
| Ciudad Sandino | 3 | 28 | 16 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Comalapa | 91 | 190 | 290 | 74 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Condega | 0 | 0 | 0 | 0 | 173 | 141 | 74 | 14 | 0 | 0 | 0 |
| Corinto | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cua-Bocay | 0 | 0 | 1 | 297 | 278 | 152 | 46 | 15 | 13 | 0 | 0 |
| Desembocadura | 1732 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dipilto | 0 | 0 | 0 | 0 | 0 | 14 | 55 | 33 | 2 | 0 | 0 |
| Diria | 0 | 2 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diriamba | 144 | 69 | 60 | 52 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diriomo | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table E–1 continued.

| | Elevation Zones (Height Above MSL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
| Dolores | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Almendro | 23 | 466 | 514 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Ayote | 4 | 541 | 280 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Castillo | 502 | 789 | 350 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Coral | 0 | 277 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Crucero | 0 | 2 | 63 | 80 | 58 | 20 | 0 | 0 | 0 | 0 | 0 |
| El Jicaral | 227 | 48 | 87 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Jicaro | 0 | 0 | 0 | 114 | 263 | 52 | 1 | 0 | 0 | 0 | 0 |
| El Rama | 2334 | 1212 | 189 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Realejo | 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Rosario | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Sauce | 26 | 282 | 221 | 115 | 37 | 12 | 0 | 0 | 0 | 0 | 0 |
| El Tortuguero | 2257 | 804 | 20 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Tuma - La Dalia | 0 | 0 | 64 | 225 | 186 | 109 | 49 | 12 | 1 | 0 | 0 |
| El Viejo | 844 | 193 | 94 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Esquipulas | 0 | 0 | 39 | 99 | 43 | 37 | 1 | 0 | 0 | 0 | 0 |
| Esteli | 0 | 0 | 0 | 13 | 41 | 318 | 303 | 110 | 5 | 0 | 0 |
| Granada | 367 | 103 | 74 | 22 | 11 | 5 | 1 | 0 | 0 | 0 | 0 |
| Jalapa | 0 | 0 | 0 | 34 | 365 | 129 | 64 | 49 | 4 | 0 | 0 |
| Jinotega | 0 | 0 | 0 | 7 | 57 | 214 | 406 | 97 | 49 | 0 | 0 |
| Jinotepe | 94 | 84 | 45 | 50 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Juigalpa | 318 | 245 | 116 | 38 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kukrahill | 1143 | 34 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Concepcion | 0 | 0 | 0 | 32 | 31 | 4 | 0 | 0 | 0 | 0 | 0 |
| La Concordia | 0 | 0 | 0 | 0 | 7 | 70 | 35 | 35 | 3 | 0 | 0 |
| La Conquista | 0 | 36 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Cruz de Rio Grande | 2443 | 877 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Libertad | 0 | 48 | 340 | 356 | 31 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Paz Centro | 357 | 234 | 51 | 23 | 12 | 7 | 0 | 0 | 0 | 0 | 0 |

Table E–1 continued.

| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Elevation Zones (Height Above MSL) | | | | | | | | | | |
| La Paz de Carazo | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Trinidad | 0 | 0 | 0 | 14 | 126 | 100 | 28 | 9 | 0 | 0 | 0 |
| Laguna de Perlas | 1898 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Larreynaga | 448 | 215 | 67 | 10 | 4 | 1 | 0 | 0 | 0 | 0 | 0 |
| Las Sabanas | 0 | 0 | 0 | 0 | 0 | 1 | 27 | 29 | 9 | 0 | 0 |
| Leon | 516 | 157 | 91 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Macuelizo | 0 | 0 | 0 | 2 | 66 | 111 | 72 | 12 | 0 | 0 | 0 |
| Managua | 65 | 65 | 89 | 37 | 9 | 1 | 0 | 0 | 0 | 0 | 0 |
| Masatepe | 0 | 0 | 13 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Masaya | 7 | 50 | 85 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Matagalpa | 0 | 0 | 20 | 73 | 233 | 160 | 83 | 72 | 5 | 0 | 0 |
| Mateare | 85 | 60 | 150 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Matiguas | 0 | 47 | 917 | 402 | 113 | 27 | 21 | 1 | 0 | 0 | 0 |
| Morrito | 468 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mosonte | 0 | 0 | 0 | 0 | 98 | 40 | 18 | 22 | 18 | 14 | 2 |
| Moyogalpa | 36 | 21 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mulle de los Bueyos | 95 | 656 | 645 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Murra | 0 | 0 | 0 | 45 | 88 | 171 | 119 | 6 | 0 | 0 | 0 |
| Muy Muy | 0 | 0 | 283 | 57 | 29 | 9 | 1 | 0 | 0 | 0 | 0 |
| Nagarote | 367 | 194 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nandaime | 149 | 178 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nandasmo | 0 | 0 | 4 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nindiri | 14 | 68 | 63 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Niquinohomo | 0 | 0 | 11 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nueva Guinea | 341 | 1610 | 720 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ocotal | 0 | 0 | 0 | 0 | 72 | 22 | 2 | 0 | 0 | 0 | 0 |
| Paiwas | 134 | 1317 | 888 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Palacaguina | 0 | 0 | 0 | 0 | 156 | 9 | 0 | 0 | 0 | 0 | 0 |
| Posoltega | 42 | 47 | 45 | 11 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |

Table E–1 continued.

