THESIS

DESCRIPTION AND DISTRIBUTION OF HELMINTH PARASITES OF WHITE-BELLIED GRASS MICE (*AKODON ALBIVENTER)* AND ANDEAN VESPER MICE (*CALOMYS LEPIDUS)* OF THE ALTIPLANO REGION OF BOLIVIA

Submitted by

Bretton Skyler Griffin

Department of Biology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2016

Master's Committee:

Advisor: Janice Moore

William Black IV
Karen Raines

ABSTRACT

DESCRIPTION AND DISTRIBUTION OF HELMINTH PARASITES OF WHITE-BELLIED GRASS MICE (*AKODON ALBIVENTER)* AND ANDEAN VESPER MICE (*CALOMYS LEPIDUS)* OF THE ALTIPLANO REGION OF BOLIVIA

Akodon and *Calomy*s genera are among the most speciose of South American rodents. Within Bolivia, these genera inhabit multiple environments of varied geomorphology, elevation, vegetation and climates, including the Bolivian altiplano, which consists of complex ecosystems at elevations of 3,000 meters and above. A number of species like the White-Bellied Grass Mouse (*Akodon albiventer*) and Andean Vesper Mouse (*Calomys lepidus)* primarily reside at the high elevations of the altiplano. Because of their habitat location, relatively few studies have addressed *A. albiventer* and *C. lepidus* parasites, specifically their helminth parasites.

To gain further knowledge about the parasite fauna infecting these rodents, a biodiversity survey was conducted. This biodiversity survey is the first that describes and reports the distribution of helminth parasites occurring in *A. albiventer* and *C. lepidus* collected from the altiplano region of Bolivia. Gardner and colleagues collected *A. albiventer* and *C. lepidus* hosts during expeditions in 1984-1993 from five departments in Bolivia: Oruro, Chuquisaca, Tarija, La Paz, and Cochabamba. From these collections, 27 *A. albiventer* and 11 *C. lepidus* were randomly chosen for parasite analysis, and yielded a total of 702 helminth parasite specimens, representing five helminth taxa. Oxyurids were the most common, followed by protospirurids, rictulariids, trichostrongylids, and cestodes. These are the first parasite records from *A. albiventer* and *C. lepidus* within the altiplano region of Bolivia.

Many of the oxyurid parasites recovered from *A. albiventer* and *C. lepidus* were unidentifiable to species due to the overlap of morphological measurements and features. In order to evaluate accuracy the of manual identification techniques through the examination of morphological relationships between species, statistical analyses were performed on male and female oxyurid specimens (*Syphacia* spp.) separated into three species groups. The analyses indicated that two species groups from the host, *A. albiventer*, shared more morphological measurement similarities than the species group from *C. lepidus*. Though further analysis is necessary, it is possible that the species group derived from *C. lepidus* is a new *Syphacia* species.

ACKNOWLEDGEMENTS

First and foremost, I give tremendous thanks to Dr. Janice Moore. Dr. Moore paved the way for my project by providing me with an incredible amount of connections, support and guidance. Not only did Dr. Moore critique my project and tediously edit my thesis, she fostered my independence and challenged me to think critically and work resourcefully. Her influence has sharpened my work ethic and bolstered my self-confidence. I am honored and grateful to have worked with her.

I would also like to thank my committee members, Dr. Karen Raines, who gave me valuable suggestions on problems I encountered, and Dr. William Black IV, who spent hours teaching and sharing his vast knowledge on statistical analysis. I also extend a special thanks to Dr. Scott L. Gardner, who generously invited me to collaborate on this project. Dr. Gardner has given me an immeasurable amount of insight, guidance, and encouragement. I owe much of what I know about parasitological laboratory techniques to Dr. Gardner and the Manter lab.

I thank my aunt, Dr. Sherri Lawless, who inspired me to pursue my passion through hard work and dedication. She has been a great support system through her editing skills, encouragements, and humorous motivations.

A special thanks goes to my mentor and parasitology professor at Southern Methodist University, Dr. John Ubelaker. Dr. Ubelaker introduced me to parasitology, inspiring me and nurturing my goals of pursuing a degree in science. Without him, I would not have considered entering into the biology graduate program at CSU to pursue a career in parasitology.

I thank my family and friends, who have been nothing but patient and supportive throughout my graduate career. Specifically, I thank my parents, Bretton Russell Griffin and Tim

Griffin, and my boyfriend, Jordan Graham; who have been my number one advocates and listeners, even though my parasitological rants were equivalent to speaking in a foreign language.

My lab mates, Paige Littman, Charles Stone and Broox Boze, have been instrumental in much needed advice and procrastination, but most importantly, my success as a graduate student. I truly appreciate all of their suggestions, encouragements, and friendships.

I would also like to thank Kyle Taitt, Suzanne Royer, and Barb Andre. Kyle lent his personal microscope camera to me and my lab with no questions asked, Suzanne not only taught me crucial microscope techniques, she also calibrated my own laboratory microscope, and Barb spent hours patiently evaluating my statistical analyses and extending her much needed recommendations. Without these three kind and intelligent individuals, I would not have been able to complete my project.

Lastly, I want to thank the parasites. Not for the headaches, tantrums, and frequently dropped obscenities but for piquing my interest through their extraordinary biological significance and for challenging me to become a better scientist.

TABLE OF CONTENTS

CHAPTER 1: Literature Review

Bolivia is a country of diverse terrain and rich ecosystems. Though many of these areas of richness have been extensively studied, the faunal parasite communities have not, particularly in rodent species of the genera *Akodon* and *Calomys*. Based on prior collections, a biodiversity survey was conducted to describe and investigate the distribution of the helminth species that infect *Akodon albiventer* and *Calomys lepidus* inhabiting the altiplano of Bolivia.

To further our understanding of the distribution of species of helminths found within these host species, I reviewed the literature pertaining to the background and attributes of *Akodon albiventer* Thomas, 1897, *Calomys lepidus* Thomas, 1884, and the Bolivian altiplano. The search strategy for the literature review used Web of Science, BioOne, Science Direct, CAB abstracts, and Google Scholar (1969- mid-2015) and was aimed at capturing literature relevant to *Akodon* and *Calomys* genera taxonomy, history, distribution, characteristics and parasite communities, and any background surrounding the biogeography and ecology of the Bolivian altiplano. Since *A. albiventer* and *C. lepidus* have not been widely studied, only a few reports of the species were found; therefore, the search was broadened to include other species in their genera. Known parasite communities of the hosts and their congeners were additionally reviewed for insight in morphological descriptions, life cycles, hosts, and distributions.

The review begins with the introduction to the biogeography of the Bolivian altiplano, and leads into an overview of that area's rodent history and taxonomy. I will conclude this chapter with a detailed introduction to *A. albiventer* and *C. lepidus*, along with synopses of the evolutionary history of these genera and a review of recorded parasite communities.

Bolivian Altiplano Biogeography

The country of Bolivia is bordered by Peru, Brazil, Paraguay, Argentina and Chile; it is one of two countries in South America that is landlocked. Bolivia spans roughly 1,100,000 km^2 ranging in elevations of 200 m to over 6,500 m, with a third of its territory lying in the highlands of the Andean Mountains (Arnade, 2014; Anderson, 1997). Bolivia comprises nine departments. The five departments in this study are Oruro, Tarija, La Paz, Chuquisaca, and Cochabamba. These departments, which are geographically adjacent to one another, are situated on and in between two parallel cordilleras on the western and eastern range of the Andes (Arnade, 2014; Brandt and Townsend, 2006; Brush, 1982). The area between these cordilleras is known as the great altiplano, or "high plateau", an arid ecological transitional zone about 500 km wide with high altitude habitats (Preston et al., 2003; Anderson, 1997; Brush, 1982; Unzueta, 1975).

The altiplano region extends from northern Argentina through the length of Bolivia to southern Peru, expanding into Chile (Támalo et al., 2010; Anderson, 1997; Unzueta, 1975). It is an eroded flat-floored depression lying at elevations of 3,500 m and above, occasionally interrupted by mountainous ridges and rocky hills with tundra- like vegetation (Arnade, 2014; Brandt and Townsend, 2006; Anderson, 1997). Central and south of the Andes, the altiplano range becomes wider and transforms into an environment known as the puna. The puna is a tilted Andean grassland embedded in a system of mountain basins and valleys and ranging in elevations from 3,000 m to 5,200 m (Arnade, 2014; Gardner and Perez-Ponce de León, 2002). Depending on the location within the altiplano, the puna can be moist or arid (Brush, 1982).

The high elevations, location, and exposure to wind greatly influence the precipitation and temperature of the altiplano region (Brandt and Townsend, 2006; Brush, 1982). The average annual precipitation of the altiplano ranges from 350 mm to 500 mm with 85% falling between

November and March (Brandt and Townsend, 2006). The average annual air temperature is roughly 8° C, but fluctuates diurnally with frosts typically occurring nightly. Meanwhile, for every 1,000 m of elevation the temperature lapse rate is approximately 6° C, dictating the presence and growth of vegetation (Brush, 1982).

Altiplano Flora

Large expanses of the high-altitude altiplano terrain consist of arid, rocky soil interspersed with scrub vegetation. Around the salt flats and rocky outcrops, patches of thorny low-statured shrubs, coarse bunchgrasses, grassy steppes, and tussocks (e.g., *Stipa ichu, Festuca orthophylla*, and *Calamagrosti*ss) are scattered across the remote landscape (Arnade, 2014; Monteiro and Körner, 2013; Tálamo et al., 2010; Brush, 1982). Areas that lack vegetation result from extreme elevations, long dry seasons, poor soil suitability, solar radiation, topographic variability, and cold harsh climatic conditions that impede plant growth (Tálamo et al., 2010; Brandt and Townsend, 2006; Anderson, 1997; UNESCO, 1981).

Within the alpine meadows of the milder puna region, a variety of plant species have adapted to the environmental constraints, potentiating an increase in the density and volume of vegetation (Anderson, 1997; Brush, 1982; UNESCO, 1981). Many of these plants "...display disproportionate development of underground organs..." (Anderson, 1997: 69) resulting in an abundance of pilose, spinescent and succulent plant species (Anderson, 1997; UNESCO, 1981). Other plants that inhabit this region include wind-resistant shrubs, moss-like pads, tree ferns and large, spiny cacti (Arnade, 2014; Preston et al., 2003; Gardner and Perez-Ponce de León, 2002). Some trees inhabit the lower-altitude puna terrain; these include the native quishura, alder, and khena trees, and introduced species of eucalyptus and pine (Arnade, 2014; Gardner and Perez-Ponce de León, 2002; Anderson, 1997). In areas where soil and topographic conditions are fertile

and fair, the land has been converted into crop and pasture fields for local agriculture (Brandt and Townsend, 2006; Preston et al., 2003).

Altiplano Fauna

Bolivia has rich biological diversity, particularly in its mammalian fauna (Wilson and Reeder, 2005; Eisenberg and Redford, 1999; Anderson, 1997). Over 330 mammalian species, 316 of them native, have been documented in Bolivia (Notarnicola et al., 2012; Anderson, 1997). Within the altiplano, there are over 50 mammalian species; a high proportion of those represent endemic rodent species. The endemic rodent community consists of Ctenomyidae, Chinchillidae, Caviidae, and Sigmodontinae subfamilies. Of the Sigmodontinae genera, species of *Abrothix, Akodon, Ausliscomys, Bolomys, Calomys, Chinchillula, Eligmodontia, Galenomys, Lenoxus, Necromys, Neotomys, Oligoryzomys, Oxymycterus* and *Phyllotis* inhabit the high Andes (Patton et al., 2015; Anderson, 1997). Aside from rodents, other mammals that typically inhabit the altiplano include carnivores like Andean foxes (*Lycalopex culpaeus*), Andean cats (*Felis concolor*), Geoffrey's cats (*Felis geoffroyi*), and Pampas cats (*Leopardus pajeros*), marsupials, lagomorphs and camelids (Vicunas, Llamas, Alpacas) which graze on the low-nutrient grass fodder (Monteiro and Körner, 2013; Anderson, 1997; Brush, 1982). Environmental conditions contribute to the low carrying capacity for most macrofauna, allowing small rodents to dominate the highland landscape.

Rodent History and Taxonomy

It is hypothesized that ancestors of caviomorph rodents first migrated to South America from Africa (via sweepstakes dispersal) in the Eocene to early Oligocene epochs and underwent a long and intensive adaptive diversification (Pascual, 2006). Other genera, including descendants of Eurasian muroid rodents, are hypothesized to have immigrated to North America

from Asia 19-17 MYA (Webb, 2006). The fossil record and morphology indicate that many more rodent migratory events, diversifications, and dispersals occurred, in turn generating many more, and sometimes competing, hypotheses on how and when rodent diversifications began. Notably, during the rise of Panamanian land bridge 2.7 MYA, there occurred a large biotic interchange between the Americas that was initially hypothesized to have started the immigration of North American muroid rodents and resulting lineage radiations in South America (Pascual, 2006; Webb, 2006; Smith and Patton, 1999,1993). However, some researchers argue that the lineage radiations of muroids preceded that initial muroid entry into South America (Patton et al., 2015; Coyner et al., 2013; Almeida et al., 2007; D'Elia, 2003; Salazar et al., 2001; Pardiñas and Tonni, 1998; Smith and Patton, 1999, 1993).

