TRANSMISSION OF *TRYPANOSOMA* SPECIES AND THE HOST ROLE OF *PROECHIMYS SEMISPINOSUS* IN PANAMA

By Ehren W. Snyder

Parasites, particularly those that utilize a vector, play an important role throughout tropical forests. Their prevalence and distribution depend on the distribution of their vectors. Wherever these vectors are found, there also has to be a suitable host for the parasite to persist in the environment. Due to their abundance, diversity, and ubiquity throughout tropical forest, rodents present an ideal host group for scientific inquiry. The Central American spiny rat, *Proechimys semispinosus*, has been the target of many such studies because of its abundance in disturbed forest, susceptibility to infection, and ease of sampling. Previous studies have documented its endo- and ectoparasites, some of which infect humans. One such parasite is *Trypanosoma cruzi* (causal agent of Chagas' disease). It has been identified infecting *P. semispinosus* in Central Panama, but preliminary findings indicate another trypanosome also may be present. My study sought evidence to identify the trypanosomes that were infecting *P. semispinosus* in Central Panama, the putative vector, and the potential role of the spiny rat as a host.

Trypanosoma lewisi and *T. renjifoi*, parasites closely related to *T. cruzi*, have been described infecting various *Proechimys* species in Central and South America but have never been found in Panama. Recent studies have identified a parasite similar to *T. lewisi* infecting *P. semispinosus*, but its transmission cycle is largely unknown. I studied how this trypanosome persists in the environment and the conditions that promote its transmission. *Proechimys semispinosus* was sampled by live-trapping in degraded forest fragments in Central Panama. A blood sample, two blood smears, and fleas (if present) were collected from each captured individual. Blood samples were used for molecular tests, blood smears for light microscopic scans, and fleas for *T. renjifoi* infection.

I hypothesized that *T. renjifoi* would be present in degraded forests near people, that *P. semispinosus* would be a competent reservoir, and that fleas would be the vector. Preliminary results showed that all 14 captured rats were negative for both trypanosome infection and flea infestation during the first sampling season. The second season resulted in 13 total captures of rats, of which 6 were infested with fleas. One collected flea from the rats was infected with a trypanosome (presumably *T. renjifoi*). However, more flea sampling needs to be conducted to further understand conditions that promote transmission.

This study provides a model for how a vector-borne parasite can be described and as a basis for further studies of the transmission of *T. renjifoi*. It also begins to describe one of the many factors that may affect *T. cruzi* in its natural environment. Understanding the entirety of this ecosystem is necessary prior to implementation of disease control methods and for conservation purposes.

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by

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I dedicate this work first and foremost to Nature. May its boundless beauty flourish, and may we find our place. Also to my parents Gene & Millie for giving me the opportunity to practice science, my brother Stefan, and the rest of my family for endless support. And to Christina, for always being there.

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Introduction To the Thesis: The Role of Spiny Rats, *Proechimys semispinosus*, as Parasite Hosts

Introduction

Across evolutionary time, parasitism has become the most common lifestyle in the biological world (Price, 1980). The vast majority of parasites are microscopic and have been coevolving for millions of years with their hosts (Stevens et al., 1999). Due to the diversity and ubiquity of parasites, they greatly impact organisms in virtually all environments (Tompkins & Begon, 1999). Studying these impacts and the relationships within are of great interest to ecologists who attempt to describe interactions within ecosystems.

Parasites receive a benefit at the expense of their host (Bush et al., 2001). Most parasites do not cause disease in their hosts but by definition lower host fitness. Some parasites infect humans, and a portion of those are pathogenic (i.e., cause disease). Examples include *Plasmodium* spp. (malaria), *Schistosoma* spp. (schistosomiasis), and *Trypanosoma* spp. (Chagas' disease and sleeping sickness). Scientists estimate the impact of parasitic diseases by calculating the Years of Life Lost (YLL) due to mortality and Years Lost due to Disability (YLD) for those living with a parasite. The sum of these calculations is the Disability Adjusted Life Year (DALY) (WHO, 2017a). Recent estimates showed humans experienced a total of 96 million DALY's due to parasitic

disease in 2014 (Jensen, 2015). This figure illustrates the need to gather as much scientific knowledge as possible about these organisms, but challenges arise during these studies when attempting to study the parasite.

Due to the evolutionary longevity of parasite-host relationships, many have become incredibly complex. Parasites are under selection pressures to maintain the relationship, while their hosts are under selection pressures to dissociate from the relationship. An evolutionary trend toward specialization of parasites has made it difficult to prevent them from causing infection. Because of the small size and poor mobility of many parasites, a common adaptation is the use of a vector for dispersal to a new host individual. A vector transmits a parasite between hosts, and it determines the host range of the parasite (Craig et al., 1999). The use of a vector further complicates disease prevention and control. Such programs aim to slow transmission by limiting or avoiding the vector, targeting a specific life stage, or preventing infection of intermediate or definitive hosts (Lardans & Dissous, 1998). Previous control methods include spraying insecticides (e.g., DDT) to kill mosquitoes (vectors of many pathogenic parasites, including those that cause malaria) and black flies (vectors of the cause of river blindness) (Christophers, 1949; Resh et al., 2004). Studies such as that by McLaren (1989) assessed potential disease control by targeting different life stages in *Schistosoma* spp. Thus after identifying several possible control strategies, scientists can then focus on the most effective methods and can implement one or multiple control strategies.

Because of the high disease burden on humans and economic importance of the Panama Canal, Central Panama has been the location of many disease-transmission studies (Lips et al., 2006; Pineda et al., 2008; Campbell-Lendrum et al., 2001). Such studies documented, for instance, the life cycles and transmission of Chagas' disease, leishmaniasis, filariasis, and malaria. During these studies, many mammals were identified as important parasite reservoirs. Those identified as reservoirs were mammals that harbored the parasite, often asymptomatically, prior to the parasite infecting another host (e.g., humans). Examples of reservoirs included common opossums (*Didelphis marsupialis*), two-toed sloths (*Choloepus hoffmanni*), and many species of rodents (Saldaña, pers. comm.). In situations where pathogenic parasites utilize such a diverse reservoir community, it is unlikely that disease transmission can be prevented by targeting the reservoirs.

Many such reservoirs simultaneously harbor more than one parasite and are unable to rid themselves of infection. In such situations, simply treating the human population for the disease will not be successful because the parasite is still in the environment. Once vectors feed on infected reservoirs, they can reinfect humans. Such reservoirs are more effective if they are abundant across a range of environments and live in close proximity to other host populations (Haydon et al., 2002).

One such reservoir for multiple parasites is the Central American spiny rat (*Proechimys semispinosus*). It is abundant in forests throughout Central America, including Panama, and it is susceptible to infection by many different parasites (Travi et

al., 2002; Mendez, 1993), some of which may use it not only as a definitive host but also as a reservoir. While the spiny rat's life history (Adler & Beatty, 1997; Adler, 2000) and role in forest regeneration (Carvajal & Adler, 2008; Dittel et al., 2015) have been well documented, its role as a definitive host and reservoir has been less quantified. Because of its abundance and ubiquity throughout lowland forests in Central Panama and the diversity of parasites that it harbors, the spiny rat is an ideal host, and it is therefore important to determine its impact in such ecosystems. Of particular interest are endoparasites that are pathogenic to humans, and quantifying their impact on human health is of paramount importance.

In addition to hosting ectoparasites such as fleas, ticks, lice, and mites, (Mendez, 1993; Winker, 2013), spiny rats also harbor endoparasites. These include trypanosomes, filarial worms, *Leishmania* spp., *Plasmodium* spp., and multiple bacterial and viral pathogens (Lisboa et al., 2009; Mackenzie, 1972; WHO, 1990; and Winker, 2013). Of these, it carries species of trypanosomes (*T. cruzi*), *Leishmania* (*L. guyanensis*, *L. mexicana*, and *L. amazonensis*), and bacteria (*Rickettsia rickettsi*) that are pathogenic to humans; therefore, any organism that is capable of carrying such a variety of pathogens needs to be recognized as a major participant in both sylvatic and domestic transmission. It is beyond the scope of this study to include viral and bacterial pathogens in spiny rats, but its capacity to carry organisms such as *Salmonella* spp. (Kourany et al., 1976), *Rickettsia* spp. (Londoño et al., 2014), and viruses (e.g., vesicular stomatitis Indiana virus) (Tesh et al., 1970) should still be recognized. Not only is *P. semispinosus*

important because of the diversity of its parasitic burden but also because of where it lives.

Spiny rats often live close to humans, are frequently abundant, and may harbor multiple potentially-zoonotic parasites; thus, they may be an important species in disease emergence within their geographical distribution. Obtaining information on such reservoirs will be paramount as new pathogens infect humans (Cuervo et al., 2015). As a representative species among those that have such high parasite loads, spiny rats may offer clues toward future disease prevention research.

Previous Studies

Previous studies have sampled spiny rats and documented their parasites in multiple locations throughout Panama. The first comprehensive ectoparasite study on *P. semispinosus* in Panama was conducted by Tipton & Mendez (1966), where they documented all species of fleas, lice, ticks, and mites that were known to infest the rats. Mendez (1993) sampled throughout Central Panama and recorded ectoparasite numbers on *P. semispinosus* as part of a broad Panamanian rodent study. He found seven species of fleas infesting *P. semispinosus*, of which 99% were *Polygenis klagesi*, and Winker (2013) found only *P. klagesi* infesting the rats. These studies found eight different tick species infesting spiny rats, and one (*Amblyomma cajennense*) is known to vector *Rickettsia rickettsi*, which causes Rocky Mountain spotted fever (Kolonin, 2009). Also, nine species of mites were found on spiny rats, and 99% were *Tur uniscutatus.* Of the

total louse species, 70.5% were *Gyropus setifer* in the Winker (2013) study, but these are not known pathogen vectors. Winker (2013) characterized ectoparasitic burdens in relation to microhabitat characteristics in which the rats were sampled and also documented filarial worm and trypanosome infections using light microscopy. Sampling occurred along Pipeline Road in Soberania National Park, near Gamboa, Panama. This sampling site was primarily second-growth tropical moist forest and had been regenerating for approximately 70 years.

Proechimys semispinosus is also a suitable definitive host for bot fly larvae (*Cuterebra* spp.). Bots infest many domesticated and sylvatic mammals, including rodents (Catts, 1982). Adler et al. (2003) examined the prevalence of spiny rat bot infestation in Central Panama. Over a nine-year sampling period, bots infested 4.6% of rats, with as many as four bots infesting a single rat. While bots do not cause major disease in humans, they are a pest in livestock, sometimes causing death (Colwell et al., 2006). Bots are typically host-specific (Catts, 1982), and those that infest *P. semispinosus* are not known to cause disease. However, it presents an opportunity to investigate their relationship with other rat parasites and to find differences with other bot species.