| | Elevation Zones (Height Above MSL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
| Potosi | 144 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prinzapolka | 6763 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pueblo Nuevo | 0 | 0 | 0 | 0 | 92 | 47 | 34 | 17 | 6 | 1 | 0 |
| Puerto Cabezas | 5619 | 156 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Puerto Morazan | 400 | 35 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Quezalguaque | 44 | 26 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Quilali | 0 | 0 | 36 | 122 | 124 | 61 | 4 | 0 | 0 | 0 | 0 |
| Rancho Grande | 0 | 15 | 176 | 135 | 156 | 91 | 17 | 5 | 0 | 0 | 0 |
| Rio Blanco | 0 | 178 | 341 | 111 | 25 | 10 | 6 | 2 | 0 | 0 | 0 |
| Rivas | 223 | 53 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rosita | 1770 | 347 | 74 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Carlos | 934 | 403 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Dionisio | 0 | 0 | 17 | 67 | 60 | 27 | 0 | 0 | 0 | 0 | 0 |
| San Fernando | 0 | 0 | 0 | 0 | 59 | 82 | 50 | 20 | 15 | 6 | 1 |
| San Francisco de Cuapa | 0 | 32 | 131 | 75 | 36 | 1 | 0 | 0 | 0 | 0 | 0 |
| San Francisco del Norte | 0 | 8 | 51 | 46 | 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco Libre | 374 | 149 | 94 | 27 | 7 | 2 | 0 | 0 | 0 | 0 | 0 |
| San Isidro | 0 | 0 | 0 | 173 | 76 | 37 | 7 | 0 | 0 | 0 | 0 |
| San Jorge | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Jose de Bocay | 0 | 158 | 2203 | 1074 | 250 | 43 | 5 | 2 | 0 | 0 | 0 |
| San Jose de Cusmapa | 0 | 0 | 13 | 32 | 29 | 35 | 18 | 4 | 0 | 0 | 0 |
| San Jose Los Remates | 0 | 0 | 10 | 142 | 70 | 53 | 6 | 0 | 0 | 0 | 0 |
| San Juan de Limay | 0 | 10 | 160 | 103 | 77 | 50 | 24 | 9 | 2 | 0 | 0 |
| San Juan de Oriente | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Juan del Norte | 1241 | 263 | 67 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Juan del Rio Coco | 0 | 0 | 0 | 18 | 38 | 78 | 38 | 15 | 0 | 0 | 0 |
| San Juan del Sur | 142 | 143 | 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Lorenzo | 246 | 64 | 155 | 70 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Lucas | 0 | 0 | 0 | 0 | 27 | 69 | 35 | 16 | 5 | 0 | 0 |

Table E–1 continued.

| | Elevation Zones (Height Above MSL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
| San Marcos | 0 | 4 | 18 | 43 | 53 | 1 | 0 | 0 | 0 | 0 | 0 |
| San Miguelito | 394 | 659 | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Nicolas | 0 | 0 | 1 | 28 | 57 | 49 | 25 | 14 | 0 | 0 | 0 |
| San Pedro de Lovago | 0 | 22 | 368 | 61 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Pedro Del Norte | 0 | 0 | 11 | 20 | 19 | 9 | 4 | 2 | 1 | 0 | 0 |
| San Rafael del Norte | 0 | 0 | 0 | 0 | 28 | 26 | 118 | 55 | 8 | 0 | 0 |
| San Rafael del Sur | 178 | 95 | 70 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Ramon | 0 | 0 | 39 | 93 | 123 | 108 | 54 | 3 | 0 | 0 | 0 |
| San Sebastian de Yali | 0 | 0 | 0 | 37 | 89 | 181 | 73 | 19 | 5 | 0 | 0 |
| Santa Lucia | 0 | 3 | 45 | 31 | 29 | 18 | 0 | 0 | 0 | 0 | 0 |
| Santa Maria | 0 | 0 | 0 | 62 | 61 | 24 | 3 | 0 | 0 | 0 | 0 |
| Santa Maria de Pantasma | 0 | 0 | 22 | 328 | 98 | 77 | 24 | 0 | 0 | 0 | 0 |
| Santa Rosa del Penon | 0 | 0 | 104 | 89 | 27 | 6 | 0 | 0 | 0 | 0 | 0 |
| Santa Teresa | 38 | 52 | 105 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Domingo | 0 | 136 | 443 | 88 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Tomas | 1 | 16 | 371 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Tomas del Norte | 1 | 22 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sebaco | 0 | 0 | 0 | 167 | 96 | 23 | 4 | 1 | 0 | 0 | 0 |
| Siuna | 752 | 2413 | 1111 | 555 | 150 | 69 | 24 | 8 | 0 | 0 | 0 |
| Somotillo | 611 | 94 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somoto | 0 | 0 | 0 | 8 | 254 | 136 | 55 | 6 | 0 | 0 | 0 |
| Telica | 106 | 114 | 109 | 46 | 14 | 1 | 0 | 0 | 0 | 0 | 0 |
| Telpaneca | 0 | 0 | 0 | 0 | 217 | 92 | 34 | 11 | 0 | 0 | 0 |
| Terrabona | 0 | 0 | 11 | 122 | 66 | 40 | 6 | 0 | 0 | 0 | 0 |
| Teustepe | 6 | 116 | 317 | 156 | 40 | 3 | 0 | 0 | 0 | 0 | 0 |
| Ticuantepe | 0 | 0 | 27 | 25 | 10 | 1 | 0 | 0 | 0 | 0 | 0 |
| Tipitapa | 604 | 107 | 149 | 95 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tisma | 80 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tola | 278 | 146 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix E. Area in square kilometers of elevation zones within municipalities.