For some muroid rodent lineages, like sigmodontines, technical and theoretical studies performed on speciation patterns, divergence times and dispersal movements are contradictory. It is evident that these complex evolutionary patterns are not completely understood (Carrizo and Catalano, 2015; Parada et al., 2015; Patton et al., 2015; Coyner et al., 2013; Almeida et al., 2007; Costa, 2003; D'Elia, 2003; Salazar-Bravo et al., 2001; Smith and Patton, 1999, 1993). Salazar-Bravo et al. (2001) explain the temporal divergence discrepancies by stating that based on the fossil record, some of the oldest muroid rodents known in South America were found to date back to 3.5-5 MYA; in contrast, Smith and Patton (1999) used genetic testing to date the estimated basal radiation of South American sigmodontines at about 10-14 MYA, thus challenging the land bridge theory. Others have hypothesized that sigmodontine lineage radiations coincided with climatic and ecological events like the uplift phase of the Andes and the grassland expansion in South America prior to the Panamanian land bridge (Coyner et al., 2013; Almeida et al., 2007; Salazar-Bravo et al., 2001; Smith and Patton, 1993). More recently,

Parada et al. (2015) estimated that the initial Sigmodontinae radiation began at about 7-9.36 MYA using taxonomic, odontologic and genetic analyses. Due to the conflicting support and lack of fossil evidence, research continues to be conducted to understand the geographical and temporal evolutionary events of sigmodontines. Recent studies agree that the immediate ancestor of sigmodontines did not originate in South America, but the geographic location of the basal sigmodontine radiation and the timing of the sigmodontine invasion of South America still elude resolution (Patton et al., 2015; D'Elia, 2003; Pardiñas et al., 2002; D'Elia, 2000). One fact is evident: the magnitude and diversity of muroid rodents exploded once they colonized South America, producing one of the most diverse taxonomic families on the continent (Webb, 2006).

At present, order Rodentia is considered the largest living mammalian order, containing 33 families and 481 genera (Wilson and Reeder, 2005). Subfamily Sigmodontinae of family Cricetidae and superfamily Muroidea contains 86 extant genera and about 400 species (Patton et al., 2015). In South America alone, there are around 60 endemic sigmodontine genera (D' Elia, 2003). Approximately 42% of South American terrestrial mammals are rodents, half of which are sigmodontines (Eisenberg and Redford, 1999). The large number of genera in Sigmodontinae have been subdivided and assembled into ten tribes: the larger tribes Oryzomyini, Akodontini, and Phyllotini, along with the smaller tribes Abrotrichini, Thomasomyini, Euneomyini, Ichthyomyini, Reithrodontini, Sigmodontini, and Wiedomyini (Pardiñas et al., 2015; Salavor-Bravo et al., 2013; D'Elia et al., 2007).

The focal species in this study come from two tribes of the Cricetidae: Akodontini and Phyllotini. Both tribes are the result of large adaptive diversifications of sigmodontines; tribe Akodontini consists of 14 genera and roughly 95 species, and tribe Phyllotini consists of approximately 11 genera and roughly 50 species (Patton et al., 2015; Salazar- Bravo et al., 2013;

D'Elia et al., 2007; Musser and Carleton 2005; Chiappero et al., 2002; Smith and Patton, 1999). Because of the diversity and magnitude of morphological diversification of sigmodontines, many researchers have debated their phylogenetic relationships and cladistic classifications, particularly within these two tribes (Carrizo and Catalano, 2015; Parada et al., 2015; Salazar-Bravo et al., 2013; Almeida et al., 2007; D'Elia et al., 2007; Wilson and Reeder, 2005; D'Elia, 2003; Chiappero et al., 2002; Salazar-Bravo et al., 2001; Smith and Patton, 1999, 1993). After many cytogenetic, phylogenetic, odontologic, taxonomic, and molecular sequencing analyses, the most recent estimated basal radiation of tribe Akodontini is between 3.33−4.77 MYA, while tribe Phyllotini is estimated to have diverged between 5.33−7.03 MYA (Parada et al. 2015; Almeida et al., 2007; Salazar-Bravo et al., 2001).

To set context for the current study, it is important to understand the evolutionary history, geographic distribution and phylogenetic relationships of *Akodon* and *Calomys*. Hosts that share similar traits and live in similar ecological areas have a higher probability of being infected by similar, if not the same, species of parasites (Poulin, 2014; Navone et al., 2009). Also, parasites that have infected ancestral host species can coevolve with the host species through multiple generations and thus share similar patterns of evolutionary history (Holmes et al., 1980; Sprent, 1969). By determining whether a parasite is found in a mammalian host because of association by descent or host-switching, one can infer ecological and evolutionary factors that may have influenced the assembly and ultimate composition of the host's parasite community (Brooks et al., 2014; Brooks, 1980; Holmes et al., 1980). Additionally, many of these host species are sympatric, increasing the probability of sharing similar environmentally influenced host- parasite features. For example, the sympatric rodents *Calomys callosus* Rengger, 1830 and *Akodon azarae* Fisher, 1829 inhabit the same Amazonian province of Argentina and are parasitized by

the tick, *Amblyomma triste* Koch, 1844 (Nava et al., 2006). Exploring host traits, phylogenetic relationships, and host-parasite associations may help predict aspects of helminth fauna within the focal hosts (Navone et al., 2009, but see Simões et al. 2011). Such studies may also test the hypothesis that the species richness of different parasite groups is correlated with different host characteristics (Vitone et al., 2002).

I have reviewed the biogeography of the Bolivian altiplano and rodent history and taxonomy of that region. In summary, the altiplano is a high altitude region inhabited largely by endemic rodents that have adapted to the harsh environmental conditions. Next, I will consider the two genera, *Akodon* and *Calomys*. I will individually discuss the history and background of each genus then review the history and background of each focal host. I will conclude each review with a description of the recorded parasite community of that genus.

Akodon: History and Background

History

Thomas (1916, 1918) first recognized and assembled tribe Akodontini Vorontsov, 1959 (see D'Elia 2003). The Akodontini tribe contains 15 genera, including *Akodon* Meyen, 1833 (Patten et al., 2015; D'Elia et al., 2007; Musser and Carleton 2005; Smith and Patton, 1999). It is hypothesized that species of the *Akodon* genus diverged from other genera of its tribe around 3.7 MYA, commencing diversification within the genus (Coyner et al., 2013; Salazar-Bravo et al., 2001; Smith and Patton, 1999). Species allocated to the genus *Akodon* genus have been commonly misallocated to numerous genera, including: *Abrothrix, Bolomys, Chalcomys, Chroeomys, Deltamys, Hypsimys, Microxus, Thalpomys, and Thaptomys* genera, mostly due to what appears to be inadequate documentation and analysis (Wilson and Reeder, 2005, 1993; Smith and Patton 1999, 1993). With more explicit specimen documentation (museum voucher

specimen deposition) and phylogenetic analysis, researchers have been able to precisely define the genera and its subsequent groups, though some distinctions vary (Coyner et al., 2013; D' Elia, 2003; Smith and Patton, 1999, 1993).

Background

The *Akodon* genus is one of the largest of the sigmodontines (Coyner et al., 2013; Smith and Patton, 1999, 1993). Forty-five species of *Akodon* are described from South America (Coyner et al., 2013; Gardner and Perez-Ponce de León, 2002; Anderson, 1997; Smith and Patton, 1993; Eisenberg and Redford, 1992). The 45 species were organized into five species groups: *boliviensis* group, *dolores* group, *cursor* group, *varius* group, and *aerosus* group (Patton et al., 2015; Coyner et al., 2013; Musser and Carleton, 2005). Of these groups, roughly twelve species of *Akodon* are currently known from Bolivia, including the focal species, *Akodon albiventer*, an old lineage in species group *aerosus* (Coyner et al., 2013; Gardner and Perez-Ponce de León, 2002; Anderson, 1997).

Patton et al. (2015) indicate that *Akodon* inhabit terrestrial niches primarily in the Andean environment, where it is assumed that speciation occurred. The rodents of the *Akodon* genus live throughout South America and occupy habitats at altitudes ranging from 200 m to over 5,000 m, but most species reside on the eastern side of the Andes (Patton et al., 2015; Eisenberg and Redford, 1992). Members of the *Akodon* genus are fossorial or cursorial, and engage in crepuscular activity (Gettinger, 2015; Patton et al., 2015). They seem to be opportunistic foragers, with diets consisting of seeds, leaves, and insects, a diet that varies based on habitat (e.g., some species occasionally eat mollusks or small vertebrates while others primarily eat fruit) (Navone et al., 2009; Vieira et al., 2006; Eisenberg and Redford, 1999).

There is no literature pertaining to the reproduction or behavior of the genus as a whole due to lack of studies and the variability of the genus. The life histories of most *Akodon* species have been poorly studied, though there are a few exceptions, particularly *A. azarae* and *Akodon cursor* Winge, 1887 (Patton et al., 2015). It is unknown if *A. albiventer*, one focus of this study, shares any traits with its better studied congeners.

I will now narrow my scope and review one of the focal host species, *A. albiventer*. I will examine the taxonomy, natural history, and description of *A. albiventer* then focus on the recorded parasite community of the *Akodon* genus.

A Synopsis of *Akodon albiventer*

White-Bellied Grass mice (*Akodon albiventer*) belong to order Rodentia, the superfamily Muroidea, the family Cricetidae, the subfamily Sigmodontinae, and the tribe Akodontini (Coyner et al., 2013; Wilson and Reeder, 2005; Anderson, 1997). In 1897, Thomas collected the White-bellied Grass Mouse in the Salta Province of Argentina and named it *Akodon albiventer* (see Musser and Carleton, 2005; Anderson 1997). It was initially thought to belong to the genus *Bolomys*, but its inclusion in *Akodon* was better supported (Patton et al., 2015; Wilson and Reeder, 2005; D'Elia et al., 2003; Smith and Patton, 1993).

The distribution of White-Bellied Grass mice ranges widely from Southeast Peru, through west Bolivia, to north Argentina and Chile (Wilson and Reeder, 2005,1993; Eisenberg and Redford, 1999; Mares et al., 1989). Within Bolivia, the mice inhabit the high slopes of the altiplano at elevations of about 3,000 m to 4,500 m (Figure 1.1: Patton et al., 2015; Eisenberg and Redford, 1999; Anderson, 1997; Wilson and Reeder, 1993). Their habitat ranges from boggy areas and semi-arid alpine meadows with dense vegetation to rocky areas on the broad Andean plain (Eisenberg and Redford, 1999; Wilson and Reeder, 1993; Mares et al., 1989). Though they

have been caught in open grass-land, Patton et al. (2015) noted that *A. albiventer* seem to prefer more protective, sheltered habitats under shrubs and rock walls. These mice are (unlike some of its congeners) omnivorous and ground dwelling (Eisenberg and Redford, 1999; Musser and Carleton, 1993; Mares et al., 1989).

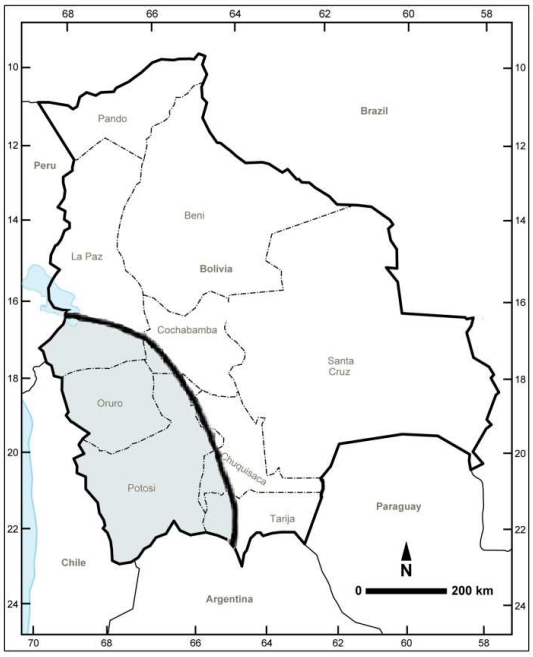


Figure 1.1: *Akodon albiventer* geographic distribution (shaded region) in Bolivia.

White-Bellied Grass mice weigh approximately 21-36 grams with a total body length of 156-183mm, tail length of 69-77mm, ear length of 13-13.5mm, and hind foot length with claw of 20-22mm (Patton et al., 2015; Anderson, 1997). They also have large auditory bullae compared to other *Akodon* species, and their pelage is short and coarse, with a grizzled texture; the dorsum is a slate gray, with a white ventral side and feet (Patton et al., 2015; Anderson, 1997). There is no detailed information pertaining to the behavior, reproduction, diet, and habits of *A. albiventer*.