Besides documenting ectoparasites, endoparasites in spiny rats have also been investigated. While spiny rats are known to carry *Plasmodium* spp. and multiple species of filarial nematodes, their role has not been thoroughly investigated. *Plasmodium* infections are rare (<1%) in *P. semispinosus* (Saldaña, pers. comm.), and there is no existing literature pertaining to reservoir competency. While it is unlikely that these

rodents play a critical role in malaria transmission, it would be of interest to definitively establish the efficacy of spiny rats in such transmission. Filarial nematodes, however, infect spiny rats more frequently. Mendez (1993) identified *Litomosoides hoplomyis* and *Dipetalonema raposoensis* infecting *P. semispinosus* in Panama. Winker (2013) reported a 15.8% infection rate in Panama, and other studies documented filarial infection in different *Proechimys* spp. from South America. Guerrero and Bain (2001) found four spp. of filarial nematodes infecting two *Proechimys* spp. in Venezuela, and Notarnicola et al. (2012) found infected *Proechimys* in Bolivia (all *Molinema* spp.). This is consistent with the vectors of these parasites (mosquitoes, Loiseau et al., 1996 and mites, Guerrero et al., 2011) because both parasitize spiny rats and likely help maintain filarial nematodes in rat populations. However, present literature does not contain work on their transmission efficacy. While it is probable that *P. semispinosus* affects the persistence and transmission of filarial nematodes, more evidence is necessary to draw this conclusion. Despite the lack of research on these two endoparasites, more work has been conducted on their role as definitive hosts of *Leishmania* spp.

After Anon (1957 & 1959) first isolated a *Leishmania* species from *P. semispinosus*, studies such as Herrer et al. (1971) characterized the importance of spiny rats as reservoirs of the disease. These leishmanial protozoans are vectored through sandfly bites during blood meals (WHO, 2017d), and infect an estimated 12 million people worldwide (WHO, 2017b). Spiny rats are susceptible to leishmanial infection, and up to 15% harbor the parasite (Travi et al., 2002; Lainson et al., 1994; Saldaña, pers.

comm.). They are ideal for sandflies to feed on because they are terrestrial (Herrer et al., 1971), and Travi et al. (2002) demonstrated that rats infected with *Leishmania panamensis* were infective to vectors (*Lutzomyia* spp.). Six anthropophilic *Lutzomyia* spp. have been found in Panama, and these may be able to transmit leishmanial protozoans to both humans and spiny rats (Dutari & Loaiza, 2014). Anon (1957 & 1959) and Travi et al. (2002) noted that infected individuals did not appear to be substantially affected, indicating greater reservoir suitability. These factors have all led to their incrimination as competent reservoirs of *Leishmania*. The ubiquity, abundance, and proximity of spiny rats to humans further compound their impact on the persistence of sylvatic and domestic leishmaniasis in Panama.

Although *P. semispinosus* harbors many endoparasites, *Trypanosoma cruzi* has received more attention because of its insidious impact on human health (Schmunis & Yadon, 2010). This parasite causes Chagas' disease and infects 6 to 7 million people in Central and South America (WHO, 2017c). Numerous studies have identified trypanosomes naturally infecting spiny rats (Yeo et al., 2005; Telford et al., 1975; Sousa, 2016; Solís & Carlos, 2016; Whitlaw & Chaniotis, 1978), but Vasquez et al. (2004) did not find rat DNA in blood meal samples from a major *T. cruzi* vector (*Rhodnius pallescens*). Infection rates were 1-5% in these studies, but none tested reservoir competency. Thus, further studies are necessary to elucidate their transmission efficacy of *T. cruzi*. Such work is of greater importance if these rodents are suitable definitive hosts for *T. cruzi*. Despite the intense focus on *T. cruzi* infections, this lack of knowledge

leaves many unanswered questions. For instance, other species of *Trypanosoma* may also infect *P. semispinosus*, which may hinder our ability to fully understand the transmission dynamics of *T. cruzi.*

Research Goals

In addition to the aforementioned studies, trypanosomes were also found by both Saldaña (pers. comm.) and Winker (2013) in *P. semispinosus* in Central Panama but were not completely described or identified to species. I used results from both of those studies and collected additional data to more precisely describe this trypanosome. Saldaña (pers. comm.) sampled spiny rats in multiple studies and noted that some of the trypanosomes in blood did not match the morphology of *T. cruzi* (Hoare, 1972; Auxiliadora de Sousa, 2014). Furthermore, molecular techniques failed to find evidence of infection by trypanosomes, despite trypanosomes being found by light microscopy by Winker (2013). My study accounted for such discrepancies and provided more evidence toward identifying this trypanosome, determining the vector, and establishing the likelihood of spiny rats as a definitive host.

Saldaña (pers. comm.) examined ectoparasitic infestations and infection by trypanosomes, leishmanial protozoans, and filarial worms. Saldaña (pers. comm.) sampled in Trinidad de las Minas near Capira, Panama, and in the town of Las Pavas. The former is approximately 70 km southwest of Gamboa, and the latter is approximately

30 km west of Gamboa (Fig.1-1). My study focused on both areas and accounted for forest degradation and human proximity to sampling sites.

Prior to my study, general changes during life stages of trypanosomes and their modes of transmission have mostly been established, but the vector and definitive host(s) of this unidentified species have not been confirmed. While the trypanosomes were found in spiny rats, this is insufficient evidence to establish the rats as definitive hosts (Haydon et al., 2002). The transmission pathway must first be established to provide sufficient evidence to support this claim. Such evidence includes establishing multiplication within the host, finding infected vectors living on the host, and showing transmission between vector and host. Documenting each of these parts together is needed to fully characterize the transmission cycle (Dsouli et al., 2006).

The purpose of my study was therefore to obtain evidence to identify the trypanosome, vector, and definitive host. Finding this information is important because it will help scientists understand the host role in the environment. It also may help disease researchers to identify a new trypanosome vector in the region, and it can contribute to a better understanding of the dynamics of *T. cruzi* and coinfections with other trypanosomes. My study will further our knowledge of parasite life cycles and expand our knowledge of the role of *P. semispinosus* in the transmission dynamics of infectious agents.

Chapter II

Transmission Dynamics of *Trypanosoma renjifoi* in Disturbed Forest

Introduction

Throughout human history, parasites have played an important role in how humans and other organisms adapt to and exploit different environments. However, we have not had the technology nor information to study the ecology or the effects of parasites until recently. Using modern scientific tools such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), western blotting, electron microscopy, and ArcGIS, scientists can make in depth analyses of their findings. These tests and others like them have applications in fields such as pathology, epidemiology, and molecular biology.

Many studies focus on parasites that utilize vectors; a vector is an organism that transmits the parasite between hosts, and its geographical distribution limits that of the parasite that depends on it (Craig et al., 1999). Vector-borne parasites and their life cycles can be extremely complex, thereby complicating our ability to decipher their ecology. Parasites such as *Plasmodium falciparum* and *Wuchereria bancrofti* (causative agents of malaria and lymphatic filariasis, respectively) undergo dramatic morphological, physiological, and behavioral changes when passing between the vector and host (Florens et al., 2002 & Ash & Schacher, 1971). These situations require extensive efforts when developing effective methods for controlling or avoiding the parasite.

One such parasite in Central and South America is *Trypanosoma cruzi*, the causative agent of Chagas' disease. The parasite uses a vector, and knowledge of the vector's ecology led to the control method of using tin roofing instead of thatch roofing (using leaves, or fronds, from various species of palm trees) (Schofield, 1985). Thatch roofing provides excellent habitat for the vector, so residents sleeping underneath were more likely to be infected with *T. cruzi* (Rassi et al., 2010). To identify transmission cycles of vector-borne parasites, the vector, the hosts, and the parasite itself all need to be identified (Oliver et al., 2003). Such identification is accomplished in field studies whereby the vector needs to be in contact with the host, and both have to be sampled to search for the presence of the parasite of interest. For Chagas' disease, the vector (various triatomine insects) can be collected, and the blood it ingests can be screened to identify the source of the blood meal (Rabinovich et al., 2011). Reservoirs of this disease are often identified using capture studies, whereby the putative reservoir's blood is taken and tested for the presence of the parasite (Travi et al., 1994). Once the vectors and the reservoirs are identified, control methods and laboratory experiments can be conducted, as is the case with *T. cruzi*.

Another trypanosome (*Trypanosoma lewisi*) has been studied extensively in the laboratory as a model for finding strategies to prevent or treat Chagas' disease due to its similarity and its low infectivity to humans (Pizzi & Taliaferro, 1960 and Truc et al., 2013). In North and South America, it infects multiple *Rattus* spp. (Hoare, 1972) and Norway rats (*Rattus norvegicus*) in Brazil (Linardi & Botelho, 2002). While many

diseases are transmitted through the vector's salivary glands, *T. lewisi* and *T. cruzi* are transmitted through the feces of their vectors (fleas and triatomines respectively) (Hoare, 1972).

In Central Panama, *T. cruzi* is endemic, but *T. lewisi* has yet to be found. Because this region has many species of rodents and fleas (the definitive hosts and vectors of *T. lewisi*), there may be an undocumented trypanosome. Previous studies in Central Panama have identified trypanosomes in blood smears from *Proechimys semispinosus* (Central American spiny rat) (Winker, 2013), but the same samples have tested negative for *T. cruzi* and *Trypanosoma rangeli* when amplified using PCR (Saldaña, pers. comm.). I suggest that this trypanosome is a different species and is most likely *Trypanosoma renjifoi*. To fully characterize this parasite, it needs to be examined morphologically, physiologically, and molecularly (Hoare, 1972).

My study is part of a preliminary effort to describe this trypanosome. Of paramount importance is the mode of transmission because it can be used for identification, control, and further characterization. Accordingly, this chapter focuses on the vector and potential modes of transmission. The putative vector is a flea and most likely *Polygenis klagesi* (Hoare, 1972). This flea was one of three species of fleas that infested spiny rats in Panama and was the overwhelmingly most abundant species, constituting 98% of the total flea burden on *P. semispinosus* (Mendez, 1993). Fleas are also vectors for other parasites, including *T. lewisi* and *Yersinia pestis* (causative agent of

plague) and can transmit through either stercorarian or salivarian transmission (Hoare, 1972; Edman, 2004).

Trypanosomes that use stercorarian transmission are differentiated from those that use salivarian transmission through their developmental characteristics. They are thus separated into two broad groups: Stercoraria and Salivaria. Those in Stercoraria develop in the vector's hindgut and undergo posterior station development. More attention was given to how *T. lewisi* develops in the vector because it was most likely related to it. In its flea vector, *T. lewisi* becomes stouter, and its nucleus and kinetoplast move closer together in the first 4-6 hours. After 6-7 hours, the trypomastigotes invade epithelial cells in the flea's stomach to reproduce using multiple fission (species such as *T. cruzi* use binary fission). Following multiple fission, the trypanosomes become spherical, undergo fission again, and move into the lumen of the stomach.

After reaching the stomach lumen, *T. lewisi* assumes a crithidiomorphic form where it is long and its kinetoplast is close behind the nucleus and has a wide posterior end. These trypomastigotes then pass through the colon and into the flea's rectum where they transform into epimastigotes (shortened body and flagellum, with kinetoplast in front of the nucleus). Some remain in the lumen, and some attach themselves to the rectum wall where they multiply using binary fission. Subsequently, some of these will reattach to the walls, while others transform into metacyclic trypomastigotes and become infective. Other Stercoraria undergo the same basic development in the vector with only

minor differences (Hoare, 1972). Because salivarian trypanosomes are generally related to the African *T. brucei*, it was not focused on during this study but is worth noting.