| | | | | | Elevation Zones (Height Above MSL) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 0-100 | 100-200 | 200-400 | 400-600 | 600-800 | 800-1000 | 1000-1200 | 1200-1400 | 1400-1600 | 1600-1800 | 1800-2000 |
| Totogalpa | 0 | 0 | 0 | 0 | 92 | 50 | 5 | 0 | 0 | 0 | 0 |
| Villa Carlos Fonseca | 281 | 165 | 77 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villa Sandino | 0 | 58 | 535 | 78 | 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villanueva | 651 | 134 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Waslala | 0 | 100 | 756 | 256 | 138 | 72 | 10 | 0 | 0 | 0 | 0 |
| Waspan | 4646 | 2543 | 1156 | 179 | 7 | 2 | 0 | 0 | 0 | 0 | 0 |
| Wiwili de Jinotega | 0 | 166 | 610 | 866 | 500 | 201 | 59 | 36 | 16 | 0 | 0 |
| Wiwili de Nueva Segovia | 0 | 0 | 15 | 210 | 109 | 65 | 8 | 0 | 0 | 0 | 0 |
| Yalaguina | 0 | 0 | 0 | 0 | 62 | 8 | 0 | 0 | 0 | 0 | 0 |

# APPENDIX F

Table F–1. Area in square kilometers of soil zones within municipalities.

| | Soil Type Zones | | | | | | |
|---|---|---|---|---|---|---|---|
| Municipality | Heavy Clay | Clay | Loamy Sand | Sandy | Loam | Clay Loam | Sandy Loam |
| Achuapa | 0 | 0 | 0 | 0 | 1 | 389 | 0 |
| Acoyapa | 745 | 90 | 0 | 0 | 16 | 524 | 0 |
| Altagracia | 0 | 83 | 0 | 0 | 23 | 63 | 32 |
| Belen | 69 | 149 | 0 | 0 | 24 | 1 | 0 |
| Bluefields | 0 | 4410 | 0 | 0 | 0 | 0 | 0 |
| Boaco | 40 | 445 | 0 | 0 | 0 | 599 | 0 |
| Bonanza | 0 | 1918 | 0 | 0 | 0 | 0 | 0 |
| Buenos Aires | 4 | 0 | 0 | 0 | 13 | 19 | 14 |
| Camoapa | 7 | 1013 | 0 | 0 | 0 | 401 | 63 |
| Cardenas | 0 | 202 | 0 | 0 | 0 | 0 | 0 |
| Catarina | 0 | 0 | 0 | 0 | 0 | 9 | 0 |
| Chichigalpa | 25 | 0 | 0 | 0 | 132 | 3 | 61 |
| Chinandega | 46 | 150 | 0 | 0 | 197 | 46 | 218 |
| Cinco Pinos | 0 | 0 | 0 | 0 | 0 | 61 | 0 |
| Ciudad Antigua | 0 | 0 | 0 | 0 | 70 | 53 | 3 |
| Ciudad Dario | 145 | 0 | 0 | 0 | 0 | 585 | 0 |
| Ciudad Sandino | 0 | 0 | 11 | 0 | 0 | 26 | 12 |
| Comalapa | 131 | 138 | 0 | 0 | 38 | 332 | 6 |
| Condega | 0 | 33 | 0 | 0 | 41 | 328 | 0 |
| Corinto | 9 | 0 | 0 | 0 | 0 | 30 | 0 |
| Cua-Bocay | 0 | 802 | 0 | 0 | 0 | 0 | 0 |
| Desembocadura | 0 | 1731 | 0 | 0 | 0 | 0 | 0 |
| Dipilto | 0 | 0 | 0 | 0 | 0 | 63 | 41 |
| Diria | 0 | 0 | 0 | 0 | 6 | 18 | 0 |
| Diriamba | 0 | 187 | 0 | 0 | 34 | 130 | 0 |
| Diriomo | 0 | 0 | 0 | 0 | 46 | 6 | 1 |
| Dolores | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| El Almendro | 147 | 815 | 0 | 0 | 0 | 41 | 0 |
| El Ayote | 0 | 825 | 0 | 0 | 0 | 0 | 0 |
| El Castillo | 0 | 1642 | 0 | 0 | 0 | 0 | 0 |
| El Coral | 0 | 312 | 0 | 0 | 0 | 0 | 0 |
| El Crucero | 0 | 1 | 0 | 0 | 138 | 84 | 0 |

Table F–1 continued.