Parasites of the genus *Akodon*

My literature review revealed no record of helminth parasites within *A. albiventer*. However, there is a record of the species' ectoparasites. Three flea species have been collected from *A. albiventer*: *Neotyphloceras crassispina hemisus* Jordan, 1936 and *Ectinorus (Ectinorus) pearsoni* Johnson, 1957 in Bolivia, and *Agastyopsylla* sp. in Chile (Beaucournu et al., 2011; Anderson, 1997). A variety of other ectoparasites have been found on eleven of the 45 documented *Akodon* species, particularly *Akodon dolores* Thomas, 1916 and *A. azarae.* Ectoparasites can be intermediate hosts of endoparasites and increase parasite transmission; the majority of ectoparasites found on *A. azarae* are probably generalists, given their association with a variety of hosts and lack of specific host-parasite features (Navone et al., 2009).

Though endoparasite records *of A. albiventer* are lacking, there is an extensive record of helminth parasites infecting congeners. Several helminth species, with direct and indirect life cycles, have been reported from numerous *Akodon* species. I have established a list of 41 possible endoparasites that might be found within White-Bellied Grass mice, given their occurrence in a total of 14 congeners (Table 1.1); these hosts are from Brazil, Argentina, Peru, Chile and Bolivia, and include parasites from phylum Nemata, class Cestoda, class Trematoda, class Kinetoplasta, class Conoidasida and Class Trichomonada. Of phylum Nemata, 30 parasites were yielded from surveys separately conducted on a total of nine *Akodon* species, with the most common hosts being *A. azarae*, *A. cursor*, and *Akodon montensis* Thomas, 1913. Four species of cestodes were collected independently in a combination of these same three species of *Akodon*. This is not surprising since these three host species are some of the most abundant *Akodon* found within the three study areas (Mino et al., 2012; Musser and Carleton, 2005). In Argentina, two trematode species were collected from *A. azarae*. Another trematode species was found in *A.*

cursor, *A. montensis*, and *Akodon arviculoides* Wagner, 1842 in Brazil, and in Bolivia, only one trematode species was found in both *Akodon fumeus* Thomas, 1902 and *Akodon mimus* Thomas, 1901. Of the parasitic protozoans, one coccidian species and a *Trichomonas* sp. were collected separately in *A. montensis*, and only one trypanosome species was recorded in a five *Akodon* species. A variety of ectoparasites also infect *Akodon* including ticks, fleas, lice, mites, and chiggers (Table 1.1). See Table 1.1 for a complete list of parasites of *Akodon* species.

Table 1.1: A checklist of parasites that have been shown to infect species in the genus *Akodon.*

Parasite Taxa			*Akodon* Host	Locality	References
Class	**Family**	**Species**			
Nemata^	Oxyuridae	*Syphacia carlitosi*	*Akodon azarae* *Akodon cursor* *Akodon montensis*	Brazil	Robles and Navone, 2007 ; Navone et al., 2009; Herrera et al 2011; Pinto et al., 2011; Miño et al., 2012; Simões et al. 2012
		Syphacia obvelata	*Akodon cursor*	Brazil	Gomes et al. 2003
		Syphacia kinsellai	*Akodon cursor* *Akodon montensis*	Brazil	Robles and Navone 2007; Simões et al., 2011; Pinto et al., 2011
	Onchocercidae	*Litomosoides chagasfilhoi*	*Akodon cursor*	Brazil	Pinto et al, 2011; Brant and Gardner, 2000
		Litomosoides odilae	*Akodon cursor* *Akodon montensis*	Brazil	Pinto et al, 2011; Brant and Gardner, 2000
		Litomosoides silvai	*Akodon montensis* *Akodon cursor*	Brazil	Pinto et al, 2011; Simões et al. 2012
	Rictularidae	*Pterygodermatites azarai*	*Akodon azarae*	Argentina	Mino et al., 2012
	Spiruridae	*Protospirura numidica criceticola*	*Akodon azarae* *Akodon montensis* *Akodon jelskii* *Akodon boliviensis*	Argentina Brazil Peru	Sutton 1989; Miño, 2008 ; Miño et al. 2012; Simões et al. 2012
	Trichuridae	*Trichuris laevitestis*	*Akodon azarae* *Akodon montensis*	Argentina	Navone et al. 2009; Miño et al., 2012
		Trichuris navonae	*Akodon montensis*	Argentina	Robles, 2011
	Heligmonellidae	*Stilestrongylus azarai*	*Akodon azarae*	Argentina	Navone et al., 2009
		Stilestrongylus sp.	*Akodon azarae* *Akodon longipillis* *Akodon olivaceus*	Argentina Chile	Miño et al., 2012; Pérez-Ponce de León et al., 2000.
		Stilestrongylus aculeata	*Akodon cursor* *Akodon montensis*	Brazil	Gomes et al. 2003; Pinto et al., 2011; Simões et al. 2012; Simões et al., 2014
		Stilestrongylus eta	*Akodon montensis* *Akodon cursor*	Brazil	Simões et al., 2012; Simões et al., 2014
		Stilestrongylus lanfrediae	*Akodon cursor* *Akodon montensis*	Brazil	Souza et al. 2009a; Simões et al., 2011; Pinto et al., 2011;Simões et al. 2012
		Hassalstrongylus epsilon	*Akodon cursor*	Brazil	Gomes et al. 2003
		Hassalstrongylus multiovatus	*Akodon simulator*	Argentina	Digiani et al., 2007
		Hassalstrongylus zeta	*Akodon cursor*	Brazil	Gomes et al. 2003
		Hassalstrongylus sp.	*Akodon montensis*	Brazil	Kuhnen et al., 2012
		Guerrerostrongylus sp.	*Akodon cursor* *Akodon montensis* *Akodon simulator*	Brazil Argentina	Sutton & Durette-Desset, 1991; Pinto et al., 2011; Simões et al., 2011; Digani et al., 2007
		Guerrerostrongylus uruguayiensis	*Akodon cursor* *Akodon montensis* *Akodon simulator*	Brazil Argentina	Sutton & Durette-Desset, 1991; Pinto et al., 2011; Simões et al., 2011; Digani et al., 2007
		Guerrerostrongylus zetta	*Akodon cursor* *Akodon montensis*	Brazil	Sutton & Durette-Desset, 1991; Pinto et al., 2011; Simões et al. 2012
		Trichofreitasia lenti	*Akodon cursor* *Akodon montensis*	Brazil	Sutton & Durette-Desset 1991; Pinto et al. 2011;Simões et al. 2011; Simões et al. 2012
		Longistriata sp.	*Akodon montensis*	Brazil	Kuhnen et al., 2012
		Avellaria sp.	*Akodon cursor* *Akodon montensis*	Brazil	Simões et al. 2012
	Strongyloididae	*Strongyloides sp.*	*Akodon montensis*	Brazil	Kuhnen et al., 2012
	Viannaiidae	*Viannaia viannai*	*Akodon cursor*	Brazil	Gomes et al. 2003
	Metastrongylidae	*Akodonema luzsarmientae*	*Akodon mollis*	Peru	Morales et al., 2012
		Angiostrongylus lenzii	*Akodon montensis* *Akodon cursor*	Brazil	Souza et al. 2009b; Pinto et al., 2011; Morales et al., 2012; Simões et al. 2012
		Angiostrongylus morerai	*Akodon azarae*	Argentina	Morales et al., 2012

Table 1.1: Continued...

Parasites Taxa			*Akodon* Host	Locality	Reference
Class	**Family**	**Species**			
Cestoda	Cyclophyllidea[+]	unknown	*Akodon azarae*	Argentina	Mino et al., 2012
	Hymenolepididae	*Hymenolepis* sp.	*Akodon montensis*	Brazil	Kuhnen et al., 2012
		Rodentolepis akodontis	*Akodon arviculoides* *Akodon montensis* *Akodon cursor*	Brazil	Rêgo, 1967; Simões et al. 2011; Simões et al. 2012
	Taeniidae	*Taenia taeniaeformis*	*Akodon azarae*	Argentina	Mino et al., 2012
Trematoda	Dicrocoeliidae	*Zoonorchis oxymycterae*	*Akodon azarae*	Argentina	Navone et al. 2009; Miño et al., 2012
		Canaania obesa	*Akodon cursor* *Akodon montensis* *Akodon arviculoides*	Brazil	Gardner and Perez-Ponce de León, 2002; Simões et al. 2012
		Yungasicola travassosi	*Akodon fumeus* *Akodon mimus*	Bolivia	Gardner and Perez-Ponce de León,2002
	Echinostomatidae	*Echinoparyphium scapteromae*	*Akodon azarae*	Argentina	Navone et al., 2009
Kinetoplastida	Trypanosomatidae	*Trypanosoma cruzi*	*Akodon boliviensis,* *Akodon dolores,* *Akodon montensis* *Akodon toba* *Akodon molinae*	Argentina, Bolivia Brazil	Brigada et al., 2010; Orozco et al., 2014; Messenger et al., 2015
Conoidasida	Sarcocystidae	*Besnoitia* sp.	*Akodon montensis*	Brazil	Grisard et al., 1997
Trichomonada	Trichomonadidae	*Trichomonas* sp.	*Akodon montensis*	Brazil	Kuhnen et al., 2012
Insecta	Ctenophthalmidae	*Agastopsylla* sp.	*Akodon albiventer*	Chile	Beaucournu et al., 2011
		Neotyphloceras crassispina hemisus	*Akodon albiventer*	Bolivia	Anderson, 1997; Lareschi et al., 2010
	Hoplopleuridae	*Hoplopleura aitkeni*	*Akodon azarae* *Akodon dolores*	Argentina Patagonia	Patton et al., 2015; Navone, 2009; Nava et al., 2003
		Hoplopleura varia	*Akodon dolores*	Patagonia	Patton et al., 2015
		Hoplopleura sp.	*Akodon varius*	Paraguay	Patton et al., 2015
	Pulicidae	*Hectopsylla gracilis*	*Akodon caenosus,* *Akodon albiventer*	Argentina	Lareschi et al., 2010
	Rhopalopsyllidae	*Ectinorus barrerai*	*Akodon dolores* *Akodon molinae*	Argentina	Patten et al., 2015; Lareschi et al., 2004
		Ectinorus (Ectinorus) pearsoni	*Akodon albiventer*	Bolivia	Anderson, 1997
		Gephyropsylla klagesi	*Akodon mollis*	unknown	Pucu et al., 2014
		Polygenis sp.	*Akodon varius*	Paraguay	Patton et al., 2015
		Polygenis (Polygenis) acodontis	*Akodon simulator*	Argentina	Lareschi et al., 2010
		Polygenis bohlsi bohlsi	*Akodon dolores* *Akodon molinae*	Argentina	Patton et al., 2015; Lareschi et al., 2004
		Polygenis platensis cisandinus	*Akodon molinae*	Argentina	Lareschi et al., 2004
		Polygenis platensis	*Akodon dolores*	Argentina	Patton et al., 2015
		Polygenis puelche	*Akodon dolores* *Akodon molinae*	Argentina	Patton et al., 2015; Lareschi et al., 2004
		Polygenis rimatus	*Akodon dolores*	Argentina	Patton et al., 2015
		Tetrapsyllus (Phylliver) bleptus	*Akodon albiventer*	Argentina	Lareschi et al., 2010
		Tetrapsyllus rhombus	*Akodon dolores*	Patagonia	Patton et al., 2015
		Tetrapsyllus tantillus	*Akodon dolores*	Patagonia	Patton et al., 2015

Table 1.1: Continued...