Salivarian transmission occurs when vectors feed on the host and release the parasite through the bite. Vectors are first infected by taking a blood meal from an infected host where the trypanosomes go to the posterior end of the midgut to multiply as trypomastigotes. These then migrate forward through the foregut, esophagus, pharynx, and hypopharynx to enter the salivary glands. Once in the salivary glands, they transform into epimastigotes and attach to the walls of the glands. After undergoing several generations of binary fission, they transform into metacyclic trypomastigotes and can infect new hosts (Roberts & Janovy Jr., 2009).

Because *T. renjifoi* is probably most closely related to *T. lewisi* (Deane, 1961), the focus of this study was the fleas (and their feces) collected from the putative definitive host, *P. semispinosus*. Because the major trypanosomes (*T. cruzi* and *T. lewisi*) in North and South America both use stercorarian transmission, the presumptive *T. renjfoi* was treated as such. If confirmed as a member of Stercoraria, I expected to see epimastigotes in the midgut (possibly undergoing binary fission), intermediate stages of trypomastigotes, and metacyclic trypomastigotes. Because there could be a new infection, and development can take 10 days or less, it is possible that an infected flea only has one of these stages present.

One of the main factors that led this study toward *T. renjifoi* was the number of positive tests when viewing with a light microscope compared to the number of positive

tests using PCR. In one study, one third of captured rats tested positive using this viewing method, but none were positive in PCR tests using *T. cruzi* and *T. rangeli* primers (Saldaña, pers. comm.). Concordant measurements have also been made on the obtained trypanosomes, and preliminary morphological results suggest that they are *T. renjifoi* (Deane, 1961). To accurately characterize this trypanosome, all of the aforementioned factors must be considered.

Methods

Study area. Sampling was conducted in summer 2014 and in summer 2015 in Central Panama (IACUC protocol A2010 05-087-Y3-A1). The two sampling locations were degraded forest patches in Las Pavas in 2014 and in Capira in 2015 (Figure 1-1). Both areas are rural farming communities with severe forest degradation and deforestation, and remnant forests are small patches of second growth. In Las Pavas, remaining forest remnants were sparse and existed only in thin strips between properties. However, such patches maintained rodent, marsupial, primate, and other mammalian populations. The forest in Trinidad de las Minas (town of Capira) was also disturbed and regenerating (less than 30 years old), but was more contiguous, with thicker undergrowth. Rainfall in the study areas is highly seasonal, with the rainy season lasting eight months (late April to December) and a severe dry season persisting through the remaining months (January-April). The study areas are classified as lowland tropical moist forest (Holdridge, 1967).

Sampling procedures. Individuals were live-trapped along transects in June 2014, July 2014, and late May 2015. The size and number of transects in each area were based on the length and width of forest patches. In Las Pavas (2014), one transect approximately 900 meters long containing 45 Tomahawk live-traps (40.6 x 12.7 x 12.7 cm) was established. Using multiple parallel transects was not practical because the fragment was of insufficient width. Each trap was baited with fresh cut ripe plantain 5-7 cm in length, and traps were placed approximately 20 m apart. Traps were set for 10 consecutive nights and checked each morning during the first trapping session and set and checked for 5 consecutive nights the second trapping session. During the second trapping session, the transect had 40 Tomahawk live-traps. In July 2014, four 80-m transects were established, sampling stations were 10 m apart, and traps were baited with fresh-cut ripe plantain. Traps were set for 10 consecutive nights and checked each morning.

During the 2015 sampling season in Trinidad de las Minas, four 80-m and one 160-m transects were established. Sampling stations within a transect were 10 m apart. Each trap again was baited with fresh cut ripe plantain. Transect length and the number of trapping stations was determined by the width and length of the forest patch. Traps were checked every morning.

Each captured *P. semispinosus* was ear-tagged with a uniquely-numbered metal tag, weighed, placed into age and sex categories, and the capture stations were recorded. The age of each individual was determined using the stage of pelage development (Adler,

1994). Fleas were taken from each individual using forceps and a flea comb after applying nontoxic flea powder (except in July 2014). Fleas were placed in 1.5 mL microcentrifuge tubes with a small piece of cotton soaked in water. Fleas infesting the same individual were placed in the same tube. Individual rats were also bled by taking a small ear snip, and the blood was used to make blood smears. Slides were brought back to the lab and fixed with 95% ethanol for 60 seconds and then stained with Giemsa stain for 15 minutes on the day that they were collected. The slides were rinsed with sterile water and allowed to air dry. Prepared slides were then scanned using light microscopy for presence of trypanosomes and other endoparasites such as filarial worms. In July 2014, samples were used to make trypanosome cultures and to run PCR. Kinetoplastid cultures were made using blood agar, 10% whole blood, and saline overlay (Yeo et al., 2007).

An assay developed by Chiurillo et al. (2003) and used by Gottdenker et al. (2012) was performed for PCR testing. It targeted the 189 base pair telomeric junction of *T. cruzi* and a subtelomeric region of *T. rangeli*. The *T. cruzi* primers were T189Fw2 (5’ -CCAAC GCTCCGGGAAAAC-3’) and Tc189Rv3 (5’ -GCGTCTTCTCAGTATGGACT T-3’). *Trypanosoma rangeli* was detected by targeting a conserved subtelomeric region with primers TrF3 (5’ -CCCCATACAAAACACCCTT-3’) and TrR8 (5’-TGGAATGACGG TGCGGC GAC-3’). Loading dye was mixed with 5 µl of PCR product and electrophoresed on a 1.5% agarose gel stained with ethidium bromide. Bands

were evaluated for *T. cruzi* (100 bp) and *T. rangeli* (170 bp) using ultraviolet light, and controls were used in each reaction.

Flea processing. Fleas were combed off of live rats into microcentrifuge tubes, and a small piece of dampened cotton was included in the tubes to prevent desiccation and to keep them alive until processing in the lab. Fleas were maintained in the tubes until feces were observed. Once found, the fleas were removed and anesthetized in a -20°C freezer for five minutes prior to dissection (Tam et al., 2014). Fleas were then identified to species at 40x magnification using the dichotomous key in Tipton & Mendez (1966). Fleas were dissected with two probes, and their foregut, midgut, and hindgut were separated according to Lizundia et al. (2011). Once fleas had been dissected, their gut contents were separated onto different slides and labeled foregut, midgut, and hindgut. These slides were then visually examined with a compound light microscope at 400x and 1000x to search for the presence of microorganisms. Videos and photographs were taken using a camera phone and a handheld Canon camera.

Fecal samples from the flea tubes were suspended in phosphate buffer solution (PBS) and then allowed to dry. After drying, the slides were fixed with methanol for 10 minutes, rinsed, and then stained with Giemsa stain for 25 minutes (Beard et al., 1989). Prepared slides were then visually scanned for trypanosomes under a compound light microscope. Two slides per flea were made using the gut contents from the dissection slide.

Trypanosome measurements. Morphological measurements of trypanosomes were taken using blood smears collected from spiny rats by Winker (2013). A total of 50 trypanosomes was measured, and measurements were taken according to Hoare (1972). Measurements were taken at 1000x magnification using polylines on CellSens software. This procedure was used to obtain proper measurements and for comparison with previous trypanosome morphological papers.

Structures and distances measured were total length, lengths from the posterior end to the kinetoplast, the kinetoplast to the nucleus, and the nucleus to the anterior end, the lengths of the free flagellum and the nucleus, and the diameter of the kinetoplast. The length from the posterior end to the nucleus was found by adding the posterior end to the kinetoplast and the kinetoplast to the nucleus. The free flagellum length was found by subtracting the body length from the total length of each individual. Because no individuals appeared to be distinctly different, all were included in final calculations. Mean, standard deviation, and range were calculated for each measurement. Nuclear index (NI) was the ratio of the distance from the posterior end of the body to the nucleus and the distance from the nucleus to the anterior end (PN/NA). Kinetoplastic index (KI) was the ratio of the posterior end to nucleus and the kinetoplast to the nucleus (PN/KN) (Hoare, 1972). Both of these calculations were used for comparison with morphological measurements of other species.

Results

In Las Pavas during summer 2014, 870 trap nights were accumulated, and 13 individual *P. semispinosus* were captured 19 times. Also captured was one *Didelphis marsupialis*. In Trinidad de las Minas during summer 2015, 248 trap nights were accumulated, and 9 *P. semispinosus* were captured 13 times. During the 2014 season in Las Pavas, 0% of individuals were infested with fleas, and 0% were positive for trypanosome infection. One spiny rat (7.1%) was positive for a filarial worm infection, and the lone *D. marsupialis* captured was infected with filarial worms. Besides those two individuals, no captures in the Las Pavas study site were infected with blood parasites or infested with fleas. In Trinidad de las Minas, 67% of captured individuals were infested with fleas and none were positive for trypanosome infection.

In summer 2015 in Trinidad de las Minas, 6 of 9 *P. semispinosus* (67%) were infested with 4 to 8 fleas, with a mean infestation of 6 fleas. Counts were conducted by performing a visual and manual scan while combing to capture fleas. Because some fleas fled the rats and some may have avoided detection, counts only included those that were conclusively different individuals. Other ectoparasites (mites, ticks, and lice) also infested the rats. Mites were present on 100% of captured rats, 2 (22%) had ticks, and 5 (56%) had lice. Precise counts and collections of these parasites were not included in this study.

All fleas were identified as *Polygenis klagesi.* Of the 14 captured fleas, 1 (7.1%) was positive for trypanosomes using a visual scan of the flea's hindgut under a light

microscope. Observed trypanosomes were alive and free-swimming; some were still associated with gut contents. Images of the parasites in the hindgut are in Figures 1-2, 1-3, and 1-4. The undulating membrane, kinetoplast, central nucleus, and characteristic movements were all present, thereby confirming their identity as trypanosomes. The trypanosomes were observed in the trypomastigote stage, and other life stages were not found during the scan.

Trypanosome measurements are noted in Table 1-5, including means, standard deviations, and ranges. Table 1-6 shows measurements from this study and those reported for *T. lewisi, T. rangeli,* and *T. cruzi* according to Hoare (1972). Total length and free flagellum length varied the most, while nuclear length and kinetoplastic diameter were the most consistent with respect to both standard deviation and range. Images of trypanosomes from these measurements are in Figure 1-5 and Figure 1-6.

Discussion

Trypanosoma cruzi and *T. rangeli* have been documented in spiny rat blood in Central Panama. Studies by Mendez (1993) and Hoare (1972) showed that *P. semispinosus* harbored *T. cruzi* but did not investigate vector transmission. Trypanosomes also have been documented in the study area and in similar habitats (Winker, 2013); however, the species and vector responsible were not investigated. While it is possible that some of these infections were *T. cruzi* or *T. rangeli* (vectored by kissing bugs), there are compelling reasons that some of these were likely *T. renjifoi*.