| | Soil Type Zones | | | | | | |
|---|---|---|---|---|---|---|---|
| Municipality | Heavy Clay | Clay | Loamy Sand | Sandy | Loam | Clay Loam | Sandy Loam |
| El Jicaral | 125 | 82 | 0 | 0 | 70 | 151 | 0 |
| El Jicaro | 0 | 57 | 2 | 0 | 210 | 161 | 0 |
| El Realejo | 26 | 0 | 0 | 0 | 58 | 12 | 0 |
| El Rosario | 0 | 0 | 0 | 0 | 2 | 11 | 0 |
| El Sauce | 76 | 0 | 0 | 0 | 0 | 617 | 0 |
| El Tortuguero | 0 | 3082 | 0 | 0 | 0 | 0 | 0 |
| El Tuma - La Dalia | 0 | 646 | 0 | 0 | 0 | 0 | 0 |
| El Viejo | 155 | 62 | 302 | 0 | 256 | 413 | 28 |
| Esquipulas | 0 | 78 | 0 | 0 | 0 | 141 | 0 |
| Esteli | 23 | 168 | 0 | 0 | 271 | 328 | 0 |
| Granada | 241 | 46 | 0 | 0 | 21 | 130 | 138 |
| Jalapa | 34 | 77 | 77 | 0 | 204 | 251 | 0 |
| Jinotega | 0 | 518 | 0 | 0 | 0 | 297 | 0 |
| Jinotepe | 30 | 159 | 0 | 0 | 35 | 64 | 0 |
| Juigalpa | 220 | 172 | 0 | 0 | 133 | 193 | 0 |
| Kukrahill | 0 | 1184 | 0 | 0 | 0 | 0 | 0 |
| La Concepcion | 0 | 0 | 0 | 0 | 39 | 0 | 28 |
| La Concordia | 0 | 112 | 0 | 0 | 0 | 38 | 0 |
| La Conquista | 0 | 66 | 0 | 0 | 0 | 23 | 0 |
| La Cruz de Rio Grande | 0 | 3326 | 0 | 0 | 0 | 0 | 0 |
| La Libertad | 0 | 749 | 0 | 0 | 21 | 5 | 0 |
| La Paz Centro | 204 | 73 | 50 | 0 | 73 | 230 | 51 |
| La Paz de Carazo | 0 | 0 | 0 | 0 | 0 | 17 | 0 |
| La Trinidad | 2 | 14 | 0 | 0 | 0 | 261 | 0 |
| Laguna de Perlas | 0 | 1931 | 0 | 0 | 0 | 0 | 0 |
| Larreynaga | 324 | 123 | 0 | 0 | 31 | 173 | 94 |
| Las Sabanas | 0 | 0 | 0 | 0 | 0 | 66 | 0 |
| Leon | 200 | 72 | 61 | 12 | 146 | 166 | 149 |
| Macuelizo | 0 | 2 | 0 | 0 | 0 | 176 | 85 |
| Managua | 0 | 0 | 3 | 0 | 32 | 148 | 83 |
| Masatepe | 0 | 0 | 0 | 0 | 31 | 12 | 16 |
| Masaya | 0 | 0 | 0 | 0 | 0 | 124 | 22 |
| Matagalpa | 4 | 345 | 0 | 0 | 0 | 297 | 0 |
| Mateare | 0 | 0 | 7 | 0 | 0 | 271 | 13 |

Table F−1 continued.

| | Soil Type Zones | | | | | | |
|---|---|---|---|---|---|---|---|
| Municipality | Heavy Clay | Clay | Loamy Sand | Sandy | Loam | Clay Loam | Sandy Loam |
| Matiguas | 73 | 1455 | 0 | 0 | 0 | 0 | 0 |
| Morrito | 367 | 223 | 0 | 0 | 0 | 76 | 0 |
| Moyogalpa | 0 | 5 | 0 | 0 | 20 | 0 | 37 |
| Mulle de los Bueyos | 0 | 1396 | 0 | 0 | 0 | 0 | 0 |
| Murra | 0 | 253 | 0 | 0 | 0 | 176 | 0 |
| Muy Muy | 164 | 174 | 0 | 0 | 0 | 41 | 0 |
| Nagarote | 201 | 0 | 0 | 0 | 99 | 295 | 5 |
| Nandaime | 109 | 118 | 0 | 0 | 9 | 87 | 47 |
| Nandasmo | 0 | 0 | 0 | 0 | 3 | 7 | 4 |
| Nindiri | 0 | 0 | 0 | 0 | 12 | 35 | 106 |
| Niquinohomo | 0 | 0 | 0 | 0 | 0 | 32 | 0 |
| Nueva Guinea | 0 | 2672 | 0 | 0 | 0 | 0 | 0 |
| Ocotal | 0 | 0 | 0 | 0 | 0 | 58 | 38 |
| Paiwas | 0 | 2324 | 0 | 0 | 0 | 29 | 0 |
| Palacaguina | 0 | 0 | 0 | 0 | 0 | 165 | 0 |
| Posoltega | 7 | 0 | 0 | 0 | 45 | 0 | 98 |
| Potosi | 110 | 0 | 0 | 0 | 16 | 10 | 10 |
| Prinzapolka | 0 | 6789 | 0 | 0 | 0 | 0 | 0 |
| Pueblo Nuevo | 0 | 2 | 0 | 0 | 18 | 177 | 0 |
| Puerto Cabezas | 0 | 5771 | 0 | 0 | 0 | 0 | 0 |
| Puerto Morazan | 259 | 0 | 0 | 0 | 89 | 120 | 0 |
| Quezalguaque | 34 | 0 | 0 | 0 | 21 | 6 | 21 |
| Quilali | 0 | 192 | 0 | 0 | 73 | 82 | 0 |
| Rancho Grande | 0 | 595 | 0 | 0 | 0 | 0 | 0 |
| Rio Blanco | 0 | 673 | 0 | 0 | 0 | 0 | 0 |
| Rivas | 110 | 48 | 0 | 0 | 33 | 83 | 0 |
| Rosita | 0 | 2199 | 0 | 0 | 0 | 0 | 0 |
| San Carlos | 0 | 1358 | 0 | 0 | 0 | 0 | 0 |
| San Dionisio | 0 | 64 | 0 | 0 | 0 | 107 | 0 |
| San Fernando | 0 | 14 | 92 | 0 | 95 | 18 | 14 |
| San Francisco de Cuapa | 0 | 64 | 0 | 0 | 0 | 208 | 3 |
| San Francisco del Norte | 0 | 4 | 0 | 0 | 0 | 116 | 0 |
| San Francisco Libre | 304 | 58 | 0 | 0 | 125 | 156 | 0 |