Parasite Taxa			*Akodon* Host	Locality	References
Class	**Family**	**Parasite Species**			
Insecta	Stephanocircidae	*Barreropsylla excelsa*	*Akodon dolores*	Patagonia	Patton et al., 2015
		Craneopsylla minerva	*Akodon toba*	n/a	Patton et al., 2015
		Craneopsylla minerva wolffhuegeli	*Akodon toba* *Akodon molinae*	n/a	Patton et al., 2015; Lareschi et al., 2004
		Plocopsylla wolffsohni	*Akodon dolores*	Patagonia	Patton et al., 2015
		Sphinctopsylla ares	*Akodon dolores*	Patagonia	Patton et al., 2015
Arachnida	Amblyommidae	*Amblyomma tigrinum*	*Akodon dolores* *Akodon molinae* *Akodon oenos*	Argentina	Nava et al., 2006
		Amblyomma triste	*Akodon azarae*	Argentina	Nava et al., 2006
	Ixodidae	*Ixodes loricatus*	*Akodon azarae*	Argentina	Navone et al., 2009
	Macronyssidae	Ornithonyssus bacoti	*Akodon dolores*	Argentina	Patton et al., 2015
	Laelapidae	*Androlaelaps fahrenholzi*	*Akodon azarae* *Akodon varius*	Argentina Paraguay	Patton et al., 2015; Navone, 2009; Nava et al., 2003
		Androlaelaps rotundus	*Akodon azarae* *Akodon toba* *Akodon varius*	Argentina Paraguay	Patton et al., 2015; Navone, 2009; Nava et al., 2003
		Androlaelaps philipmyers	*Akodon toba* *Akodon philipmyers*	Argentina	Patten et al., 2015; Lareschi, 2011
		Androlaelaps ulysespardinasi	*Akodon toba*	n/a	Patton et al., 2015
		Laelaps manguinhosi	*Akodon azarae*	Argentina	Nava et al., 2003
	Leeuwenhoekiidae	*Paraguacarus callosus*	*Akodon varius*	Paraguay	Patton et al., 2015
	Listrophoridae	*Prolistrophorus (Prolistrophorus) argentinus*	*Akodon affinis*	Columbia	Bochkov et al., 2014
		Prolistrophorus (Aprolistrophorus) parabidentatus	*Akodon azarae*	Argentina	Bochkov et al., 2014
	Trombiculoidea	*Andalgalomacarus paraguayensis*	*Akodon varius*	Paraguay	Patton et al., 2015
		Eutrombicula alfreddugesi	*Akodon azarae*	Argentina	Navone, 2009
		Paratrombicula enciscoensis	*Akodon varius*	Paraguay	Patton et al., 2015

^= Phylum $^{+}$= Order

Viruses

Hantaviruses are the only known viruses to infect *Akodon* species. Strains of hantavirus are the causative agents of zoonotic rodent-borne hemorrhagic fevers that manifest as a variety of infectious hemorrhagic diseases in humans [e.g., hantavirus cardiopulmonary syndrome (HPS), hemorrhagic fever with renal syndrome (HFRS)] (Oliveira et al., 2012). Muroid rodents are the

primary reservoir hosts for hantavirus strains, with each strain mainly associated with a single species of muroid rodent host (Oliveira et al., 2014; Mills and Childs, 1998). Once established in the rodent host through horizontal or vertical transmission, the hantavirus maintains a subclinical infection, rarely causing disease in the host (Mills and Childs, 1998). There are multiple hantavirus strains, but only the Jabora virus (JABV) has been recorded in *A. montensis* and *Akodon paranaensis*, Christoff, Fagundes, Sbalqueiro, Mattevi & Yonenaga Yassuda, 2000 with no documented transmission to humans (Oliveira et al., 2014; Oliveira et al. 2012).

Parasitic protozoa

Three parasitic protozoan species of different phyla have been recorded in multiple *Akodon* species. *Trypanosoma cruzi* Chagas, 1909 is a single-celled flagellated kinetoplastid parasite transmitted in domestic and sylvatic habitats between triatomine insect vectors and mammalian hosts. The transmission in sylvatic cycles occurs through reservoir, secondary, and/or dead-end hosts (Orozco et al., 2014; Brigada et al., 2010). Orozco et al. (2014) reported *T. cruzi* in five *Akodon* species (Table 1.1: see Messenger et al., 2015; Brigada et al., 2010). *Trypanosoma cruzi* typically causes subclinical infections in reservoir hosts; however, it is the etiological agent of Chagas disease in humans (Brigada et al., 2010). In addition, species of the coccidian genus *Besnoitia* have been found to naturally infect *A. montensis* (Grisard et al., 1997). A *Trichomonas* sp. was also collected from *A. montensis* (Kuhnen et al., 2012).

Helminths

Known helminths of other species of *Akodon* from similar habitats or nearby locations may indicate the parasite composition of *A. albiventer* since patterns of spatial variation and abundance in host species can predict wild rodent helminths (Behnke, 2008). As stated above,

the helminth parasite fauna of *A. albiventer* are unknown, but a variety of helminths have been documented in other *Akodon* species (Table 1.1). The majority of the studies were performed on prevalent and accessible *Akodon* species, such as *A. azarae*, *A. cursor*, and *A. montensis*. These studies found parasites from phylum Nemata, class Cestoda, and class Trematoda. In my study, I focused on helminth parasites, so I will explore these helminths in greater depth below.

Nematodes

Phylum Nemata is one of the largest phyla containing parasites. Though there are a few exceptions, the general characteristics of nematodes include bilateral symmetry and cylindrical shape with tapering ends, lack of segmentation, cuticular covering, sexual dimorphism and dioecy (Roberts and Janovy, 2009). Nematodes have direct and indirect life cycles with varying modes of transmission. Also, some nematode parasites are pathogenic.

The genus *Akodon* is rich in nematode parasites (Table 1.1), hosting oxyurids, filarioids, rictulariids, spirurids, trichurids, and strongylids, with strongylids being the most common. Roughly 20 species of strongylids have been recorded in *Akodon*, of which 15 species are from family Heligmonellidae (Simões et al., 2014; Kuhnen et al., 2012; Mino et al., 2012; Simões et al. 2012; Pinto et al., 2011; Simões et al., 2011; Navone et al., 2009; Souza et al., 2009a; Sutton and Durette-Desset, 1991; Gomes et al., 2003).

Oxyurids, also known as pinworms, of the genus Sy*phaci*a are commonly found in the cecum of rodents, have direct life cycles, and are considered to have coevolved with their rodent hosts (Robles and Navone, 2007; Okamato et al., 2007; Quentin, 1969). Only three *Syphacia* species have been documented in a combination of three *Akodon* species from Brazil. All three *Syphacia* species have been reported in *A. cursor*; two *Syphacia* species have been reported in *A. montensis*, and only one *Syphacia* species has been documented in *A. azarae* (Simões et al.,

2012; Miño et al., 2012; Pinto et al., 2011; Herrera et al., 2011; Navone et al., 2009; Robles and Navone, 2007; Gomes et al., 2003). Filarioids, which require an insect intermediate host via vector transmission, have also been found in *Akodon*.

Two species of filarioids have been identified in *A. cursor* and *A. montensis* in Brazil (Simões et al. 2012; Pinto et al., 2011; Brant and Gardner, 2000). One other filarioid of genus *Litomosoides* has also been identified in *A. cursor* of Brazil. The spirurid, *Protospirura numidica criceticola* Quentin, Karimi & Rodriguez de Almeida, 1968, is the only spirurid commonly found to parasitize *A. azarae* and *A. montensis* from Central Argentina to Brazil (Simões et al., 2012; Miño et al., 2012; Miño, 2008). The rictulariid, *Pterygodermatites azarai* Sutton, 1984, has been reported from *A. azarae* inhabiting pampean grasslands in Argentina (Mino et al., 2012). In addition, two species of trichurids have been recorded in a combination of two *Akodon* species in Argentina. *Trichuris laevitestis* Suriano and Navone, 1994 was collected from *A. azarae* and *A. montensis,* and *Trichuris navonae* Robles, 2011 was collected from *A. montensis* (Miño et al., 2012; Robles, 2011; Navone et al. 2009).

Cestodes

Cestodes, commonly referred to as tapeworms, are flat, "segmented" worms that absorb nutrients directly from their hosts' intestines. The tapeworm's body is made up of a scolex, which attaches to the intestinal mucosa of its host, a neck, and strobila (Roberts and Janovy, 2009). The strobila is formed from asexually produced units known as proglottids; these contain the reproductive organs and when gravid, are shed in the host's feces. (Roberts and Janovy, 2009).

A total of four cestode species have been found in *Akodon* (Table 1.1). Cestodes that infect rodents typically have an indirect life cycle and need an intermediate host, whether it be an

arthropod or the rodent itself (Miño et al., 2012). Cestode infections are typically asymptomatic, but death can occur from a blockage of knotted tapeworms within the intestines (Wiger, 1977).

Trematodes

Class Trematoda contains flatworms known as flukes. Though flukes vary in shape and size, most are typically flattened and oval shaped with oral and ventral suckers (Roberts and Janovy, 2009). Four species of trematodes infect a variety of *Akodon* species (Table 1.1). All four of these species are digenetic trematodes, which have complex life cycles, alternating between sexual and asexual reproduction in definitive and intermediate hosts, respectively (Roberts and Janovy, 2009). The life cycle of these trematodes includes a mollusk as a first intermediate host, an invertebrate or vertebrate as a second intermediate host, and a vertebrate as the definitive host (Gardner and Perez-Ponce de León, 2002).

Calomys: History and Background

History

Of sigmodontines, tribe Phyllotini contains approximately 11 genera, including the genus *Calomys* Waterhouse, 1837 (Patton et al., 2015; Salavor-Bravo et al., 2013; Smith and Patton, 1999). Phyllotini genera are thought to have primarily diversified in the central Andes (Smith and Patton, 1999; Steppan, 1995). However, there is much controversy over the divergence times and biogeographical history of *Calomys* (Salavor-Bravo et al., 2013; Almeida et at., 2007; Salazar-Bravo et al., 2001; Smith and Patton, 1999). The following account illustrates some of the conflicting hypotheses:

> Reig (1986) proposed that the genus originated in the Andes with posterior invasion of lowland habitats, as suggested by the high diversity of Phyllotini rodents in this cordillera. Alternatively, Braun (1993) proposed, based on Marshall's hypothesis (Marshall, 1978),

> that the genus originated in the northern part of its range in Venezuela and diversified as it expanded its distribution southward. According to Marshall (1978), a corridor of open vegetation appeared on the eastern slopes of the Andes at around 3.5 million years ago allowing the southward migration of savanna/grassland sigmodontine rodents that inhabited northern South America. Salazar-Bravo et al. (2001) suggested that the genus appeared directly south of Amazonia and at about 8.5 – 9 Mya split into two branches: one that originated the high-mid altitude clade and another that gave rise to *Calomys hummelincki*, through long range dispersal, and the ancestor of all other lowland species. These authors also linked the evolution of the genus to the spread of the C4 grasses in South America. (Almeida et al., 2007: 451)

In an effort to gain a more accurate resolution of *Calomys* divergence and phylogenetic relationships, Almeida et al. (2007) conducted cytogenetic analysis on a large geographic and species sampling of *Calomys* and other taxa, and found that the divergence of *Calomys* from other Phyllotini was estimated at 2.8–7.1 MYA. Many authors hypothesized that the evolution of the genus was possibly spurred by climatic and biotic events, such as the uplift phase of the Andes and the grassland expansion in South America during the late Miocene and early Pliocene epochs (Almeida et al., 2007; Salazar-Bravo et al., 2001; Smith and Patton, 1993, but see Parada et al., 2015). Even the divergence of *Calomys lepidus* Thomas, 1884 from other *Calomys* species has been debated (Almeida et al., 2007; Wilson and Reeder, 2005; Salazar-Bravo et al., 2001).

Background

The *Calomys* species are small bodied with a diet that is mostly granivorous but some species have been known to be herbivorous or omnivorous (Gettinger, 2015; Almeida et al., 2007; Eisenberg and Redford, 1999; Wilson and Reeder, 1993; Mares et al., 1989). The *Calomys* genus comprises three main species groups that represent highland, lowland, and Amazonian clades (Almeida et al., 2007; Salazar et al., 2001). *Calomys lepidus* is in the highland group along with *Calomys sorellus* Thomas, 1900 and *Calomys musculinus* Thomas, 1913 (Carrizo and

Catalano, 2015; Haag et al., 2007; Salazar- Bravo et al., 2001). Chiappero et al. (2002) reported that *C. lepidus* is most closely related to *C. musculinus*, with *Calomys laucha* Fischer, 1814 as a sister group.

Similar to the conflicting hypotheses about the *Calomys* divergence from other Phyllotines, there are also tentative hypotheses about the divergence timeline of *Calomys* species. Some authors suggest that the initial cladogenesis of *Calomy*s species occurred 9 MYA (Wilson and Reeder, 2005). Other authors propose that the highland and lowland clades diverged less than 5.3 MYA following the Andean uplift (Almeida et al., 2007). And Salazar- Bravo et al. (2001) argue that *C. lepidus* split from the other species of its highlands group roughly 2 MYA. Though there are many hypotheses about the divergence timeline of *Calomys* species, these timeline hypotheses are weakly supported and ultimately lack consensus among authors.

Unlike many sigmodontine rodents, the genus *Calomys* has a large geographical distribution in South America, even though they are exclusively found in dry habitats (Patton et al., 2015; Almeida et al., 2007; Espinosa et al., 1997). Within Bolivia, *Calomys* species have been found in all nine departments (Anderson, 1997).

A Synopsis of *Calomys lepidus*

Calomys lepidus, known as Andean Vesper mice, belong to order Rodentia, the superfamily Muroidea, the family Cricetidae, the subfamily Sigmodontinae, and the tribe Phyllotini (Wilson and Reeder, 2005; Anderson, 1997). In 1884, Thomas discovered and described the mouse in San Antonio, Peru (Anderson, 1997).