In Soberania National Park, 17.5% of sampled spiny rats hosted trypanosomes (Winker, 2013), and 57.1% hosted fleas. Of the rats infected with trypanosomes, 50% (5/10) were actively hosting adult fleas. The trypanosomes did not have the characteristically-large 1.2-µm kinetoplast that often extends across the width of many *T. cruzi* specimens (Hoare, 1972). Instead, the kinetoplasts were smaller (0.47 µm), and the parasite body types were slender, as in *T. lewisi.* However, Winker's trypanosomes were shorter than *T. lewisi* and *T. rangeli* (19.9 µm, 30.6 µm, and 27.0-32.2 µm respectively) but close in length to *T. cruzi* (21.4 µm) (Hoare, 1972). Measurements were most similar to *T. renjifoi* found by Deane (1961) and Mello (1978). This could provide preliminary evidence for the identity of this trypanosome, but it is difficult to confirm identity based solely on morphological measurements. These observations suggest a possible causal link between flea infestation and trypanosome infection, with fleas serving as the vector.

Saldaña (pers. comm.) found trypanosomes in 33% (15/46) of spiny rats; 6 tested positive for *T. cruzi* and 1 for *T. rangeli* using PCR. However, 8 were positive in culture but were not positive using the *T. cruzi* and *T. rangeli* primers. While this may be attributed to false-negative PCR results, it is not likely for two reasons. First, PCR's are reliable, and such a high rate of false-negatives cannot be solely attributed to PCR error. Second, while spiny rats can harbor *T. cruzi*, infection rates are low (<5%) in most populations. In the same area of Panama, Vasquez et al. (2004) did not detect *P. semispinosus* DNA in 466 *Rhodnius pallescens* (the main vector of *T. cruzi*) blood meal samples. Saldaña (pers. comm.) found spiny rat DNA in blood meals, but only at a 2%

infection rate. These results were of further interest because similar results were obtained earlier in spring 2014. Of 30 spiny rats captured, 11 were positive for trypanosomes using light microscopy (37%), but measurements showed that they were morphologically most similar to *T. lewisi*, and PCR tests were once again negative for the presence of *T. cruzi* (using *T. cruzi*-specific primers). These two studies further argue for the need to accurately describe this trypanosome. I suggest that these negative PCR results were obtained because the trypanosomes grown in culture were neither *T. cruzi* nor *T. rangeli* but were instead *T. renjifoi*.

Using previous studies that were conducted at various sites in Central and South America, one can infer how this species (putatively *T. renjifoi*) persists within Central Panama. Deane (1961) first described *T. renjifoi*, when it was infecting *Proechimys longicaudatus* in Brazil. He performed laboratory tests on laboratory-born house mice (*Mus musculus*) to determine if they were susceptible to the trypanosome and if triatomines were able to become infected from the mice using xenodiagnosis, but these tests were negative for trypanosomes. The negative results were probably due to using mice that could not become infected with *T. renjifoi* and triatomines that could not vector it. However, there was no comment on the supposed vector. If *T. renjifoi* is similar to *T. lewisi* in Panama, it would stand to reason that it would have a similar transmission cycle. Because *T. lewisi* is a common parasite of rodents in labs (Desquesnes et al., 2002), its life cycle has been well-documented, and fleas are the known vector (Molyneux, 1969). Although the relationship between fleas and trypanosomes has been studied in North and

South America, it has never been investigated in Panama (Pinto et al., 2006, Doran, 1954).

Based upon this gap in knowledge and past studies by Tipton & Mendez (1966) and Winker (2013), I hypothesized that the trypanosome was transmitted between definitive rodent hosts (*P. semispinosus*) by the flea (*P. klagesi*). Tipton & Mendez (1966) found that 99% of fleas collected from *P. semispinosus* were *P. klagesi,* and 91% of this flea species were collected from *P. semispinosus*. Similarly, Winker (2013) found that 100% of fleas infesting spiny rats were *P. klagesi*, and 92.9% of the total fleas collected were from *P. semispinosus.* These two studies provide evidence for host specificity of *P. klagesi* for *P. semispinosus*.

I collected 14 fleas, all of which were *P. klagesi,* from *P. semispinosus*, which was consistent with previous studies and supportive of (but not confirming) both of my hypotheses. It is also consistent with Deane (1961) and Mello (1978) in that the definitive hosts are *Proechimys* spp. My study and those of Winker (2013) and Saldaña (pers. comm.) noted that trypanosome infections were found when fleas were also present, suggesting a possible causal link between the two. Las Pavas has yielded no trypanosome infections and no flea infestations, which does not provide evidence of a vector-host relationship, but is worth noting. This correlation is best supported by finding trypanosomes in the fleas infesting the putative host.

Observed trypanosomes in the gut of the flea were in the trypomastigote stage of their life cycle, consistent with *T. lewisi* observations (Hoare, 1972), where

trypomastigotes were in the lumen of the gut, while other life stages were still attached to the walls. Different life stages may have been present, but I was unable to detect them. I also did not find trypanosomes undergoing binary fission, despite scanning the entire specimen. This is a key part of the life cycle and provides evidence of multiplication and progression through the life cycle (Hoare, 1972). However, this evidence is less conclusive than also finding trypanosomes in the flea's feces.

Another possibility involves mechanical transmission. For instance, MacNeal (1904) found that *T. lewisi* can be mechanically vectored by lice. Lice became infected through feeding on an infected host, but no trypanosome development was found. In this way, lice spread the trypanosome between hosts but were not passing infective trypanosomes through their feces. In my study, fleas may have played a similar role where development did not occur, and infective trypanosomes were not passed through their feces.

My other hypothesis, that *T. renjifoi* would be more abundant in close proximity to humans, was not supported. There was a low infection rate in summer 2015, no infections in summer 2014, and both study sites were close to people. I hypothesized this because spiny rats have high abundances in degraded forests (Lambert & Adler, 2000), and this can also affect flea abundance in the area. Degraded forests in the area are mostly caused by human activity, thereby providing suitable habitat for spiny rats near human habitations. The presence of *P. semispinosus* also provides a host for fleas, and the fleas provide a vector for trypanosomes.

Unfortunately, it was beyond the scope of this study to determine the factors that most strongly influence *T. renjifoi* infection rates. In the 2014 dry season sampling, there was a 37% (11/30) infection rate, but the rate was only 18% (7/38) during the wet season. There were similar seasonal effects in Winker's (2013) study, where 22% (8/37) of *P. semispinosus* were infected in the dry season, and 11% (2/22) were infected in the wet season. While the peridomiciliary setting had a higher infection rate, this higher rate may be more due to the difference in forest size. In the National Park near Gamboa, there was much more available forest space and may have led to less burrow sharing between rats and consequent flea host switching. This may indicate seasonal effects on trypanosome infections, but larger sample sizes are necessary to draw more rigorous conclusions.

By contrast, studies have shown that fleas are more abundant during the wet season (Winker, 2013; Saldaña, pers. comm.). During this time, spiny rats use and share more burrows (Endries & Adler, 2005). The combination of more fleas and more opportunity for host switching (Krasnov et al., 2006) may propagate the transmission of *T. renjifoi.* However, the present data suggest that infection rates are higher during the dry season. A more focused seasonal study is needed to determine when infection rates are highest. Along these lines, following studies can potentially elucidate factors such as these that may help promote the transmission of *T. renjifoi*.

One of the goals of this study was to provide a basis for identifying a vector based on previous knowledge of a parasite. This was accomplished by identifying the presence of the parasite within the putative vector. Although my study fell short in identifying the

same parasite in the definitive host and in the feces of the vector, there are now steps toward achieving this identification. Steps included drawing from past studies in the area and on similar species and identifying where knowledge was lacking. The missing piece was in fully explaining trypanosome infections, where previous studies only noted the infections but did not investigate patterns or processes of infection.

Furthering knowledge on this trypanosome species can have potential applications to our understanding of other species of *Trypanosoma*, including *T. cruzi, T. rangeli,* and *T. lewisi*. Similarly, future studies can use a similar procedure in finding other parasites in their natural life cycles; this can also be helpful in future disease work. Thus, encroachment by humans into tropical ecosystems can unwittingly insert humans into the natural transmission cycles of potential pathogens. It is not only important to identify which organisms can be pathogenic but also how to determine their life cycles quickly. This information can be used to slow transmission to vectors and hosts, thereby preventing a potential disease outbreak.

Chapter III

Limitations, Future Research, and Implications for *Trypanosoma cruzi* Dynamics

This research has implications for future work on *Trypanosoma renjifoi* and potentially for Chagas' disease. Central inquiries remaining include determining the pathogenicity of *T. renjifoi,* its relationship to *T. cruzi*, and its role in the environment. *Trypanosoma renjifoi* has not been documented in humans, and its definitive hosts and vectors are restricted primarily to forest. However, present research indicates that it is most similar to *T. lewisi,* which is infective to humans in isolated cases (Sarataphan et al., 2007; Verma et al., 2011). Saldaña and colleagues (pers. comm.) sequenced the genome of *T. renjifoi,* and they confirmed that it is a rodent trypanosome. This is the first such documentation of this species and requires further characterization. This should include characterizing the species and its ecological relationships. Studies similar to this one will increase in importance as more pathogens reach humans, and they need to be understood prior to causing serious disease outbreaks.

Although humans could potentially be infected, the sylvatic components of the local mammalian community could also be vulnerable to disease. Cuervo et al. (2015) raised concerns about parasite spillback involving fluke (nematode) infection of brown hares. The process of parasite spillback involves a parasite infecting an invasive species (e.g., brown hares), multiplying, and then infecting native organisms (Kelly et al., 2009). A similar situation could occur in this region, with invasive species such as *Rattus rattus*

(black rat) and *Rattus norvegicus* (Norway rat). These exotics occur in the region and may have already caused spillback into natural populations (Morand et al., 2015). Native species may be susceptible to such spillback, and trypanosome infection rates could increase.

Presently, reservoir infection rates are between 1-10% (Saldaña, pers. comm.), but a severe spillback situation could affect both native organisms and the ecosystem. Spiny rats are one such native species; they would be ideal in studies involving spillback and trypanosome communities because they live in *T. cruzi* endemic areas, are infected with *T. renjifoi,* are abundant, and are easily sampled (Whitlaw & Chaniotis, 1978). Scientists can then assess the relationship between *T. cruzi* and the other endemic blood parasites in the area and the potential for the increase of trypanosome infections. Finding such answers has thus far involved using less virulent species and making comparisons with those that cause more debilitating disease. Such answers can be found by developing a better understanding of entire ecosystems.