Table F–1 continued.

| | Soil Type Zones | | | | | | |
|---|---|---|---|---|---|---|---|
| Municipality | Heavy Clay | Clay | Loamy Sand | Sandy | Loam | Clay Loam | Sandy Loam |
| San Isidro | 137 | 0 | 0 | 0 | 0 | 156 | 0 |
| San Jorge | 0 | 0 | 0 | 0 | 15 | 8 | 0 |
| San Jose de Bocay | 0 | 3735 | 0 | 0 | 0 | 0 | 0 |
| San Jose de Cusmapa | 0 | 47 | 0 | 0 | 0 | 84 | 0 |
| San Jose de Los Remates | 0 | 66 | 0 | 0 | 0 | 215 | 0 |
| San Juan de Limay | 10 | 93 | 0 | 0 | 36 | 296 | 0 |
| San Juan de Oriente | 0 | 0 | 0 | 0 | 0 | 8 | 0 |
| San Juan del Norte | 0 | 1568 | 0 | 0 | 0 | 0 | 0 |
| San Juan del Rio Coco | 0 | 0 | 0 | 0 | 119 | 68 | 0 |
| San Juan del Sur | 135 | 284 | 0 | 0 | 0 | 0 | 0 |
| San Lorenzo | 252 | 124 | 0 | 0 | 0 | 175 | 0 |
| San Lucas | 0 | 79 | 0 | 0 | 0 | 71 | 0 |
| San Marcos | 0 | 4 | 0 | 0 | 95 | 17 | 3 |
| San Miguelito | 150 | 942 | 0 | 0 | 0 | 12 | 0 |
| San Nicolas | 26 | 0 | 0 | 0 | 0 | 148 | 0 |
| San Pedro de Lovago | 1 | 349 | 0 | 0 | 33 | 77 | 0 |
| San Pedro Del Norte | 0 | 0 | 0 | 0 | 0 | 66 | 0 |
| San Rafael del Norte | 0 | 156 | 0 | 0 | 0 | 79 | 0 |
| San Rafael del Sur | 0 | 103 | 0 | 0 | 100 | 157 | 0 |
| San Ramon | 6 | 349 | 0 | 0 | 0 | 65 | 0 |
| San Sebastian de Yali | 0 | 295 | 0 | 0 | 0 | 109 | 0 |
| Santa Lucia | 6 | 51 | 0 | 0 | 0 | 59 | 0 |
| Santa Maria | 0 | 0 | 0 | 0 | 0 | 90 | 59 |
| Santa Maria de Pantasma | 0 | 496 | 0 | 0 | 0 | 53 | 0 |
| Santa Rosa del Penon | 20 | 0 | 0 | 0 | 0 | 206 | 0 |
| Santa Teresa | 4 | 124 | 0 | 0 | 0 | 82 | 0 |
| Santo Domingo | 0 | 676 | 0 | 0 | 0 | 0 | 0 |

Table F−1 continued.

| Municipality | Soil Type Zones<br>Heavy Clay | Clay | Loamy Sand | Sandy | Loam | Clay Loam | Sandy Loam |
|---|---|---|---|---|---|---|---|
| Santo Tomas | 1 | 467 | 0 | 0 | 0 | 24 | 0 |
| Santo Tomas del Norte | 0 | 0 | 0 | 0 | 0 | 41 | 0 |
| Sebaco | 76 | 0 | 0 | 0 | 0 | 215 | 0 |
| Siuna | 0 | 5082 | 0 | 0 | 0 | 0 | 0 |
| Somotillo | 416 | 15 | 0 | 0 | 76 | 206 | 0 |
| Somoto | 0 | 216 | 0 | 0 | 63 | 86 | 94 |
| Telica | 81 | 3 | 0 | 3 | 48 | 33 | 222 |
| Telpaneca | 0 | 0 | 0 | 0 | 29 | 325 | 0 |
| Terrabona | 27 | 0 | 0 | 0 | 0 | 218 | 0 |
| Teustepe | 77 | 284 | 0 | 0 | 80 | 197 | 0 |
| Ticuantepe | 0 | 0 | 0 | 0 | 22 | 3 | 38 |
| Tipitapa | 473 | 197 | 0 | 0 | 166 | 117 | 0 |
| Tisma | 22 | 0 | 0 | 0 | 0 | 92 | 0 |
| Tola | 255 | 178 | 0 | 0 | 32 | 12 | 0 |
| Totogalpa | 0 | 7 | 0 | 0 | 0 | 132 | 8 |
| Villa Carlos Fonseca | 64 | 119 | 0 | 0 | 6 | 373 | 0 |
| Villa Sandino | 1 | 576 | 0 | 0 | 0 | 106 | 0 |
| Villanueva | 336 | 53 | 0 | 0 | 1 | 406 | 0 |
| Waslala | 0 | 1332 | 0 | 0 | 0 | 0 | 0 |
| Waspan | 0 | 8521 | 0 | 0 | 0 | 0 | 0 |
| Wiwili de Jinotega | 0 | 2454 | 0 | 0 | 0 | 0 | 0 |
| Wiwili de Nueva Segovia | 0 | 384 | 0 | 0 | 0 | 23 | 0 |
| Yalaguina | 0 | 0 | 0 | 0 | 0 | 70 | 0 |