The distribution of Andean Vesper mice ranges from the altiplano of central Peru through west Bolivia, to northeast Chile and northwest Argentina (Eisenberg and Redford, 1999; Wilson and Reeder, 1993; Mares et al., 1989). Their ecotonal habitats consist of high altitude grasslands

and rocky areas at about 3,200 m and above (Eisenberg and Redford, 1999; Anderson, 1997; Wilson and Reeder, 1993; Mares et al., 1989). Within Bolivia, *C. lepidus* has been found in five departments along the Andean cordilleras (Figure 1.2). These mice are typically omnivorous, fossorial and ground dwelling, similar to the White-Bellied Grass Mice (Gettinger, 2015; Eisenberg and Redford, 1999; Wilson and Reeder, 1993; Mares et al., 1989). The Andean Vesper mouse weighs 10-18 grams at a total length of 103-128 mm with notably large ears and a very short tail (< 50mm) (Patton et al., 2015; Anderson, 1997). The pelage is soft with a marbled grayish fawn dorsum, while the ventral side is whitish-gray (Patton et al., 2015). Based on recent literature searches, there are no other detailed reports of the behavior, reproduction, diet, and habits of *Calomys lepidus.*

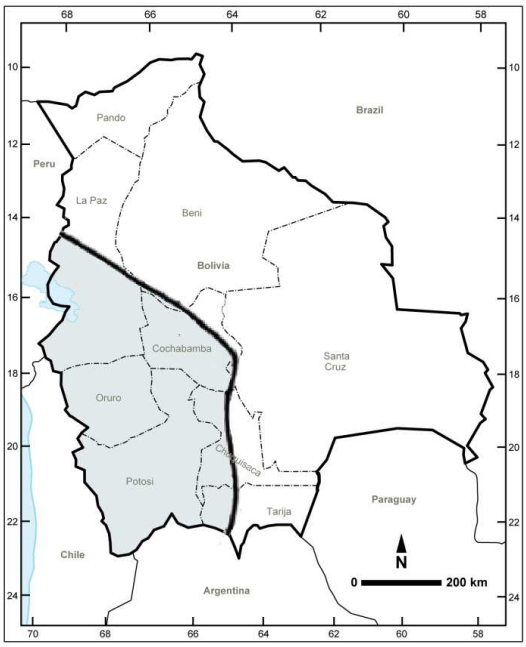


Figure 1.2: *Calomys lepidus* geographic distribution (shaded region) in Bolivia.

Parasites of the genus *Calomys*

The helminth record of the *Calomys* genus includes only one parasite known to infect *Calomys lepidus*. From this record of parasites of *Calomys*, I have constructed a list of twelve endoparasites that might be found within *C. lepidus* (Table 1.2). Studies of five species of *Calomys* from Brazil, Argentina, Uruguay, and Bolivia found parasites from phylum Nemata, class Cestoda, class Kinetoplasta and class Conoidasida. Of phylum Nemata, parasites of five taxonomic families have been documented in *Calomys*. Two *Syphacia* species were collected separately in *C. laucha* and *C. callosus*. *Calomys laucha* also is the host of one protospirurid species, and *C. callosus* hosts one physalopteran species. Two filarioid species were collected separately from *C. lepidus* and one unknown *Calomys* species. Lastly, two strongylids of the *Hassalstrongylus* genus were documented separately from two *Calomys* species in Brazil (Pinto et al., 1982; Patton et al., 2015). One coccidian species was experimentally transmitted perorally in *C. callosus*, while one trypanosome species was recorded in three *Calomys* species. A variety of external parasites also infect *Calomys* including ticks, fleas, mites, and chiggers (Table 1.2). Below, I discuss in detail each parasite taxon that is represented, including viruses.

Viruses

Calomys species have been associated with zoonotic emerging diseases like South American hemorrhagic fevers, hantavirus pulmonary syndrome (HPS), and hemorrhagic fever with renal syndrome (HFRS) (Oliveira et al., 2014; Oliveira et al., 2012; Simone et al., 2009; Mills and Childs, 1998). Viral hemorrhagic fevers are caused by two distinct groups of viruses, hantavirus and arenavirus, resulting in hundreds of South American cases per year (Mills and Childs, 1998). *Calomys musculinus* is the reservoir host of the Junín virus (an arenavirus), which is the etiological agent of Argentine hemorrhagic fever (AHF) (Simone et al., 2009; Mills and

Childs, 1998). Out of the multiple hantavirus strains, the Laguna Negra strain has been recorded in *C. laucha* and *Calomys callidus* Thomas, 1916 in Paraguay, and the Machupo virus strain from *C. callosus* in Bolivia (Oliveira et al., 2014; Dragoo et al., 2002; Mills and Childs, 1998).

Table 1.2: A checklist of parasites that have been shown to infect species in the genus *Calomys.*

Parasite Taxa			Akodon Species	Locality	References
Class	**Family**	**Species**			
Nemata^	Oxyuridae	*Syphacia hordarae*	*Calomys laucha*	Argentina	Herrera et al., 2011
		Syphacia criceti	*Calomys callosus*	Brazil	Quentin, 1969
	Physalopteridae	*Physaloptera calnuensis*	*Calomys laucha*	Uruguay	Sutton, 1989; Herrera et al., 2011
	Spiruridae	*Protospirura numidica criceticola*	*Calomys callosus*	Brazil	Sutton, 1989; Pinto et al., 2011
	Onchocercidae	*Litomosoides circularis*	*Calomys* sp.	Brazil	Brant and Gardner, 2000
		Litomosoides esslingeri	*Calomys lepidus*	Bolivia	Notarnicola et al., 2012
	Heligmonellidae	*Hassalstrongylus epsilon*	*Calomys cerqueirai*	Brazil	Patton et al., 2015
		Hassalstrongylus hoineffae	*Calomys callosus*	Brazil	Pinto et al., 1982
Cestoda	Taeniidae	*Taenia sp.*	*Calomys cerqueirai*	Brazil	Patton et al., 2015
		Taenia taeniaeformis	*Calomys laucha* *Calomys musculinus*	Brazil	Miño et al., 2013
Conoidasida	Sarcocystidae	*Toxoplasma gondii*	*Calomys callosus*	Laboratory*	Franco et al., 2011
Kinetoplastida	Trypanosomatidae	*Trypanosoma cruzi*	*Calomys musculinus* *Calomys callosus* *Calomys laucha*	Argentina	Brigada et al., 2010; Orozco et al., 2014
Insecta	Rhopalopsyllidae	*Polygenis (Polygenis) bohlsi bohlsi*	*Calomys callosus*	Bolivia	Anderson, 1997
		Polygenis (Gephyropsylla) klagesi samuelis	*Calomys callosus*	Bolivia	Anderson, 1997
		Polygenis (Polygenis) platensis cisandinus	*Calomys musculinus*	Argentina	Lareschi et al., 2004
	Ctenophthalmidae	*Neotyphloceras crassispina hemisus*	*Calomys callosus*	Bolivia	Anderson, 1997
Arachnida	Amblyommidae	*Amblyomma* sp.	*Calomys callosus*	Bolivia	Anderson, 1997
		Amblyomma triste	*Calomys musculinus* *Calomys callosus*	Argentina	Nava et al., 2006
		Amblyomma tigrinum	*Calomys musculinus* *Calomys venustus*	Argentina	Nava et al., 2006
	Laelapidae	*Gigantolaelaps oudemansi*	*Calomys callosus*	Bolivia	Anderson, 1997
		Gigantolaelaps wolffsohni	*Calomys callosus*	Bolivia	Anderson, 1997
		Mysolaelaps heteronychus	*Calomys callosus*	Bolivia	Anderson, 1997
		Schistolaelaps mazzai	*Calomys callosus*	Bolivia	Anderson, 1997
	Macronyssidae	*Bdellonyssus vitzthumi*	*Calomys callosus*	Bolivia	Anderson, 1997
	Trombiculidae	*Miyatrombicula arandiai*	*Calomys callosus*	Bolivia	Anderson, 1997
		Euschoengastia kunsi	*Calomys callosus*	Bolivia	Anderson, 1997
		Euschoengastia mackenziei	*Calomys callosus*	Bolivia	Anderson, 1997
		Euschoengastia johnsoni	*Calomys callosus*	Bolivia	Anderson, 1997

^= Phylum *=Experimental infection

Parasitic protozoa

Two species of protozoa have been documented in *Calomys* genera: *Toxoplasmosis gondii* Nicolle & Manceaux, 1908 and *Trypansoma cruzi*. As previously described, *T. cruzi* is a single-celled flagellated protozoan that is transmitted in domestic and sylvatic habitats between triatomine insect vectors and mammalian definitive hosts through multiple stages (Orozco et al., 2014; Brigada et al., 2010). *Calomys callosus, C. laucha* and *C. musculinus* have all been documented with *T. cruzi* infections (Orozco et al., 2014; Brigada et al., 2010). *Calomys callosus* is highly susceptible to sylvatic strains of *T. cruzi*, but it can control and regulate the intensity of parasitemia (Orozco et al., 2014). *Toxoplasma gondii* is an obligate, intracellular parasite that can infect *Calomys* under experimental conditions, but natural infections have not yet been found (Franco et al., 2011).

Helminths

Currently, the helminth parasite fauna of *C. lepidus* is largely unknown, except for one filarioid. The majority of the studies were done on prevalent and accessible species, such as *C. callosus, C. laucha,* and *C. musculinus* (Table 1.2). The helminth fauna of *Calomys* includes nematodes and two cestodes from family Taeniidae, which have been separately reported in Brazil. *Taenia sp.* was reported parasitizing *Calomys cerqueirai* Bonvicino, Oliveira, and Gentile, 2010 (Patton et al., 2015) and *Taenia taeniaeformis* Batsch, 1786 was collected from *C. laucha* and *C. musculinus* (Miño et al., 2013).

Nematodes

Numerous nematodes have been documented in *Calomys* species. As shown on Table 1.2, *Calomys laucha* is host to two nematodes: *Physaloptera calnuensis* Sutton, 1989 (Herrera et

al., 2011; Sutton, 1989) and *Syphacia hordarae* Herrera, 2011 (Herrera et al., 2011; Table 1.2). *Calomys callosus* harbors the nematodes *Syphacia criceti* Quentin, 1969, and *Protospirura numidica criceticola* (Herrera et al., 2011; Pinto et al., 2011; Robles and Navone, 2007; Quentin, 1969). The filarioid, *Litomosoides circularis* von Linstow, 1899 was reported in an unknown *Calomys* species in Brazil (Brant and Gardner, 2000). Lastly, two *Hassalstrongylus* spp. were documented separately from two *Calomys* species, *C. cerqueirai* and *C. callosus*, in Brazil (Pinto et al., 1982; Patton et al., 2015). The only endoparasite to be reported from *C. lepidus* was the filarioid, *Litomosoides esslingeri* Bain, Petit, and Diagne, 1989 from Bolivia (Notarnicola et al., 2012).

Conclusion

In this chapter, I have reviewed the biogeography of the Bolivian altiplano, and rodent history and taxonomy. I then individually examined the history and background of *Akodon* and *Calomys* genera followed by a detailed review of the history, description and background of the focal hosts, *Akodon albiventer* and *Calomys lepidus*. The chapter concluded with a description of the recorded parasite community of each focal host genus.

The parasite records and background information previously described for each focal host genus suggest the possible helminth parasite assemblages infecting *A. albiventer* and *C. lepidus*. Information on the history, physical characteristics, and habitat of *A. albiventer* and *C. lepidus* provide insight on potential environmental, ecological and behavioral factors that influence their parasite community structure. For example, the length of the body and tail of the focal hosts indicate fossorial behavior (Patton et al., 2015), which increases the host's contact with specific ectoparasites and endoparasites associated with underground burrows and nests. Once the helminth parasite assemblages are determined for both focal hosts, a better understanding of host

behavior, diet and other influential host characteristics may be deduced since many morphological and ecological traits of hosts have been shown to correlate with parasite species richness (Vitone et al., 2004). The recorded parasites will also indicate host specificity, and expanded geographical distributions. This information can establish any host-parasite associations between the focal hosts and their parasites, as well as define the ecological role the focal host species plays within its environment.

REFERENCES

Almeida, F.C., Bonvicino, C. R., and Cordeiro-Estrela, P. 2007. Phylogeny and temporal diversification of *Calomys* (Rodentia, Sigmodontinae): Implications for the biogeography of an endemic genus of the open/dry biomes of South America. Molecular Phylogenetics and Evolution 42*:* 449–466.

Anderson, S. 1997. Mammals of Bolivia, taxonomy and distribution. Bulletin of American Museum of Natural History231: 1-652.

Anderson, S., and T.L. Yates. 2000. A new genus and species of Phyllotine rodent from Bolivia. Journal of Mammology 81:18-36.

Arnade, C.W. 2014.In Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2015. Web. 20 Feb. 2015 <http://www.britannica.com/EBchecked/topic/72106/Bolivia>.

Beaucournu, J.C., Moreno, L., Gonzalez-Acuna, D. 2011. Two new species of fleas (Siphonaptera: Ctenophthalmidae & Rhopalopsyllidae) from Chile. Parasite-Journal de la Societe Francaise de Parasitologie.18(3): 241-246.

Behnke, J. M. 2008. Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions . . . . or pure randomness? Parasitology 135: 751–766.