The importance of these studies has recently been understated and neglected, as most work has focused only on pathogens with zoonotic potential (Thompson et al., 2010; McCallum & Dobson, 1995; Holmes, 1996; Daszak et al., 2000; Rhyan & Spraker, 2010). While zoonotic pathogens are important, a broader understanding of their ecological role is necessary. Once this is complete, efficacy during implementation procedures is increased and can have positive effects on other organisms in the environment. In this case, *T. renjifoi* may impact other mammals or other parasites, but

research must progress properly to accurately describe its relationships. More specifically, its pathogenicity to spiny rats and other mammals needs to be tested and viewed from a conservation standpoint. It has been well documented that parasites have greatly impacted wildlife populations (Daszak et al., 2000; Harvell et al., 2002; Smith et al., 2006), and the impact of parasites such as trypanosomes should be quantified. Acquiring this information also assists in identifying scenarios in which pathogens may have a greater impact on native fauna (Smith et al., 2009). Using these methods can have positive impacts on both human disease and wildlife conservation. Thus far, trypanosome relationships in Panama require more work, but other trypanosomal comparisons have been made.

For instance, studies often use *T. lewisi* in laboratory work as a comparative species to *T. cruzi* (Chagas' disease) and *T. brucei* (African sleeping sickness) (Pizzi & Taliaferro, 1960; Dixon & Williamson, 1970). It is effective in such studies because it is less pathogenic and is well suited for infecting laboratory rodents. These comparisons are even more important in studying *T. renjifoi* due to the native trypanosome community. As shown in my study, *T. rangeli* is also present in the area and coinfects both hosts (including spiny rats) and vectors with *T. cruzi*. This unique circumstance of an animal carrying three *Trypanosoma* spp. is yet another reason to study them and gain knowledge of how these trypanosomes affect each other. However, *T. renjifoi* must be characterized before experiments can test this relationship.

Works following this study can focus on capturing rats, collecting their fleas, and sampling in both the wet and dry season to account for seasonal differences. This should include rat blood samples to use for smears, PCR, and trypanosome culture. Collected fleas can also be maintained for processing. Blood and flea samples can be used for PCR with primers for *T. cruzi, T. rangeli, T. lewisi,* and kinetoplast DNA. Blood smears and cultures can serve as a check for the PCR results, and offer morphological clues, such as length, width, and kinetoplast size to determine the *Trypanosoma* spp. (Hoare, 1972). While I was unsuccessful with electron microscopy, it is still of interest to make more precise measurements of the organism's structures (Anderson & Ellis, 1965). Experimental infections remain the best way to definitively characterize this life cycle and to accurately describe the life history of the trypanosome present.

My study also tried to account for human proximity in relation to trypanosome infection in spiny rats, but more robust sample sizes will be necessary to draw significant conclusions. Unfortunately, it was beyond the scope of this study to achieve these goals, but it has provided an adequate basis with which to continue this work. Overall goals were achieved in finding trypanosomes within the putative vector collected from a spiny rat. It also helped create a model for identifying parasite life cycles as humans contact more of them.

Table 1-1. Sampling dates, trap nights, numbers of individuals, and total captures of *Proechimys semispinosus.*

Location	Date	Trap-nights	*P. semispinosus* individuals	*P. semispinosus* captures
Las Pavas	**Jun 2014**	**670**	**5**	**10**
Las Pavas	**Jul 2014**	**200**	**8**	**9**
Capira	**Jun 2015**	**248**	**9**	**13**
TOTAL		**1,118**	**22**	**32**

Table 1-2. Sampling locations, dates, individuals captured, and corresponding blood smear and PCR results (numbers of trypanosomes denote positive results, and (-) denotes that none were tested).

Location	Date	*P. semispinosus* individuals	Trypanosomes in Blood Smears	*T. cruzi/ T. rangeli* in PCR
Las Pavas	**Jun 2014**	**5**	**0**	**0**
Las Pavas	**Jul 2014**	**8**	**0**	**0**
Capira	**Jul 2014**	**40**	**13**	**7**
Capira	**Jun 2015**	**9**	**0**	**-**
TOTAL		**62**	**13**	**7**

Table 1-3. Sampling locations, dates, number of captured individuals, and number of individuals infested with fleas, mites, lice, and ticks in each sampling period.

Location	**Date**	***P. semispinosus*** **Individuals**	**Flea** **Infestations**	**Mite** **Infestations**	**Lice** **Infestations**	**Tick** **Infestations**
Las Pavas	Jun 2014	5	0	4	3	2
Las Pavas	Jul 2014	8	0	8	3	1
Capira	Jun 2015	9	6	9	5	2
Total		22	6	21	11	5

Table 1-4. Trypanosome morphological measurements from blood smears prepared by Winker (2013). “L” is total length, “PK” is posterior end to kinetoplast, “KN” is kinetoplast to nucleus, “PN” is posterior end to nucleus, “NA” is nucleus to kinetoplast, “F” is free flagellum, “N” is nucleus length, and “K” is kinetoplast diameter. All measurements are in micrometers, and measurements were made to the center of the nucleus and kinetoplast.

	L	**PK**	**KN**	**PN**	**NA**	**F**	**N**	**K**
Mean	19.9	1.58	6.16	7.74	4.10	7.10	1.27	0.468
Std Dev	2.33	0.350	0.951	0.979	0.989	1.78	0.166	0.126
Low	14.25	0.900	8.25	4.42	2.88	3.03	0.97	0.300
High	24.75	4.00	3.34	10.83	7.60	10.07	1.81	0.740

Table 1-5. Morphological measurements of trypanosomes from Winker (2013), *T. renjifoi* (Mello, 1977), *T. lewisi* (Minchin, 1909), *T. cruzi*, and *T. rangeli* (Hoare, 1972). "L" is total length, "PK" is posterior end to kinetoplast, "PN" is posterior end to nucleus, "NI" is nuclear index (PN/NA) (NA is nucleus to anterior end), "F" is free flagellum, and "K" is kinetoplast diameter. All measurements are in micrometers (μm), measurements were made to the center of the nucleus and kinetoplast, and (-) denotes that the measurement was not available.

	Winker (2013)	***T.renjifoi***	***T. lewisi***	***T. cruzi***	***T. rangeli***
L	19.9	27.0	30.6	16.3-21.8	27-32.2
PK	0.9-4.0	3.3	4.5	-	1.8-7.0
PN	4.4-10.8	9.1	7.04	-	8.2-10.0
NI	1.9	1.9	1.6	0.9-1.7	1.6-2.0
KI	1.3	1.1	0.6	-	1.2-1.5
F	3.0-10.1	8.1	7.2-7.8	2.0-11.2	7.9-9.5
K	0.47	-	-	1.2	0.70

Figure 1-1. Plot locations in Central Panama. Panama City is marked for reference, Gamboa is the site of the Winker (2013) study, and Las Pavas and Capira are the sites of this study and the Saldaña (pers. comm.) studies. Las Pavas is roughly 35 km west of Gamboa, and Capira is roughly 70 km southwest of Gamboa.

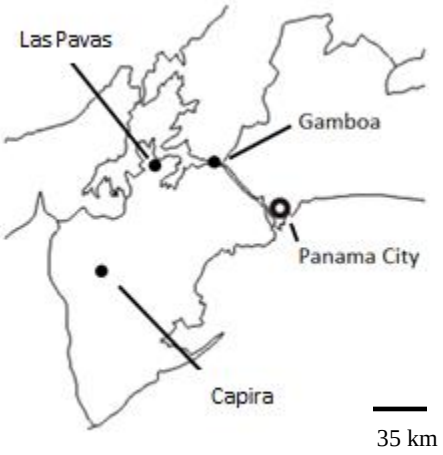


Figure 1-2. Image of trypanosome in hindgut of flea (*Polygenis klagesi*) collected from *Proechimys semispinosus*. The undulating membrane is marked below.

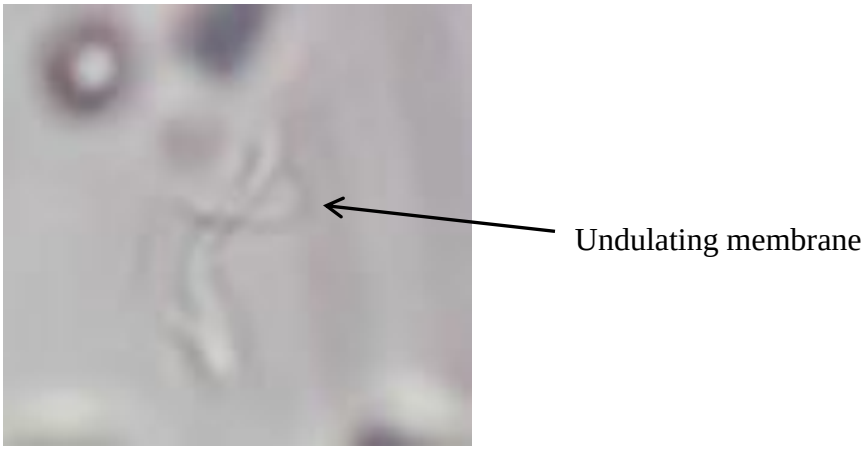


Figure 1-3. Image of trypanosome found in hindgut of *Polygenis klagesi* collected from *Proechimys semispinosus*. The kinetoplast and nucleus are marked below.

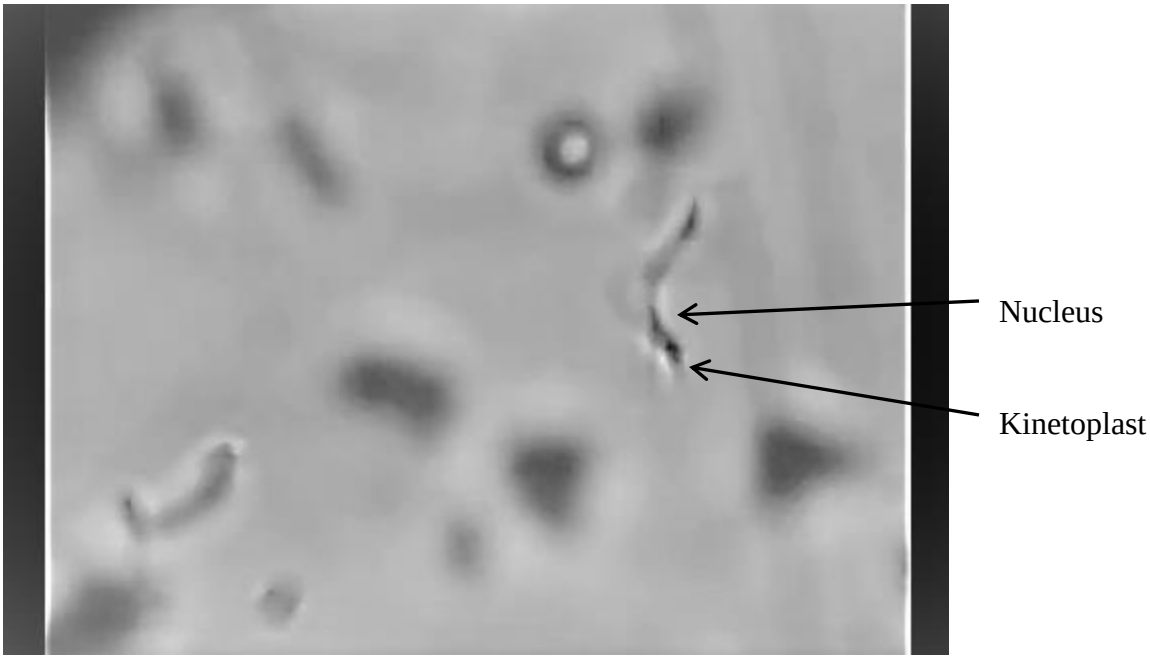


Figure 1-4. Image of trypanosome found in hindgut of *Polygenis klagesi* collected from *Proechimys semispinosus*. The kinetoplast and undulating membrane are marked below.