# APPENDIX G

Table G–1. Area in square kilometers of land cover/use classification zones within municipalities.

| | Area (km$^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Achuapa | 0 | 8 | 15 | 339 | 0 | 0 | 0 | 0 | 11 | 6 | 11 | 0 | 0 |
| Acoyapa | 0 | 27 | 247 | 906 | 0 | 0 | 0 | 71 | 0 | 93 | 0 | 0 | 0 |
| Altagracia | 0 | 9 | 57 | 36 | 0 | 0 | 0 | 0 | 0 | 91 | 0 | 0 | 3 |
| Belen | 0 | 88 | 103 | 17 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 |
| Bluefields | 2 | 0 | 2 | 150 | 19 | 49 | 32 | 0 | 0 | 0 | 8 | 1532 | 2665 |
| Boaco | 1 | 0 | 471 | 612 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bonanza | 0 | 0 | 15 | 350 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 1528 |
| Buenos Aires | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Camoapa | 0 | 0 | 788 | 688 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| Cardenas | 0 | 6 | 6 | 127 | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 | 20 |
| Catarina | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chichigalpa | 0 | 183 | 3 | 24 | 1 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| Chinandega | 6 | 303 | 6 | 52 | 24 | 0 | 11 | 0 | 0 | 252 | 3 | 0 | 0 |
| Cinco Pinos | 0 | 14 | 0 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ciudad Antigua | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 65 | 13 | 47 | 0 | 0 |
| Ciudad Dario | 0 | 65 | 93 | 485 | 0 | 0 | 0 | 72 | 0 | 15 | 0 | 0 | 0 |
| Ciudad Sandino | 0 | 27 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| Comalapa | 0 | 58 | 240 | 26 | 0 | 0 | 0 | 0 | 293 | 22 | 0 | 0 | 2 |
| Condega | 0 | 40 | 71 | 291 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corinto | 2 | 2 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cua-Bocay | 0 | 41 | 3 | 690 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 45 |
| Desembocadura | 0 | 0 | 0 | 0 | 0 | 193 | 135 | 0 | 0 | 0 | 168 | 176 | 1044 |
| Dipilto | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 100 | 0 | 0 |
| Diria | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 |

Table G–1 continued.

| | Area ($km^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Diriamba | 0 | 26 | 72 | 141 | 0 | 0 | 0 | 5 | 0 | 106 | 0 | 0 | 0 |
| Diriomo | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Dolores | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Almendro | 0 | 0 | 650 | 329 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 |
| El Ayote | 0 | 0 | 722 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| El Castillo | 0 | 0 | 61 | 135 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1446 |
| El Coral | 0 | 0 | 299 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Crucero | 0 | 0 | 0 | 140 | 0 | 0 | 0 | 0 | 0 | 83 | 0 | 0 | 0 |
| El Jicaral | 0 | 53 | 16 | 165 | 0 | 0 | 0 | 175 | 0 | 20 | 0 | 0 | 0 |
| El Jicaro | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 18 | 376 | 0 | 18 |
| El Rama | 1 | 0 | 2029 | 1436 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 23 | 235 |
| El Realejo | 0 | 75 | 0 | 0 | 11 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 |
| El Rosario | 0 | 0 | 5 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| El Sauce | 0 | 0 | 312 | 291 | 0 | 0 | 0 | 7 | 0 | 83 | 0 | 0 | 0 |
| El Tortuguero | 0 | 0 | 1546 | 394 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 21 | 1117 |
| El Tuma - La Dalia | 0 | 289 | 78 | 255 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| El Viejo | 4 | 464 | 11 | 235 | 141 | 10 | 4 | 17 | 0 | 331 | 0 | 0 | 0 |
| Esquipulas | 0 | 0 | 110 | 105 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Esteli | 7 | 15 | 79 | 605 | 0 | 0 | 0 | 0 | 38 | 0 | 37 | 0 | 9 |
| Granada | 11 | 190 | 77 | 58 | 0 | 0 | 0 | 17 | 0 | 156 | 0 | 0 | 2 |
| Jalapa | 0 | 229 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 316 | 0 | 0 |
| Jinotega | 2 | 168 | 184 | 299 | 0 | 0 | 0 | 0 | 55 | 13 | 0 | 0 | 91 |
| Jinotepe | 0 | 26 | 112 | 40 | 0 | 0 | 0 | 33 | 0 | 76 | 0 | 0 | 0 |
| Juigalpa | 1 | 180 | 201 | 223 | 0 | 0 | 0 | 43 | 10 | 17 | 0 | 0 | 25 |
| Kukrahill | 0 | 0 | 0 | 300 | 0 | 70 | 437 | 0 | 0 | 0 | 46 | 0 | 330 |
| La Concepcion | 0 | 0 | 0 | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Concordia | 0 | 0 | 105 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 |

Table G–1 continued.