Bochkov, A., Lareschi, M., and Barreto, M. 2014. New species and records of the mite genus *Prolistrophorus* (Acariformes: Listrophoridae) from rodents of the subfamily Sigmodontinae (Rodentia: Cricetidae). Acta Parasitologica 59(4): 586–595.

Brandt, J. S., and Townsend, P. 2006. Land use – land cover conversion, regeneration and degradation in the high elevation Bolivian Andes. Landscape Ecology 21:607–623.

Brant, S and Gardner, S.L. 2000. Phylogeny of the Species of the Genus Litomosoides (Nematatoda: Onchocercidae): Evidence of Rampant Host Switching. Journal of Parasitology 86(3): 545–554.

Brigada, A.M., Dona, R., Caviedes-Vidal, E., Moretti, E., Basso, B., 2010. American tripanosomiasis: a study on the prevalence of Trypanosoma cruzi and Trypanosoma cruzi-like organisms in wild rodents in San Luis province, Argentina. Rev. Soc. Bras. Med. Trop. 43: 249–253.

Brooks, D.R.1980. Allopatric Speciation and Non-Interactive Parasite Community Structure. Systematic Zoology 29 (2): 192-203.

Brooks, D, R., Hoberg, E. P., Boeger, Walter A.; Gardner, S. L., Galbreath, K. E., Herczeg, D., Mejía-Madrid, H. H., Rácz, S. E., Dursahinhan, A. T. 2014. Finding Them Before They Find Us: Informatics, Parasites, and Environments in Accelerating Climate Change. Comparative Parasitology 81(2): 155–164.

Brush, S. A. 1982. The Natural and Human Environment of the Central Andes Mountain. Mountain Research and Development 2 (1): 19-38.

Carrizo, L.V., and Catalano, A.S. 2015. First phylogenetic analysis of the tribe Phyllotini (Rodentia: Sigmodontinae) combining morphological and molecular data. Cladistics 31: 593–620.

Chiappero, M.B., Sousa, G.B., Blanco, A., and Gardenal, C.N. 2002. Evolutionary relationships among eight species of South American phyllotine rodents (Rodentia: Muridae) based on allozymic data. Zool. Syst. Evol. Research 40: 1-7.

Costa, L. P. 2003. The Historical Bridge between the Amazon and the Atlantic Forest of Brazil: A Study of Molecular Phylogeography with Small Mammals. Journal of Biogeography 30(1): 71-86.

Coyner, B.S., J.K. Braun, M. A. Mares, and R.A. Van Den Bussche. 2013. Taxonomic validity of speceis groups in the genus Akodon (Rodentia, Cricetidae). Zoologica Scripta 42):335-350.

D. Gettinger. 2015. Pers. Comm. Senior Research Fellow. Harold W. Manter Lab of Parasitology. University of Nebraska, Lincoln.

D'Elia, G. 2000. Comments on recent advances in understanding sigmodontine phylogeny and evolution. Mastozool. Neot. 7: 47–54.

D'Elia, G. 2003. Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19: 307-323.

D'Elía, G., Pardiñas, U.F.J., Teta, P. and Patton, J.L. 2007. Definition and diagnosis of a new tribe of sigmodontine rodents (Cricetidae: Sigmodontinae), and a revised classification of the subfamily. Gayana 71(2):187-194.

Digiani, M.C., Navone, G.T., and Durette-Desset, M.C. 2007. The systematic position of some nippostrongyline nematodes (Trichostrongylina: Heligmosomoidea) parasitic in Argentinean sigmodontine rodents. Systematic Parasitology 67:87–92.

Dragoo, J. W., Bravo, J., Layne, L. J., and Yates, T. L. 2002. Relationships within the Calomys callosus species group based on amplified fragment length polymorphisms. Biochemical Systematic and Ecology 31:703-713.

Eisenberg, J. F. and K. H. Redford. 1999. Mammal of the Neotropics, The Central Neotropics: Ecuador, Peru, Bolivia, Brazil, vol. 3. University of Chicago, Chicago, p.

Espinosa, M.B., Lasserrea, A., Piantanidab M. and Vitulloa, A.D. 1997.Cytogenetics of vesper mice, *Calomys* (Sigmodontinae): a new karyotype from the Puna region and its implication for chromosomal phylogeny. Cell. mol. life sci. 53: 583–586.

Franco, P.S., Costa, I.N., Gomes, A.O., Silva, A.L.N., Pena, J.D.O, Mineo, J.R., Ferro, E.A.V. 2011. Evaluation of vertical transmission of Toxoplasma gondii in Calomys callosus model after reinfection with heterologous and virulent strain. Placenta 32(2): 116 - 120.

Gardner, S. L., Perez-Ponce de León, G. 2002. *Yungasicola travassosi* gen. n., sp. n. (Digenea: Dicrocoeliidea: Eurytrematinae) from Two Species of Grass Mice of the Genus *Akodon* Meyen (Rodentia: Muridae) from the Yungas of Bolivia. Comparative Parasitology 69(1): 51-57.

Gardner, S. L., J. Salazar-Bravo, and J. A. Cool. 2012. New species of Ctenomys (Rodentia: Ctenomyidae) from Bolivia. Occasional papers of the Museum of Texas Tech University.

Gomes, D.C., R. Pereira da Cruz, J.J. Vicente, and R.M. Pinto. 2003. Nematode parasites of marsupials and small rodents from the Brazilian Atlantic Forest in the State of Rio de Janeiro, Brazil. Revista Brasileira de Zoologia 20 (4):699-707.

Grisard, E.C., Elsaidt, M.M.A., Tafurit, W.L., Limat, J.D., Pinto, C.J.C, Steindel, M., and Vitort, R.W.A.1997. Besnoitia sp. (Protozoa: Toxoplasmatinae) from Akodon montensis (Rodentia: Cricetidae) in Santa Catarina State, Brazil. Journal Parasitology 83(2):314-316.

Haag, T., Muschner, V.C., Freitas, L.B., Flamarion, L. B., Alfredo, Langguth, O.R., and Mattevi, M.S. 2007. Phylogenetic Relationships among Species of the Genus Calomys with Emphasis on South American Lowland Taxa. Journal of Mammalogy 88 (3): 769-776.

Herrera, Elba J.R., M. H. Mino, J. Notarnicola, and M.R. Robles. 2011. A new species of *Syphacia* (Nematoda: Oxyuridae) from Calomys Laucha (Rodentia: Cricetidae) in an Agroecosystem of Central Argentina. Journal of Parasitology. 97 (4): 676-681.

Holmes, J. C. and Price, P.W. 1980. Parasite communities: the roles of phylogeny and ecology. Systematic Zoology : 203-212.

Hugot, J. P. 1988. Les Nématodes Syphaciinae Parasites de Rongeurs et de Lagomorphs. Axonomie, Zoogeographie, Evolution. Bulletin du Muséum National d'Histoire Naturelle, Série A 141: 1-153.

Krasnov, B.R., Shenbrot, G.I., Medvedev, S.G., Vatschenok, V.S., and I. S. Khokhlova, I.S. 1997. Host–habitat relations as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. Parasitology 114: 159-173.

Kuhnen, V.V., Graipel, M.E., and Pinto, C.J.C. 2012. Differences in richness and composition of gastrointestinal parasites of small rodents (Cricetidae, Rodentia) in a continental and insular area of the Atlantic Forest in Santa Catarina state. Brazil Braz. J. Biol. 72 (3): 563-567.

Lareschi, M. 2011. Laelapid Mites (Parasitiformes: Gamasida), Parasites of Akodon philipmyersi (Rodentia: Cricetidae) in the Northern Campos Grasslands, Argentina, with the Description of a New Species. Journal of Parasitology (97) 5: 795-799.

Lareschi, M., Ojeda, R., and Linardi, P.M. 2004. Flea parasites of small mammals in the Monte Desert biome in Argentina with new host and locality records. Acta Parasitologica 49(1): 000-000.

Lareschi, M., Sanchez, J.P., Ezquiaga, M.C., Autino, A.G., Diaz, M.M., and Barquez, R.M. 2010. Fleas Associated with Mammals from Northwestern Argentina, with New Distributional Reports. Comparative Parasitology 77 (2): 207–213.

Mares, M.A., R. A. Ojeda, and R. M. Barquez. *Guide to the Mammals of Salta Province, Argentina*. Oklahoma: U of Oklahoma, 1989. 149, 164. Print.

Marshall, L.G., and T. Sempere. 1993. Evolution of the Neotropical Cenozoic land mammal fuana in its geochronologic, stratigraphic and tectonic context. Pages 329-392 in P. Goldblatt, ed. Biological relationships between Africa and South America. Yale Universit Press, New Haven, Connecticut, U.S.A.

Messenger, L.A., Garcia, L., Vanhove, M., Huaranca, C., Bustamante, M., Torrico, M.C., Miles, M.A., and Llewellyn, M.S. 2015. Ecological host fitting of Trypanosoma cruzi TcI in Bolivia: mosaic population structure, hybridization and a role for humans in Andean parasite dispersal Molecular Ecology 24: 2406–2422.

Mills, J.N., and Childs, J.E. 1998. Ecologic studies of rodent reservoirs: their relevance for human health. Emerging Infectious Disease.4(4):529-37.

Miño, M.H. 2008. Infection pattern of the spirurid nematode Protosprirura numidica cricetid rodent Akodon azarae on poultry farms of central Argentina. Journal of Helminthology 82: 153-158.

Miño, M.H., E.J. Rojas Herrera, J. Notarnicola, M. del R. Robles and G.T. Navone. 2012. Diversity of the helminth community of the pampean grassland mouse (Akodon azarae) on poultry farms in central argentina. Journal of Helminthology 86: 46–53.

Miño, M. H., Rojas Herrera, J., and Notarnicola, J. 2013. The wild rodent akodon azarae (Cricetidae: Sigmodontinae) as intermediate host of Taenia taeniaeformis (Cestoda: Cyclophyllidea) on poultry farms of central Argentina. Mastozoología neotropical.20 (2): 406-412.

Monteiro, J., and Körner, C. 2013. Leaf turnover and herbivory in the tall tussock grass Festuca orthophylla in the Andean Altiplano.Alpine Botany 123 (1): 13-20.

Morales, M.E., Ubelaker, J.E., and Gardner, S.L. 2012. A New Genus and Species of Lungworm (Nemata: Metastrongyloidea) from Akodon mollis Thomas,1894 (Rodentia: Cricetidae) in Peru. Journal of Parasitology 98(3): 612–616.

Musser, G.G. and Carleton, M.D. 2005. Superfamily Muroidea. In D.E. Wilson and D. M. Reeder (Eds) Mammal Species of the World: A Taxonomic and Geographic Reference, 3rd edn. Baltimore: The Johns Hopkins University Press. pp.894-1531.

Nava, S., Lareschi, M., Voglino, D. 2003. Interrelationship between Ectoparasites and Wild Rodents from Northeastern Buenos Aires Province, Argentina. Mem Inst Oswaldo Cruz, Rio de Janeiro 98: 000-000.

Nava, S., Lareschi, M., Abba, A. M., Beldomtnic, P. M., Venzal, J. M., Mangold, A. J., Guglielmone, A. A. 2006. Larvae and nymphs of Amblyomma tigrinum Koch,1844 and Amblyomma triste Koch, 1844 (Acar: Ixodidae) naturally parasitizing sigmodontinae rodents. Acarologia 46 (3-4): 135-141.

Navone, G.T., J. Notarnicola, S. Nava, M.R. Robles, C. Galliari, and M. Lareschi. 2009. Arthropods and helminths assemblage in sigmodontine rodents from wetlands of the Rio de Plata, Argentina. Mastozoologia Neotropical 16: 121-133.

Notarnicola, J.F., A. Jimenez, and S.L. Gardner. 2012. New Species of *Molina* (Nematoda: Onchocercidae) in Bolivian Rodents and Emended Description of *Litomosoides Esslingeri* Bain, Petit. and Diagne, 1989. Journal of Parasitology 98:1200-1208.

Okamoto, M., Urushima, H., Iwasa, M., Hasegawa, H. 2007. Phylogenetic Relationships of Rodent Pinworms (genus *Syphacia)* in Japan Inferred from Mitochondrial CO1 Gene Sequences. Journal of Veterinary Medical Science 69 (5): 545-547.

Oliveira R.C., Gentile, R., Guterres, Fernandes, J., Teixeira, B. R, Vaz, V., Valdez, F.P., Vicente, L.H.B., Costa-Neto, S.F., Bonvicino C.R., D'Andrea P.S., de Lemos E.R. 2014. Ecological study of hantavirus infection in wild rodents in an endemic area. Acta Tropica 131: 1–10.

Oliveira, R.C., Guterres, A., Schrago, C. G., Fernandes, J., Teixeira, B. R., Zeccer, S., Bonvicino, C.R., D'Andrea, P. S., & Lemos, E. R. S. 2012. Detection of the first incidence of Akodon paranaensis naturally infected with the Jabora virus strain (Hantavirus) in Brazil. *Memórias do Instituto Oswaldo Cruz 107*(3): 424-428.