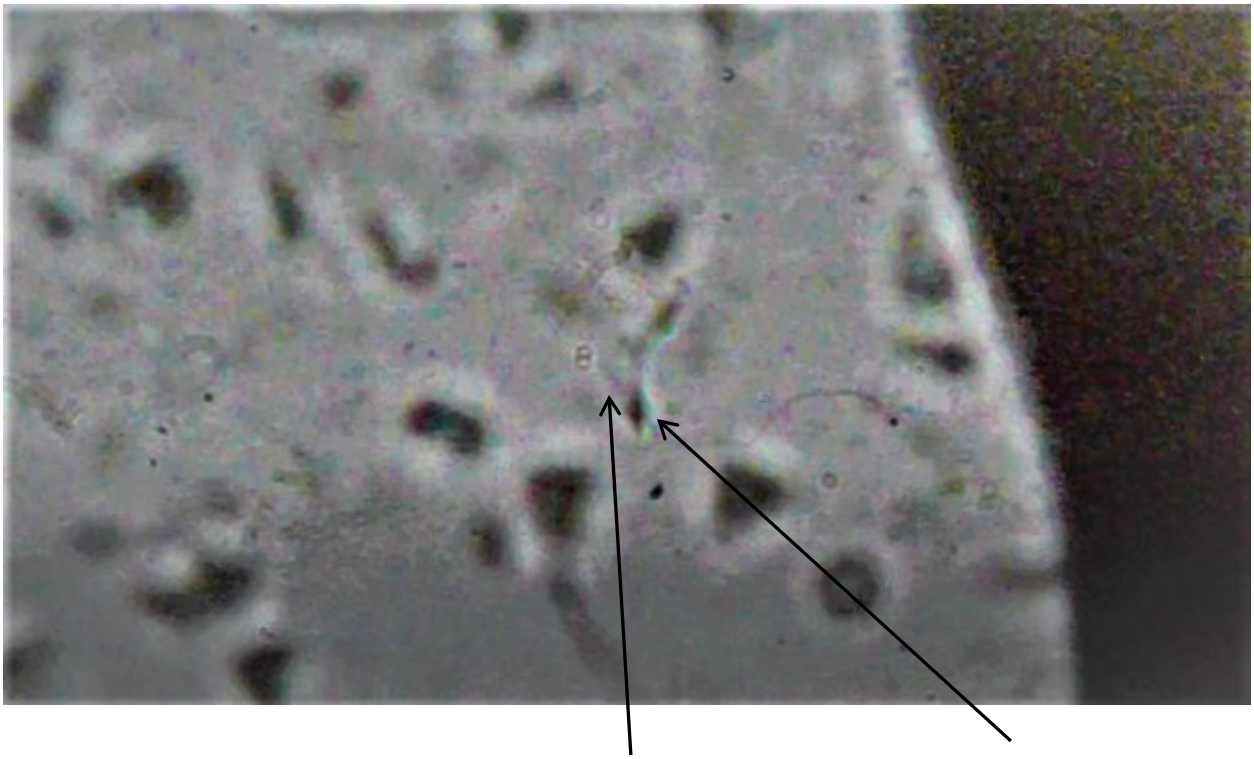


Figure 1-5. Image of trypanosome in blood smear from Winker (2013). The nucleus and kinetoplast are marked below.

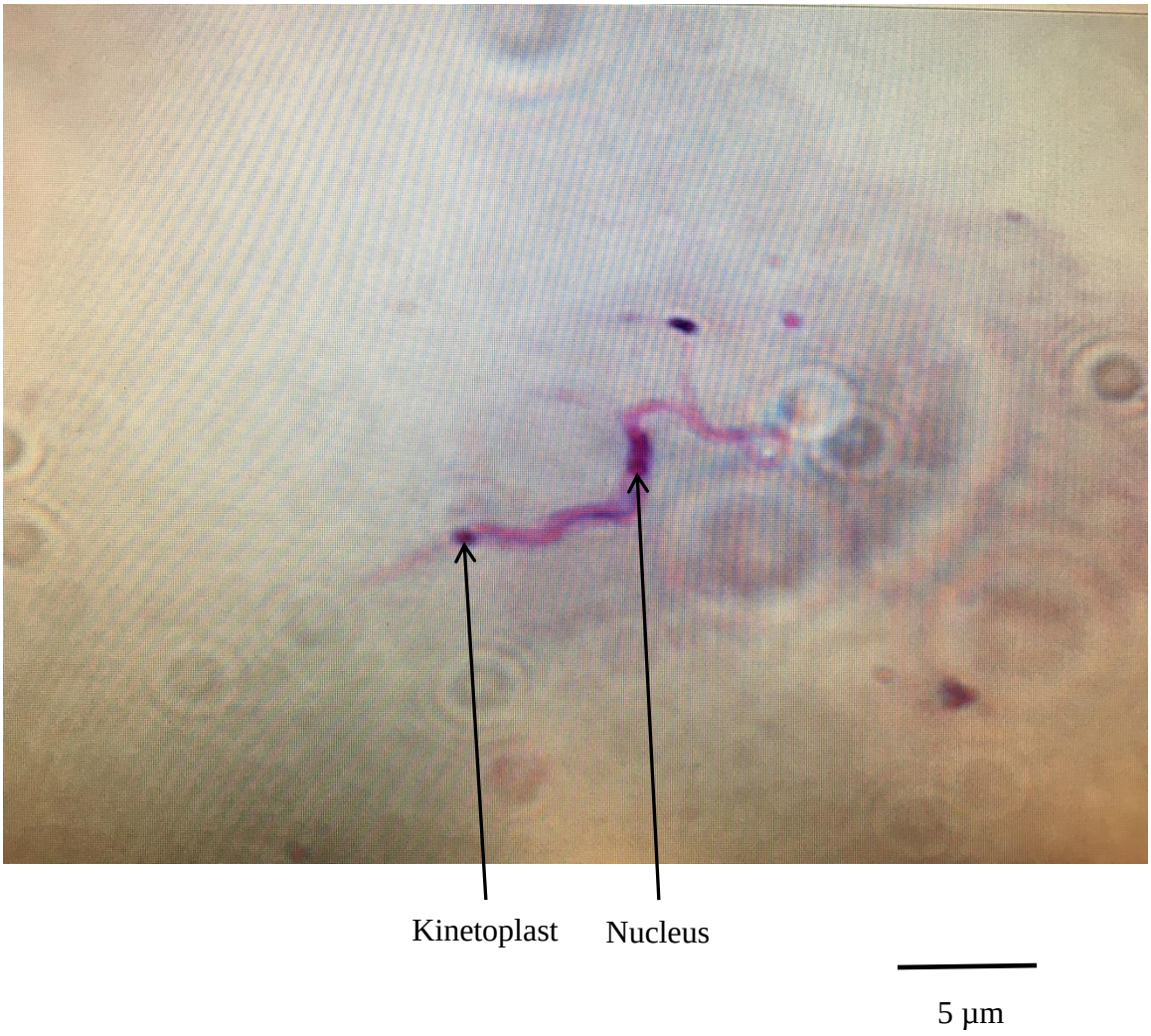
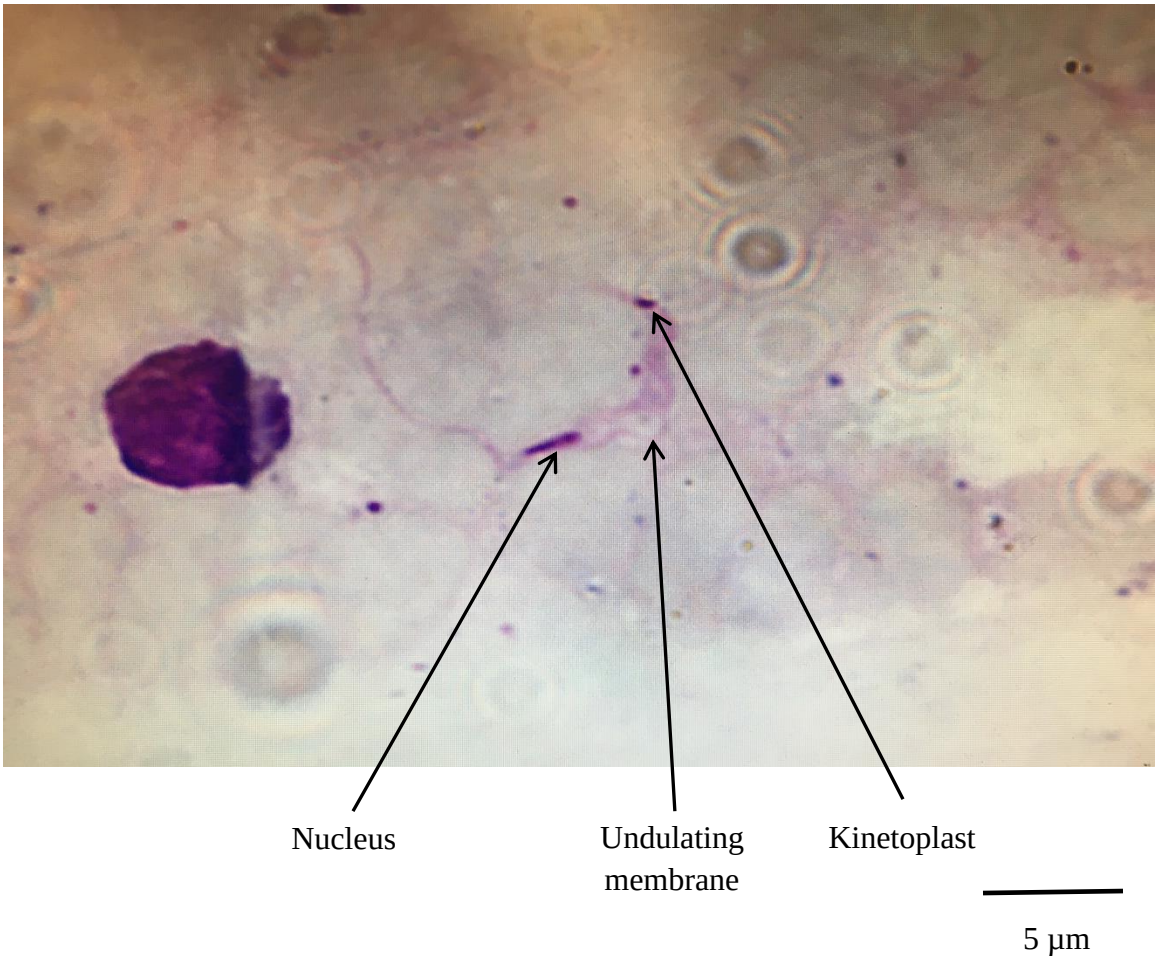


Figure 1-6. Image of trypanosome in blood smear from Winker (2013). The nucleus, undulating membrane, and kinetoplast are marked below.