| | Area (km$^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| La Conquista | 0 | 3 | 74 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 |
| La Cruz de Rio Grande | 0 | 0 | 993 | 985 | 0 | 0 | 5 | 0 | 0 | 0 | 35 | 32 | 1276 |
| La Libertad | 0 | 0 | 538 | 234 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| La Paz Centro | 2 | 137 | 106 | 95 | 0 | 0 | 0 | 25 | 0 | 313 | 0 | 0 | 0 |
| La Paz de Carazo | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| La Trinidad | 0 | 2 | 36 | 219 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 |
| Laguna de Perlas | 0 | 0 | 0 | 88 | 1 | 446 | 258 | 0 | 0 | 0 | 12 | 2 | 1128 |
| Larreynaga | 0 | 199 | 278 | 93 | 0 | 0 | 0 | 120 | 0 | 55 | 0 | 0 | 0 |
| Las Sabanas | 0 | 23 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 |
| Leon | 15 | 304 | 88 | 154 | 39 | 3 | 0 | 72 | 0 | 123 | 0 | 0 | 0 |
| Macuelizo | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 57 | 170 | 0 | 0 |
| Managua | 67 | 79 | 36 | 30 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 |
| Masatepe | 0 | 0 | 0 | 57 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Masaya | 0 | 10 | 57 | 79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Matagalpa | 1 | 0 | 338 | 124 | 0 | 0 | 0 | 0 | 4 | 136 | 0 | 0 | 43 |
| Mateare | 0 | 102 | 9 | 0 | 0 | 0 | 0 | 3 | 0 | 177 | 0 | 0 | 0 |
| Matiguas | 0 | 0 | 1056 | 409 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 63 |
| Morrito | 0 | 35 | 385 | 43 | 0 | 0 | 0 | 51 | 0 | 133 | 0 | 0 | 0 |
| Mosonte | 0 | 1 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 41 | 164 | 0 | 0 |
| Moyogalpa | 0 | 35 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 |
| Mulle de los Bueyos | 0 | 0 | 1396 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Murra | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 366 | 44 | 0 | 0 |
| Muy Muy | 0 | 0 | 293 | 86 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nagarote | 0 | 6 | 332 | 1 | 7 | 0 | 0 | 146 | 0 | 104 | 0 | 0 | 0 |
| Nandaime | 0 | 142 | 121 | 34 | 0 | 0 | 0 | 23 | 0 | 40 | 0 | 0 | 0 |
| Nandasmo | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nindiri | 0 | 28 | 97 | 11 | 0 | 0 | 3 | 0 | 3 | 14 | 0 | 0 | 0 |

Table G−1 continued.

| | Area (km$^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Niquinohomo | 0 | 0 | 1 | 26 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 |
| Nueva Guinea | 0 | 0 | 1335 | 524 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 810 |
| Ocotal | 2 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 45 | 0 | 0 |
| Paiwas | 0 | 0 | 2158 | 137 | 0 | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 4 |
| Palacaguina | 1 | 96 | 0 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Posoltega | 0 | 80 | 17 | 28 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 |
| Potosi | 0 | 129 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prinzapolka | 0 | 0 | 13 | 542 | 29 | 326 | 424 | 0 | 0 | 53 | 2296 | 469 | 2616 |
| Pueblo Nuevo | 0 | 121 | 0 | 69 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 |
| Puerto Cabezas | 0 | 0 | 222 | 120 | 124 | 89 | 912 | 0 | 0 | 15 | 2178 | 0 | 2116 |
| Puerto Morazan | 0 | 38 | 0 | 73 | 260 | 3 | 0 | 0 | 0 | 95 | 0 | 0 | 0 |
| Quezalguaque | 0 | 61 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |
| Quilali | 0 | 49 | 6 | 7 | 0 | 0 | 0 | 0 | 0 | 96 | 31 | 0 | 158 |
| Rancho Grande | 0 | 87 | 262 | 191 | 0 | 0 | 0 | 0 | 0 | 42 | 0 | 0 | 13 |
| Rio Blanco | 0 | 0 | 418 | 77 | 0 | 0 | 0 | 0 | 0 | 133 | 0 | 0 | 45 |
| Rivas | 0 | 198 | 45 | 21 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Rosita | 0 | 0 | 55 | 630 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 72 | 1425 |
| San Carlos | 0 | 0 | 589 | 400 | 0 | 0 | 167 | 0 | 0 | 0 | 0 | 18 | 148 |
| San Dionisio | 0 | 0 | 16 | 110 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 |
| San Fernando | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 229 | 0 | 0 |
| San Francisco de Cuapa | 0 | 16 | 247 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco del Norte | 0 | 2 | 0 | 118 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Francisco Libre | 0 | 94 | 59 | 105 | 0 | 0 | 0 | 265 | 0 | 102 | 0 | 0 | 0 |
| San Isidro | 0 | 94 | 71 | 73 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 0 | 0 |
| San Jorge | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Jose de Bocay | 0 | 0 | 170 | 699 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 2848 |
| San Jose de Cusmapa | 0 | 0 | 0 | 91 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 |

Table G–1 continued.