Orozco, M.M, Piccinali, R.V., Mora, M.S., Enriquez, G.F., Cardinal, M.V., and Gürtler, R.E. 2014. The role of sigmodontine rodents as sylvatic hosts of Trypanosoma cruzi in the Argentinean Chaco. Infection, Genetics and Evolution 22: 12–22.

Parada, A., D'Elía, G., Palma, R.E. 2015. The influence of ecological and geographical context in the radiation of Neotropical sigmodontine rodents. BMC Evolutionary Biology 15:172.

Pardiñas, U.F.J., Tonni, E., 1998. Procedencia estratigrafica y edad de los mas antiguos muroideos (Mammalia, Rodentia) de America del Sur. Ameghiniana 35: 473–475.

Pardiñas, U.F.J., D'Elıa, G., Ortiz, P.E., 2002. Sigmodontinos fosiles (Rodentia, Muroidea, Sigmodontinae) de America del Sur : estado actual de su conocimiento y prospectiva. Mastozoologia Neotropical 9: 209–252.

Pardiñas, U.F.J., Teta, P., Salazar-Bravo, J. 2015. A New Tribe of Sigmodontinae Rodents (Cricetidae). Mastozoología Neotropical, 22(1):171-186.

Pascual, R. 2006. The Biogeographic History of South American Land Mammals. Annals of the Missouri Botanical Garden 93(2): 209-230.

Patton, James L., Ulyses F.J. Pardiñas, and Guillermo D'Elia, eds. 2015. Mammals of South America: Rodents. University of Chicago 2: 1384.

Pérez-Ponce de León, G.L., Gardner S.L., and Falcón-Ordáz J. 2000. Phylogenetic relationships among species of Stilestrongylus Freitas, Lent and Almeida, 1937 (Trichostrongyloidea: Heligmonellidae: Nippostrongylinae), parasites of myomorph rodents (Rodentia: Muridae) in the Neotropics. Journal of Parasitology 86(6):1326-1335.

Pinto, R.M., Knoff, M., Gomes, C.D. & Noronha, D. 2011. Nematodes from mammals in Brazil: an updating. Neotropical Helminthology 5 (2): 139-183.

Pinto, R.M., Kohn, A., Fernandes, B.M.M., and Mello, D.A. 1982. Nematodes of rodents in Brazil, with description of Aspidodera vicentei n.sp. Systematic Parasitology 4: 263-267.

Poulin, R. 2014. Parasite biodiversity revisited: frontiers and constraints. International Journal for Parasitology 44: 581–589.

Preston, D., Fairbairn, J., Paniagua, N., Maas, G., Yevara, M., and Beck, S. 2003. Grazing and Environmental Change on theTarija Altiplano, Bolivia. Mountain Research and Development 23 (2): 141–148.

Pucu, E., Lareschi, M.A., and Gardner, S.L. 2014. Bolivian Ectoparasites: A Survey of the Fleas of Ctenomys (Rodentia: Ctenomyidae). Comparative Parasitology 81(1) : 114–118.

Quentin, J.C. 1969. Etude de Nematodes *Syphacia* Parasites du Rongeurs Cricetidae Sud-Americains et de Leurs Correlations Biogeographiques Avec Certaines Especes Neartiques. Bulletin du Museum National D'Histoire Naturelle 2 (4): 909-925.

Quentin, J.C. 1971. Morphologie comparée des structures céphaliques et génitales des Oxyures du genre *Syphacia*. Annales de Parasitologie 46: 15-60.

Roberts, L.S. and J. Janovy. 2009. Foundations of parasitology, 8th ed. McGraw-Hill.

Robles, M.R., Navone, G.T. 2007. A new species of Syphaci (Nematoda: Oxyuridae) from *Akodon Azarae* (Rodentia: Cricetidae) in Argentina. Journal of Parasitology. 93(2): 383-391.

Robles, M. R. 2011. New Species of Trichuris (Nematoda: Trichuridae) from Akodon montensis Thomas, 1913, of the Paranaense Forest in Argentina. Journal of Parasitology 97 (2): 319-327.

Salazar-Bravo, J., Dragoo, J. W., Tinnin, D. S. & Yates, T. L. 2001. Phylogeny and evolution of the neotropical rodent genus Calomys: inferences from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 20: 173–184.

Salazar-Bravo, J. A., Pardiñas, U. F. J., D'Elıa, G. 2013. A phylogenetic appraisal of Sigmodontinae (Rodentia, Cricetidae) with emphasis on phyllotine genera: systematics and biogeography. Zoologica Scripta 42: 250–261.

Simões, R.O., Souza, J.G.R., Maldonado-Junior, A. and Luque. 2011. Variation in the helminth community structure of three sympatric sigmodontine rodents from the coastal Atlantic Forest of Rio de Janeiro, Brazil. Journal of Helminthology 85 (2):171-178.

Simões, R.O. Maldonado-Júnior, and A, Luque, J.L. 2012. Helminth communities in three sympatric rodents from the Brazilian Atlantic Forest: contrasting biomass and numerical abundance. Brazilian Journal of Biology 72 (4): 909-914.

Simões, R.O., Luque, J.L., and Maldonado, A. 2014. Additional Data on the Synlophe of Stilestrongylus aculeata (Travassos, 1918) and Stilestrongylus eta (Travassos, 1937) (Heligmonellidae) Parasitic in Akodon montensis (Sigmodontinae) from the Atlantic Forest. Journal of Parasitology 100(1): 151-153.

Simone, I., Cagnacci, F., Provensal, C., and Polop, J. 2010. Environmental determinants of the small mammal assemblage in an agroecosystem of central Argentina: The role of Calomys musculinus. Mammalian Biology 75: 496–509.

Smith, M.F., and Patton, J.L. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol. J. Linn. Soc. 50: 149-177.

Smith, M.F. and Patton, J.L. 1999. Phylogenetic Relationships and the Radiation of Sigmodontine Rodents in South America: Evidence from Cytochrome *b*. Journal of Mammalian Evolution 6 (2): 89-128.

Souza, JGR, Digiani, MC, Simões, RO, Luque, JL, Rodrigues-Silva, R, Maldonado Jr, A .2009a. A new heligmonellid species (Nematoda) from Oligoryzomys nigripes (Rodentia: Sigmodontinae) in the Atlantic Forest, Brazil. Journal of Parasitology, vol. 95, pp. 734-738.

Souza, JGR, Simões, RO, Thiengo, SARC, Lima, WSL, Mota, EM, Rodrigues-Silva, R, Lanfredi, and R, Maldonado, Jr, A. 2009b. A new metastrongylid species (Nematoda, Metastrongylidae): a lungworm from A kodonmontens is (Rodentia: Sigmodontinae) in Brazil. Journal of Parasitology, vol. 95, pp. 1507-1511.

Sprent, J. F. A. 1969. Evolutionary aspects of immunity in zooparasitic infections. Pp. 3-62, in Immunity to parasitic animals (G. J. Jackson, R. Herman, and I. Singer, eds.), Appleton-Century-Crofts, New York, 2 vols, 1217 pp.

Steppan, S. 1993. Phylogenetic Relationships Among the Phyllotini (Rodentia: Sigmodontinae) Using Morphological Characters. Journal of Mammalian Evolution 1 (3): 187-213.

Sutton, C. 1989. Contribution to the knowledge of Argentina's parasitological fauna. XVII Spirurida (Nematoda) from neotropical Cricetideae: *Physaloptera calneunsis* n sp and *Protospirura numidica criceticola* Quentin, Karimi and Rodriguez de Almeida. Bulletin du Muséum National D'Histoire Naturelle, Paris 4° Série 11, Sección A 1:61-67.

Sutton, C. A., and M. C. Durette-Desset. 1991. Nippostrongylinae (Nematoda Trichostongyloidea) parasites d'Oryzomys flavescens em Argentine et en Uruguay. Revue Suisse de Zoologie 98: 535–553.

Tálamo, A., Tolaba, J., Trucco, C., and Acuña, E. 2010. Vegetation units and floristic composition in areas of the northwestern Altiplano, Argentina. I. Steppe environments. Ecología de Bolivia 45(1): 4-19.

UNESCO. 1981. Vegetation map of South America. 1: 5,000,000.

Unzueta, O.Q. 1975. Mapa Ecológico de Bolivia: memoria explicativa. Ministerio de Asuntos Campesinos y Agropecuarios, División de Suelos, Riegos e Ingeniera, La Paz, Bolivia.309 pp.

Vieira, E.M., Paise, G., and Machado, P.H.D. 2006. Feeding of small rodents on seeds and fruits: a comparative analysis of three species of rodents of the Araucaria forest, southern Brazil. Acta Theriol (Warsz) 51: 311–318.

Vitone, N. D., S. Altizer, and C. L. Nunn. 2004. Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates. Evolutionary Ecology Research 6:183-199.

Webb, S.D. 2006. The Great American Biotic Interchange: Patterns and Processes. Annals of the Missouri Botanical Garden 93(2):245-257.

Wiger, R. 1977. Some Pathological Effects of Endoparasites on Rodents with Special Reference to the Population. Oikos 29 (3): 598-606.

Wilson, D. E., and D. M. Reeder. 1993. *Mammal Species of the World: A Taxonomic and Geographic Reference*, 2nd ed. Washington: Smithsonian Institution: 687.

Wilson, D. E., and D. M. Reeder (editors). 2005. Mammal Species of the World. A Taxonomic and Geographic Reference (3rd ed.). Johns Hopkins University Press, Ball.

CHAPTER 2: Gastrointestinal Helminths from White-Bellied Grass Mice and Andean Vesper Mice of the Altiplano Region of Bolivia

Summary

This biodiversity survey is the first that describes and reports the distribution and composition of helminth parasites occurring in White-Bellied Grass mice *(Akodon albiventer*) and Andean Vesper mice (*Calomys lepidus*) collected from the Bolivian altiplano. Twenty-seven White-Bellied Grass mice and 11 Andean Vesper mice from Gardner and colleagues' collection expeditions during 1984-1993 in five departments of Bolivia were randomly chosen for parasite analysis. A total of 702 helminth parasite specimens were recovered, representing five helminth taxa. *Syphacia* spp. and *Hassalstrongylus* sp. were identified from *C. lepidus*. *Syphacia carlitosi*, *Syphacia* sp., *Protospirura* spp., *Raillietina* sp. and a rictulariid species were identified from *A. albiventer*. These are the first records of helminths infecting *A. albiventer* and. *C. lepidus* of the Bolivian altiplano.

Introduction

Many wild animal populations harbor diverse parasite communities. The exposure to internal parasitic worms (helminths) occurs through a variety of transmission strategies and has diverse effects on host fitness. Some helminths have strict host specificity, while other helminths are generalists. Host traits (e.g. behavior, diet and habitats) influence the strategies and assemblages of helminth parasites, and ultimately help define host-parasite associations (Vitone et al., 2004). Helminth parasite assemblages provide information about their hosts, and insight into possible evolutionary patterns between the parasite and host (Robles, 2010). To expand our knowledge of parasite communities and of the hosts they inhabit, parasite distributions and description records must be established.

During 1984-1993, the American Museum of Natural History (AMNH), the Museum of Southwestern Biology (MSB), the Bolivian National Museum of Natural History in La Paz, and the Harold W. Manter Laboratory of Parasitology (HWML) launched collection expeditions throughout Bolivia to survey and inventory sylvatic mammals and their parasites (Notarnicola et al., 2012). These collections took place in five departments of Bolivia located on the altiplano: La Paz, Oruro, Cochabamba, Tarija, and Chuquisaca. *Akodon albiventer* and *Calomys lepidus* were two of several sympatric rodent species collected in the survey; they lacked any previous record of helminth parasites. To add to the inventory of helminth parasite records of the collected hosts, *A. albiventer* and *C. lepidus* were selected for parasite analysis.

This is the first survey of helminth parasites for *A. albiventer* and *C. lepidus* captured in these five departments within the high altitude Bolivian altiplano. The biodiversity survey contributes to the biogeographical record of helminth parasite distributions and the host records. These distributional records will encourage further research on parasite compositions and characteristics of the hosts.

Methods

A total of five expeditions took place within five departments of Bolivia during the joint collection expeditions of the Bolivian Biodiversity Survey conducted by Dr. Scott L. Gardner and contributors: Oruro (1984), Tarija (1986 and 1991), Chuquisaca (1990), and Cochabamba and La Paz (1993). Rodents were collected and processed following the guidelines of the American Society of Mammalogists for the use of wild mammals in research. Details of each rodent collected, trapping localities, and additional data were recorded in the NK field collection catalog, which is maintained at the Museum of Southwestern Biology (MSB) of the University of New Mexico, Albuquerque, and within personal field notes of the collectors. Rodent and

parasite specimens are desposited at the MSB and the Harold W. Manter Laboratory of Parasitology (HWML) of the University of Nebraska, Lincoln.