Literature Cited

Adler, G.H. (1994). Tropical forest fragmentation and isolation promote asynchrony among populations of a frugivorous rodent. *The Journal of Animal Ecology*, 63:903-911.

Adler, G. H. (2000). Tropical tree diversity, forest structure and the demography of a frugivorous rodent, the spiny rat (*Proechimys semispinosus*). *Journal of Zoology*, *250*(1), 57-74.

Adler, G. H., & Beatty, R. P. (1997). Changing reproductive rates in a Neotropical forest rodent, *Proechimys semispinosus*. *Journal of Animal Ecology*, 472-480.

Adler, G. H., Davis, S. L., & Carvajal, A. (2003). Bots (Diptera: Oestridae) infesting a neotropical forest rodent, *Proechimys semispinosus* (Rodentia: Echimyidae), in Panama. *Journal of Parasitology*, *89*(4), 693-697.

Anderson, W. A., & Ellis, R. A. (1965). Ultrastructure of *Trypanosoma lewisi*: flagellum, microtubules, and the kinetoplast. *The Journal of Protozoology*, *12*(4), 483-499.

Anon (1957). The twenty-ninth annual report of the work and operation of the Gorgas Memorial Laboratory, covering the fiscal year ended June 30, 1956. United States Government Printing Office, Washington.

Anon (1959). Thirty-first annual report of the work and operation of the Gorgas Memorial Laboratory, covering the fiscal year ended June 30, 1958. United States Government Printing Office, Washington

Ash, L. R. & Schacher, J. F. (1971) Early life cycle and larval morphogenesis of *Wuchereria bancrofti* in the jird, *Meriones unguiculatus*. *The Journal of Parasitology*. 57(5): 1043-1051.

Auxiliadora de Sousa, M. (2014). On opportunist infections by *T. lewisi* in humans and its differential diagnosis from *T. cruzi* and *T. rangeli*. *The Journal of Parasitology Research.* 113:4471-4475.

Beard, C. B., Butler, J. F., & Greiner, E. C. (1989). In vitro growth characterization and host-parasite relationship of Leptomonas pulexsimulantis n. sp., a trypanosomatid flagellate of the flea Pulex simulans. *The Journal of Parasitology*, 658-668.

Bush, A. O., Fernandez, J. C., Esch, G. W., & Seed, J. R. (2001). Parasitism: The diversity and ecology of animal parasites. *Cambridge University Press.* Cambridge, UK.

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

Carvajal, A., & Adler, G. H. (2008). Seed dispersal and predation by Proechimys semispinosus and Sciurus granatensis in gaps and understorey in central Panama. *Journal of Tropical Ecology, 24*(05), 485-492.

Catts, E. P. (1982). Biology of New World bot flies: Cuterebridae. *Annual Review of Entomology* 27: 313–338.

Chiurillo M, Crisante G, Rojas A, Peralta A, Dias M, et al. (2003) Detection of *Trypanosoma cruzi* and *Trypanosoma rangeli* infection by duplex PCR assay based on telomeric sequences. *Clinical and Diagnostic Laboratory Immunology 10*: 775–779.

Christophers, S. R. (1949). DDT for Mosquitoes. *British Medical Journal*, *1*(4602), 483.

Colwell, D. D., Hall, M. J., & Scholl, P. J. (Eds.). (2006). *The oestrid flies: biology, host-parasite relationships, impact and management*. CABI.

Craig, M. H., Snow, R. W., & le Sueur, D. (1999) A climate-based distribution model of malaria transmission in sub-saharan Africa. *Parasitology Today.* 15(3): 105-111.

Cuervo, P. F., Cataldo, S. D., Fantozzi, M. C., Deis, E., Isenrath, G. D., Viberti, G., ... & Mas-Coma, S. (2015). Liver fluke (Fasciola hepatica) naturally infecting introduced European brown hare (Lepus europaeus) in northern Patagonia: phenotype, prevalence and potential risk. *Acta Parasitologica*, 60(3), 536-543.

Daszak, P., Cunningham, A.A., & Hyatt, A.D., (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287, 443-449.

Deane, L. M. (1961). Tripanosomideos de mamiferos da região Amazônica. I. Alguns flagelados encontrados no sangue de mamiferos silvestres do Estado do Pará. *Revista do Instituto de Medicina Tropical de São Paulo, 3*: 15-28.

Desquesnes, M., Ravel, S., & Cuny, G. (2002). PCR identification of *Trypanosoma lewisi*, a common parasite of laboratory rats. *Kinetoplastid Biology and Disease*, *1*(1), 2.

Dittel, J. W., Lambert, T. D., & Adler, G. H. (2015). Seed dispersal by rodents in a lowland forest in central Panama. *Journal of Tropical Ecology*, *31*(05), 403-412.

Dixon, H., & Williamson, J. (1970). The lipid composition of blood and culture forms of *Trypanosoma lewisi* and *Trypanosoma rhodesiense* compared with that of their environment. *Comparative Biochemistry and Physiology*, *33*(1), 111IN7117-116IN8128.

Doran, D. J. (1954). A catalogue of the Protozoa and helminths of North American rodents. I. Protozoa and Acanthocephala. *The American Midland Naturalist*, *52*(1), 118-128.

Dsouli, N., Younsi-Kabachii, H., Postic, D., Nouira, S., Gern, L., & Bouattour, A. (2006). Reservoir role of lizard Psammodromus algirus in transmission cycle *of Borrelia burgdorferi* sensu lato (Spirochaetaceae) in Tunisia. *Journal of Medical Entomology*, *43*(4), 737-742.

Dutari, L. C., & Loaiza, J. R. (2014). American Cutaneous Leishmaniasis in Panama: a historical review of entomological studies on anthropophilic Lutzomyia sand fly species. *Parasites & Vectors*, *7*(1), 218.

Edman, J. D. (2004). Arthropod transmission of vertebrate parasites. *In Medical Entomology* (pp. 151-163). Springer Netherlands.

Endries, M.J., & Adler, G. H. (2005). Spacing patterns of a tropical forest rodent, the spiny rat (*Proechimys semispinosus*), in Panama. *Journal of Zoology*, 265:147-155.

Florens, L., Washburn, M. P., Raine J. D., Anthony, R. M., Grainger, M. Haynes, J. D., et al. (2002) A proteomic view of the *Plasmodium falciparum* life cycle. *Nature.* 419: 520-526.

Gottdenker, N. L., Chaves, L. F., Calzada, J. E., Saldaña, A., & Carroll, C. R. (2012). Host life history strategy, species diversity, and habitat influence *Trypanosoma cruzi* vector infection in changing landscapes. *PLoS Neglected Tropical Dis*eases *6*(11), e1884.

Guerrero, R., & Bain, O. (2001). The New World filarial genus *Molinema* Freitas & Lent, 1939 (Nematoda: Onchocercidae), with a description of four new species parasitic in the Echimyidae (Rodentia). *Systematic Parasitology*, *48*(3), 203-221.

Guerrero, R., O. Bain, C. Martin, & M. Barbuto. (2011). A new species of *Litomosoides* (Nematoda: Onchocercidae), parasite of *Nectomys palmipes* (Rodentia: Cricetidae: Sigmodontinae) from Venezuela: description, molecular evidence, *Wolbachia pipientis* screening. *Folia Parasitologica*, 58:149-156.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., & Samuel, M.D., (2002). Climate warming and disease risk for terrestrial biota. *Science* 296, 2158-2162.

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases*, *8*(12), 1468-1473.

Herrer, A., Telford Jr, S. R., & Christensen, H. A. (1971). Enzootic cutaneous leishmaniasis in eastern Panama: I: Investigation of the infection among forest mammals. *Annals of Tropical Medicine & Parasitology*, *65*(3), 349-358.

Hoare, C. A. (1972) The trypanosomes of mammals. A zoological monograph. *Blackwell Scientific Publications*. Oxford, 722.

Holdridge, L. (1967) Life Zone Ecology San Jose (Costa Rica): Tropical Science Center

Holmes, J.C., (1996). Parasites as threats to biodiversity in shrinking ecosystems. *Biodiversity Conservation* 5, 975-983.

Jensen, K. (2015) Global burden of parasitic disease. (Webpage) Retrieved from http://faculty.ucmerced.edu/kjensen5/index.php/research/global-burden-of-parasitic-disease/

Kelly, D. W., Paterson, R. A., Townsend, C. R., Poulin, R., & Tompkins, D. M. (2009). Parasite spillback: a neglected concept in invasion ecology? *Ecology*, *90*(8), 2047-2056.

Kolonin, G.V. (2009). Fauna of Ixodid ticks of the world (Acari, Ixodidae). Moscow.

Kourany, M., Bowdre, L., & Herrer, A. (1976). Panamanian forest mammals as carriers of Salmonella. *The American Journal of Tropical Medicine and Hygiene*, *25*(3), 449-455.

Krasnov, B.R., Stanko, M., Miklisova D., & Morand, S. 2006. Habitat variation in species composition of flea assemblages on small mammals in central Europe. *Ecological Research*, *21*: 460-469.

Lainson, R., Shaw, J. J., Silveira, F. T., De Souza, A. A. A., Braga, R. R., & Ishikawa, E. A. Y. (1994). The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. *Memórias do Instituto Oswaldo Cruz*, *89*(3), 435-443.

Lambert, T. D., & Adler, G. H. (2000). Microhabitat use by a tropical forest rodent, *Proechimys semispinosus*, in central Panama. *Journal of Mammalogy*, *81*(1), 70-76.

Lardans, V., & Dissous, C. (1998). Snail control strategies for reduction of schistosomiasis transmission. *Parasitology Today*, *14*(10), 413-417.

Linardi, P. M. & Botelho, J. R. (2002) Prevalence of *Trypanosoma lewisi* in *Rattus norvegicus* from Belo Horizonte, State of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz*. *97*(3): 411-414.

Lips, K. R., Brem, F., Brenes, R., Reeve, J. D., Alford, R. A., Voyles, J., ... & Collins, J. P. (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(9), 3165-3170.

Lisboa, C. V., Cristina, S., Herrera, H. M., & Jansen, A. M. (2009). The ecology of the *Trypanosoma cruzi* transmission cycle: Dispersion of zymodeme 3 (Z3) in wild hosts from Brazilian biomes. *Veterinary Parasitology*. *165*: 19-24.

Lizundia, R., Newman, C., Buesching, C. D., Ngugi, D., Blake, D., Sin, Y. W., ... & McKeever, D. (2011). Evidence for a role of the host-specific flea (Paraceras melis) in the transmission of *Trypanosoma* (Megatrypanum) *pestanai* to the European badger. *PLoS One*, *6*(2), e16977.

Loiseau, P.M., Mettey, Y., & Vierfond, J. M. (1996). Antifilarial and trypanocidal properties of phenothiazines and related polycyclics as new lead structures. *International Journal for Parasitology*, *26*: 1115-1117.