| | Area (km$^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| San Jose de Los Remates | 0 | 0 | 18 | 192 | 0 | 0 | 0 | 0 | 0 | 71 | 0 | 0 | 0 |
| San Juan de Limay | 1 | 13 | 59 | 339 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 |
| San Juan de Oriente | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| San Juan del Norte | 1 | 0 | 1 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 | 1428 |
| San Juan del Rio Coco | 0 | 1 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 65 | 6 | 0 | 108 |
| San Juan del Sur | 0 | 67 | 51 | 137 | 0 | 0 | 0 | 0 | 0 | 165 | 0 | 0 | 0 |
| San Lorenzo | 0 | 74 | 144 | 57 | 0 | 0 | 0 | 4 | 189 | 71 | 0 | 0 | 0 |
| San Lucas | 0 | 110 | 2 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 |
| San Marcos | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 |
| San Miguelito | 0 | 0 | 399 | 621 | 0 | 0 | 0 | 33 | 0 | 1 | 0 | 0 | 0 |
| San Nicolas | 0 | 0 | 19 | 155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Pedro de Lovago | 0 | 0 | 374 | 86 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| San Pedro Del Norte | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 |
| San Rafael del Norte | 0 | 9 | 74 | 135 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 |
| San Rafael del Sur | 0 | 35 | 0 | 225 | 0 | 0 | 0 | 0 | 0 | 99 | 0 | 0 | 0 |
| San Ramon | 0 | 44 | 121 | 157 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 43 |
| San Sebastian de Yali | 0 | 5 | 41 | 307 | 0 | 0 | 0 | 0 | 0 | 1 | 31 | 0 | 19 |
| Santa Lucia | 0 | 0 | 0 | 81 | 0 | 0 | 0 | 0 | 10 | 35 | 0 | 0 | 0 |
| Santa Maria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 137 | 0 | 0 |
| Santa Maria de Pantasma | 0 | 168 | 0 | 359 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| Santa Rosa del Penon | 0 | 0 | 15 | 211 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santa Teresa | 0 | 0 | 149 | 22 | 0 | 0 | 0 | 0 | 0 | 39 | 0 | 0 | 0 |
| Santo Domingo | 0 | 0 | 313 | 363 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Tomas | 0 | 0 | 401 | 91 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Santo Tomas del Norte | 0 | 4 | 0 | 0 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sebaco | 0 | 56 | 168 | 18 | 0 | 0 | 0 | 4 | 45 | 0 | 0 | 0 | 0 |
| Siuna | 1 | 0 | 639 | 3186 | 0 | 0 | 0 | 0 | 0 | 342 | 0 | 0 | 914 |

Table G−1 continued.

| | Area ($km^2$) of classification zones* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Municipality | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Somotillo | 0 | 53 | 236 | 66 | 126 | 0 | 0 | 65 | 0 | 95 | 0 | 0 | 0 |
| Somoto | 1 | 126 | 18 | 4 | 0 | 0 | 0 | 132 | 146 | 0 | 32 | 0 | 0 |
| Telica | 0 | 170 | 27 | 50 | 0 | 0 | 0 | 1 | 0 | 142 | 0 | 0 | 0 |
| Telpaneca | 0 | 8 | 0 | 200 | 0 | 0 | 0 | 0 | 45 | 71 | 1 | 0 | 29 |
| Terrabona | 0 | 17 | 133 | 82 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| Teustepe | 0 | 0 | 57 | 136 | 0 | 0 | 0 | 12 | 186 | 231 | 0 | 0 | 0 |
| Ticuantepe | 0 | 2 | 3 | 56 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Tipitapa | 0 | 318 | 110 | 118 | 0 | 0 | 0 | 231 | 28 | 137 | 0 | 0 | 0 |
| Tisma | 0 | 50 | 28 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tola | 0 | 91 | 189 | 35 | 0 | 0 | 0 | 0 | 0 | 163 | 0 | 0 | 0 |
| Totogalpa | 0 | 64 | 0 | 69 | 0 | 0 | 0 | 0 | 0 | 9 | 5 | 0 | 0 |
| Villa Carlos Fonseca | 0 | 50 | 333 | 67 | 0 | 0 | 0 | 0 | 0 | 111 | 0 | 0 | 0 |
| Villa Sandino | 0 | 0 | 610 | 73 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Villanueva | 0 | 21 | 345 | 224 | 0 | 0 | 0 | 126 | 0 | 80 | 0 | 0 | 0 |
| Waslala | 0 | 0 | 509 | 639 | 0 | 0 | 0 | 0 | 0 | 184 | 0 | 0 | 0 |
| Waspan | 0 | 0 | 217 | 595 | 19 | 45 | 49 | 0 | 0 | 63 | 2077 | 428 | 5043 |
| Wiwili de Jinotega | 0 | 34 | 303 | 632 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1485 |
| Wiwili de Nueva Segovia | 0 | 2 | 149 | 66 | 0 | 0 | 0 | 0 | 0 | 117 | 0 | 0 | 73 |
| Yalaguina | 0 | 28 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* 1−Urban areas. 2−Cropland. 3−Agricultural areas with 10–25% natural vegetation. 4−Agricultural areas with 25–50% natural vegetation. 5−Mangroves. 6−Coastal transition vegetation. 7−Savannah. 8−Grassland with deciduous shrubs. 9−Shrubland. 10−Deciduous broadleaf seasonal forest. 11−Tropical evergreen pine forest. 12−Tropical evergreen swamp forest. 13−Tropical evergreen broadleaf seasonal forest.

# VITA

Russell Wayne Raymond received an A.S. in Aviation Technology from Southwest Texas Junior College in 1983. He worked for Doss Aviation as an instructor pilot in the United States Air Force Flight Screening Program and later with Northrop Grumman as a production test pilot at the T-3A assembly plant in Hondo, Texas. He received a B.S. in Biology in 1998 and an M.S. in Biology in 2000 from the University of the Incarnate Word in San Antonio, Texas where his research centered on the ecology of *Leishmania mexicana* within woodrat (*Neotoma* sp.) populations. He received a Ph.D. in 2008 from Texas A&M University in College Station, Texas. He currently is employed as an Instructor of Biology at the University of the Incarnate Word and can be reached at: University of the Incarnate Word, Biology Department CPO 311, 4301 Broadway, San Antonio, TX 78209.