Londoño, A. F., Díaz, F. J., Valbuena, G., Gazi, M., Labruna, M. B., Hidalgo, M., ... & Rodas, J. D. (2014). Infection of Amblyomma ovale by Rickettsia sp. strain Atlantic rainforest, Colombia. *Ticks and Tick-borne Diseases*, *5*(6), 672-675.

Mackenzie, R. B. (1972). Public health importance of rodents in South America. *Bulletin of the World Health Organization. 47*: 161-169.

MacNeal, W. J. (1904). The life-history of *Trypanosoma lewisi* and *Trypanosoma brucei*. *Journal of Infectious Diseases*, *1*(4), 517-543.

McCallum, H. & Dobson, A. (1995). Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology & Evolution. 10*, 190-194.

McLaren, D. J. (1989). Will the real target of immunity to schistosomiasis please stand up. *Parasitology Today*, *5*(9), 279-282.

Mello, D. A. (1978) Sobre o encontro do *Trypanosoma* (*Herpetosoma*) *renjifoi* Deane 1961, Infectando *Proechimys longicaudatus* (Rodentia-Echimyidae). *Revista de Saúde Pública*, *12*: 523-525.

Mendez, E. (1993). Los roedores de Panama. Impresora Pacifico S.A., Panama.

Minchin, E. A. (1909). The structure of *Trypanosoma lewisi* in relation to microscopical technique. *Journal of Cell Science*, *2*(212), 755-808.

Molyneux, D. H. (1969). Intracellular stages of Trypanosoma lewisi in fleas and attempts to find such stages in other trypanosome species. *Parasitology*, *59*(04), 737-744.

Morand, S., Bordes, F., CHEN, H. W., Claude, J., COSSON, J. F., Galan, M., ... & Ribas, A. (2015). Global parasite and Rattus rodent invasions: The consequences for rodent-borne diseases. *Integrative Zoology*, *10*(5), 409-423.

Notarnicola, J., Jiménez, F. A., & Gardner, S. L. (2012). A new species of *Molinema* (Nematoda: Onchocercidae) in Bolivian rodents and emended description of *Litomosoides esslingeri* Bain, Petit, and Diagne, 1989. *Journal of Parasitology*, *98*(6), 1200-1208.

Oliver, J. H., Lin, T., Gao, L., Clark, K. L., Banks, C. W., Durden, L. A., ... & Chandler, F. W. (2003). An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. *Proceedings of the National Academy of Sciences of the United States*, *100*(20), 11642-11645.

Pineda, V., Montalvo, E., Alvarez, D., Santamaría, A. M., Calzada, J. E., & Saldaña, A. (2008). Feeding sources and trypanosome infection index of *Rhodnius pallescens* in a Chagas disease endemic area of Amador County, Panama. *Revista do Instituto de Medicina Tropical de São Paulo, 50*(2), 113-116.

Pinto, C. M., Ocaña-Mayorga, S., Lascano, M. S., & Grijalva, M. J. (2006). Infection by trypanosomes in marsupials and rodents associated with human dwellings in Ecuador. *Journal of Parasitology, 92*(6), 1251-1255.

Pizzi, T., & Taliaferro, W. H. (1960). A comparative study of protein and nucleic acid synthesis in different species of trypanosomes. *The Journal of Infectious Diseases,* 100-107.

Price, P.W. (1980). Evolutionary biology of parasites. Princeton University Press, Princeton, New Jersey.

Rabinovich, J. E., Kitron, U. D., Obed, Y., Yoshioka, M., Gottdenker, N., & Chaves, L. F. (2011) Ecological patterns of blood-feeding by kissing-bugs (Hemiptera: Reduviidae: Triatominae). *Memórias do Instituto Oswaldo Cruz*. *106*(4): 479-494.

Rassi, A. R., Rassi, A., & Marin-Neto, J. A. (2010) Chagas disease. *Lancet*. 375: 1388-1402.

Resh, V. H., Lévêque, C., & Statzner, B. (2004). Long-term, large-scale biomonitoring of the unknown: assessing the effects of insecticides to control river blindness (onchocerciasis) in West Africa. *Annual Reviews in Entomology*, *49*(1), 115-139.

Rhyan, J.C., & Spraker, T.R., (2010). Emergence of disease from wildlife reservoirs. *Veterinary Pathology, 47,* 34-39.

Roberts, L. S. S., & Janovy, G. D. (2009). *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology* (No. 574.5249 R6/2009).

Sarataphan, N., Vongpakorn, M., Nuansrichay, B., Autarkool, N., Keowkarnkah, T., Rodtian, P., ... & Jittapalapong, S. (2007). Diagnosis of a *Trypanosoma lewisi*-like (Herpetosoma) infection in a sick infant from Thailand. *Journal of Medical Microbiology*, *56*(8), 1118-1121.

Schmunis, G. A., & Yadon, Z. E. (2010). Chagas disease: a Latin American health problem becoming a world health problem. *Acta Tropica*, *115*(1), 14-21.

Schofield, C. J. (1985). Control of Chagas' Disease Vectors. *British Medical Bulletin, 41*(2), 187-194.

Smith, K.F., Acevedo-Whitehouse, K., & Pedersen, A.B. (2009). The role of infectious diseases in biological conservation. *Animal Conservation*. *12*, 1-12.

Smith, K.F., Sax, D.F., & Lafferty, K.D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology*. *20,* 1349-1357.

Solís, H. M., & Carlos, N. (2016). Reservorios silvestres de Trypanosoma cruzi en cuatro localidades de las regiones Amazonas y Loreto. *Theorēma (Lima, Segunda época, En línea)*, (2), 63-73.

Sousa, O. E. (2016). Anotaciones sobre la enfermedad de Chagas en Panamá. Frecuencia y distribución de *Trypanosoma cruzi* y *Trypanosoma rangeli*. *Revista de Biologia Tropical*, *20*(2), 167-179.

Stevens, J. R., Noyes, H. A., Dover, G. A., & Gibson, W. C. (1999). The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitology*, *118*(01), 107-116.

Tam, C., Demke, O., Hermanas, T., Mitchell, A., Hendrickx, A. P. A., & Schneewind, O. (2014) YfbA, a *Yersinia pestis* Regulator Required for Colonization and Biofilm Formation in the Gut of Cat Fleas. *Journal of Bacteriology*. *196*(6): 1165-1173

Telford Jr, S. R., Tonn, R., Betancourt, P., & Gonzalez, J. (1975). New hosts of *Trypanosoma cruzi* in Venezuela. *Boletin de la Direccion de Malariologia y Saneamiento Ambiental*, *15*(5), 240-241.

Tesh, R. B., Peralta, P. H., & Johnson, K. M. (1970). Ecologic studies of vesicular stomatitis virus II. Results of experimental infection in Panamanian wild animals. *American Journal of Epidemiology*, *91*(2), 216-224.

Thompson, R.C.A., Lymbery, A.J. & Smith, A. (2010) Parasites, emerging disease and wildlife conservation. *International Journal for Parasitology*, *40*(10). pp. 1163-1170.

Tipton, V.J., & Mendez, E. (1966). The fleas (*Siphonaptera*) of Panama. Pp 289-338. *in* Ectoparasites of Panama. (Wenzel, R.L., and V.J. Tipton eds.). Field Museum of Natural History, Chicago, Illinois.

Tompkins, D. M., & Begon, M. (1999). Parasites can regulate wildlife populations. *Parasitology Today*, *15*(8), 311-313.

Travi, B. L., Arteaga, L. T., Leon, A. P., & Adler, G. H. (2002). Susceptibility of spiny rats (*Proechimys semispinosus*) to *Leishmania (Viannia) panamensis and Leishmania (Leishmania) chagasi*. *Memórias do Instituto Oswaldo Cruz*, *97*(6), 887-892.

Travi, B.L., Jaramillo, C., Montoya, J., Segura, I., Zea, A., Goncalves, A., & Velez, I.D. (1994) *Didelphis marsupialis,* an important reservoir of *Trypanosoma* (Shizotrypanum) *cruzi* and *Leishmania* (Leishmania) *chagasi* in Colombia. *The American Journal of Tropical Medicine and Hygiene. 50*(5): 557-565

Truc, P., Büscher, P., Cuny, G., Gonzatti, M. I., Jannin, J., Joshi, P., ... & Simarro, P. P. (2013). Atypical human infections by animal trypanosomes. *PLoS Neglected Tropical Diseases*, *7*(9), e2256.

Vasquez, A. M. D., Samudio, F. E., Saldaña, A., Paz, H. M., & Calzada, J. E. (2004). Eco-epidemiological aspects of Trypanosoma cruzi, Trypanosoma rangeli and their vector (Rhodnius pallescens) in Panama. *Revista do Instituto de Medicina Tropical de São Paulo*, *46*(4), 217-222.

Verma, A., Manchanda, S., Kumar, N., Sharma, A., Goel, M., Banerjee, P. S., ... & Deborggraeve, S. (2011). Trypanosoma lewisi or T. lewisi-like infection in a 37-day-old Indian infant. *The American Journal of Tropical Medicine and Hygiene*, *85*(2), 221-224.

Winker, A. (2013) Parasite communities of tropical forest rodents; influences of microhabitat structure and specialization. Unpublished Master's thesis, University of Wisconsin-Oshkosh.

Whitlaw Jr, J. T., & Chaniotis, B. N. (1978). Palm trees and Chagas' disease in Panama. *The American journal of tropical medicine and hygiene*, *27*(5), 873-881.

World Health Organization (1990). Control of the Leishmaniases, Technical report series 793, Geneva, 158 pp.

World Health Organization. (2017a) Metrics: Disability-Adjusted Life Year (DALY) (Webpage) Retrieved from http://www.who.int/healthinfo/global_burden_disease/metrics_daly/en/

World Health Organization. (2017b) Leishmaniasis: Magnitude of the Problem (Webpage) Retrieved from http://www.who.int/leishmaniasis/burden/magnitude/burden_magnitude/en/

World Health Organization. (2017c) Chagas Disease: American Trypanosomiasis-Fact Sheet (Webpage) Retrieved from http://www.who.int/mediacentre/factsheets/fs340/en/

World Health Organization. (2017d) Leishmaniasis: The Vector (Webpage) Retrieved from http://www.who.int/leishmaniasis/vector/en/

Yeo, M., Acosta, N., Llewellyn, M., Sánchez, H., Adamson, S., Miles, G. A., ... & de Arias, A. R. (2005). Origins of Chagas disease: Didelphis species are natural hosts of Trypanosoma cruzi I and armadillos hosts of Trypanosoma cruzi II, including hybrids. *International Journal for Parasitology*, *35*(2), 225-233.

Yeo, M., Lewis, M. D., Carrasco, H. J., Acosta, N., Llewellyn, M., da Silva Valente, S. A., ... & Miles, M. A. (2007). Resolution of multiclonal infections of Trypanosoma cruzi from naturally infected triatomine bugs and from experimentally infected mice by direct plating on a sensitive solid medium. *International Journal for Parasitology*, *37*(1), 111-120.