During each expedition, rodents were captured using Sherman live traps baited with a mixture of vanilla, oatmeal, and banana. The traps were placed in suitable habitats each evening within walking distance of the field sites, and checked at sunrise the following morning. Captured rodents were then taken back to the field sites, where the gastrointestinal tracts of the rodents were opened and the contents quickly examined. Helminths were collected and preserved following Gardner (1996) and Gardner and Jimenz (2009). Most parasite specimens were relaxed in distilled water, and placed directly into 10% aqueous formalin. Some specimens were preserved in vials filled with 70% aqueous ethanol. All parasite specimens were transported in their allotted solutions to the HWML and stored until further examination.

Though numerous wild rodents were collected throughout the decade of expeditions, only a total of 38 hosts, 27 *A. albiventer* and 11 *C. lepidus*, were randomly chosen for this study. Once I received the parasite specimens of the 38 hosts, I cleared them using either glycerol or lactophenol, and placed them on temporary slides for morphological analysis. Whole mounts of two tapeworms were stained with Ehrlich's acid hemtoxylin or Semichon's acetic carmine, dehydrated in ethanol, cleared in xylene, or terpineol, and mounted on glass slides in Damar gum.

I examined the morphological characters of the specimens using a Carl Zeiss Axioplan 2 imaging digital microscope with an Axiocamera accompanied with AxioVision LE64 software and Nikon compound microscope using an Amscope Toupview camera. The number of characters measured depended on the taxon of the specimens, specimen sex, specimen clarity and available equipment. Oxyurid specimens had an average of 20 characters measured, whereas

cestodes, trichostrongylids, rictulariids, and protospirurids averaged four characters measured. All measurements were in micrometers. I compared the measurements to the published literature for identification. Holotype, allotype, and paratype species' measurements found in the literature helped me determine specimen genus and /or species. Some specimens were degraded, broken, or had morphological characteristics that were not visible, and therefore, could not be identified.

Results

The 27 *Akodon albiventer* and 11 *Calomys lepidus* hosts (n=38) in this study were infected with oxyurids, protospirurids, rictulariids, trichostrongylids and cestodes (Table 2.1). The prevalence of each helminth taxon for *Akodon albiventer* and *Calomys lepidus* is reported in Table 2.1. Three of the 38 hosts did not have any helminth parasites.

Table 2.1: Prevalence of the parasite taxa of *Akodon albiventer* and *Calomys lepidus*

Helminth Taxa	*Akodon albiventer*	*Calomys lepidus*	Combined Prevalence (%)
NEMATA	**25/27 (92.59%)**	**10/11(90.90%)**	**35/38 (92.11%)**
Oxyurid			
Syphacia carlitosi	12/27 (44.44%)	-	12/38 (31.58%)
Syphacia spp.	10/27 (37.04%)	8/11 (72.73%)	18/38 (47.37%)
Unidentified sp.	-	1/11 (9.09%)	1/38 (2.63%)
Protospirurid			
Protospirura spp.	4/27 (14.82%)	-	4/38 (10.53%)
Trichostrongylid			
Hassalstrongylus sp.	-	3/11(27.28%)	3/38 (7.89%)
Rictulariid			
Unidentified sp.	2/27 (7.40%)	-	2/38 (5.26%)
CESTODA	**3/27 (11.12%)**	**-**	**3/38 (7.89%)**
Raillietina sp.	2/27 (7.41%)	-	2/38 (5.26%)
Metacestode	1/27 (3.70%)	-	1/38 (2.63%)

Nematodes

A total of 699 nematodes were collected from the *Akodon* and *Calomys* hosts, both of which were found in all five departments. Thirty-five out of 38 hosts were infected, yielding a prevalence of 92.11%. The number of nematodes per rodent host ranged from zero to 352, with a total mean intensity of 20.56 (CI: 8.54, 61.5) and median intensity of 4.0 (Figure 2.1). The mean intensity of nematodes in *Akodon albiventer* was 8.08 (SD=10.85), while the mean intensity of nematodes in *Calomys lepidus* was 49.7 (SD=108.9).

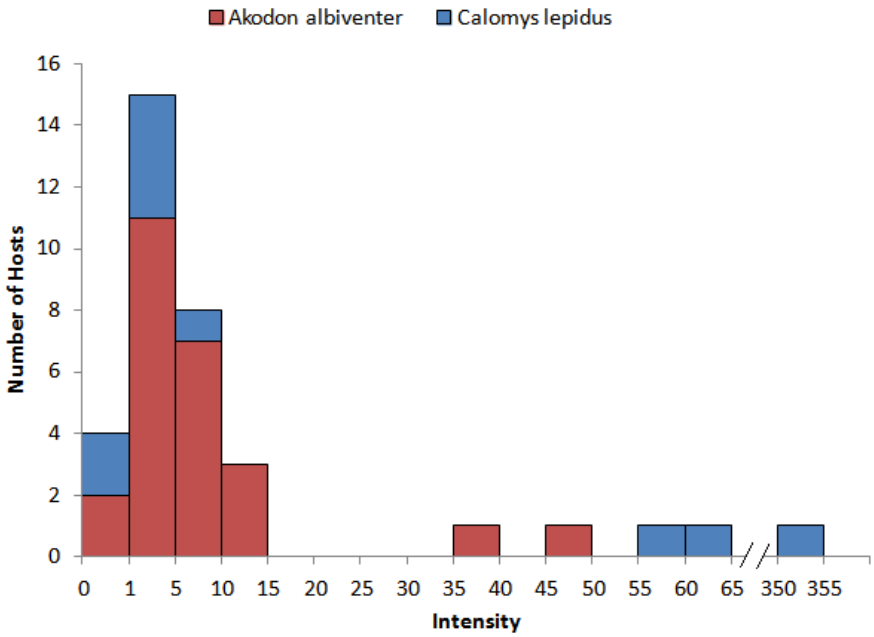


Figure 2.1: Nematode Intensity of *Akodon albiventer* and *Calomys lepidus*

Multiple nematode taxa were identified: oxyurids, rictulariids, protospirurids, and trichostrongylids. Of the 699 nematodes found, 681 oxyurids were collected in 30 hosts from all five departments. The total prevalence of oxyurids was 78.95%. Though both host species were infected with oxyurids, the majority of the oxyurids (493) were collected from *C. lepidus*, with one *C. lepidus* having a maximum intensity infection of 352 oxyurids. All oxyurids were in the

genus *Syphacia*. Eighty-nine of these were identified as *Syphacia carlitosi* Robles and Navone, 2007. The remainder of *Syphacia* specimens could not identified to species because diagnostic morphological features resembled two or more species. There was one unidentifiable nematode. Though further analysis is needed, the unknown parasite was believed to be a male oxyurid because of unique morphological features, specifically mamelons, which are used for mating.

Twelve protospirurids, identified as *Protospirura* spp., were found in four *A. albiventer* trapped in La Paz. Total prevalence was 10.53%. It is clear that the 12 protospirurids comprised two different species based on differing spicule lengths, but the species could not be determined. Two of the four *A. albiventer* infected by *Protospiruria* spp., were co-infected with other taxa. One host was co-infected with three protospirurids and a one oxyurid, while another *A. albiventer* was infected with five protospirurids and one cestode.

Four trichostrongylids (*Hassalstrongylus* spp.) were collected from three *C. lepidus* from two departments, yielding a total prevalence of 7.89%. Two *Hassalstrongylus* spp. were collected from one *C. lepidus* in Tarija with a co-infection of one oxyurid. The other two *Hassalstrongylus* spp. were also found in *C. lepidus* but the two host individuals were found in Cochabamba and each was independently infected with only one *Hassalstrongylus.*

Lastly, two rictulariids were recovered from two *A. albiventer* trapped in Oruro. The genus and species of the rictulariids could not be determined. Both of these *Akodon* hosts had co-infections with multiple oxyurids.

Cestodes

A total of three cestodes were found in three *Akodon* hosts, each from three different Bolivian departments. Two mature cestodes were collected in Oruro and La Paz, while one

metacestode was collected in Tarija. The diagnostic features of two mature tapeworms were consistent with *Raillietina* species. Total prevalence was 7.89%, with a mean intensity of 1.

Discussion

These results contribute to the knowledge of the helminth fauna of *Akodon albiventer* and *Calomys lepidus*. Since parasite-host associations result from interactions of specific parasite and host species, it is best to employ caution when identifying the specimens in order to avoid any inaccuracy and misconceptions about host specificity and geographic distribution (Robles, 2010). Therefore, most of the parasite specimens collected from the *Akodon* and *Calomys* hosts were identified only to genus due to unclear morphological characters necessary for species identification. In this discussion, I report on some of these genera.

Nematodes

Oxyurids:

Unsurprisingly, species of the *Syphacia* genus were the most prevalent nematodes found within the *Akodon* and *Calomys* hosts. Over 20 species of *Syphacia* inhabit North and South America (Robles and Navone 2010). Of these 20 species, at least 13 *Syphacia* species have been reported from over 15 species of sigmodontine rodents (Herrera et al., 2011; Robles and Navone, 2010; Robles and Navone, 2007a, b; Hugot, 1988; Dick et al., 1973; Quentin, 1971). *Syphacia* species have a direct life cycle and typically inhabit the cecum of their rodent hosts (Miño et al., 2012; Robles and Navone, 2007a; Hugot, 1988; Quentin, 1971). Many of these *Syphacia* species are host specific to certain sigmodontines (Robles, 2010). A study performed by Robles (2010) indicated signs of cospeciation between *Syphacia* parasites and Akodontini rodent hosts; the author concluded this because of on the parasites' high host specificity, prevalence, and

abundance compared to the parasites of other studied muroid rodents. Five *Syphacia* species have been previously reported from *Akodon* and *Calomys* genera. *Syphacia carlitosi* has been identified in Brazilian *A. azarae, A. cursor,* and *A. montensis, Syphacia kinsellai* Robles and Navone, 2007 has been recorded infecting Brazilian *A. cursor* and *A. montensis,* and *Syphacia obvelata* Rudolphi, 1802 has been identified in Brazilian *A. cursor.* Of *Calomys* hosts, *S. hordarae* has been found in Argentinean *C. laucha* and *Syphacia criceti* Quentin, 1969 identified in Brazilian *C. callosus* (Miño et al., 2012; Simões et al. 2012; Herrera et al., 2011; Pinto et al., 2011; Simões et al., 2011; Navone et al., 2009; Robles and Navone 2007; Gomes et al. 2003; Quentin, 1969).

In this study, *Syphacia carlitosi* was the only *Syphacia* species identified, while the other oxyurids could only be identified to genus. *Syphacia carlitosi* was identified from only *A. albiventer*. Some *Syphacia* specimens from *C. lepidus* were similar to *S. carlitosi* but one or more key morphological characters were so indistinguishable as to prevent an accurate identification. It appears that *S. carlitosi* is host specific to the *Akodon* genus. The remainder of the *Syphacia* specimens also could not be identified because their characteristics did not correspond to any morphological measurements of reported *Syphacia* species. It is probable that these indistinguishable *Syphacia* specimens are at least two new species of *Syphacia*. This is the first record of the *Syphacia* genus infecting *A. albiventer* and *C. lepidus,* and the first record *Syphacia carlitosi* parasitizing *A. albiventer*. This is also the first report of *Syphacia* in Bolivia.

Protospirurids:

The 12 protospirurids found in this study are the first record of *Protospirura* species infecting *A. albiventer.* All the protospirurids were recovered from *Akodon* hosts trapped in La Paz, approximately 20km south of Lake Titicaca. *Protospirura* have an indirect life cycle via the

ingestion of an arthropod intermediate host (Miño et al., 2012), indicating that *Akodon albiventer* include insects in their diet. Based on differing spicule lengths in two males, it is possible that the 12 *Protospirura* represent two species. Unequal spicule lengths and pseudolabia morphology with inner denticles (Smales, 2001; Sutton, 1989) suggest that one species of the 12 *Protospirura* recovered from the *Akodon* hosts is *Protospirura numidica criceticola. Protospirura numidica criceticola* is the only *Protospirura* species that has been documented in four *Akodon* species trapped in Brazil, Peru, and Argentina; *A. azarae, A. montensis, A. jelskii*, and *A. boliviensis* (Miño et al., 2012; Simões et al., 2012; Miño, 2008; Sutton, 1989). *Protospirura numidica criceticola* has also been reported infecting other cricetid rodents, including *Calomys,* and are widespread throughout the Neotropics (Pinto et al., 2011; Miño, 2008; Sutton, 1989). The other possible *Protospirura* species recovered from *Akodon* hosts has one unique morphological distinction, subequal spicule lengths. Among protospirurids, spicule morphology has been used as an informative characteristic to distinguish species, but has become a source of confusion due to varied spicule descriptions from multiple authors (Smales et al., 2009). The subequal spicule length is the only discernible difference that sets the specimens apart from the other reported protospirurids. Because of documented spicule variation among protospirurid species, it is possible that the protospirurid specimens are all the same species.

Trichostrongylids:

Hassalstrongylus sp. (subfamily Nippostrongylinae) is the first trichostrongylid to be recorded in *C. lepidus* and in Bolivia. The opacity and configuration of the specimens did not allow for further taxonomic classification. *Hassalstrongylus* spp. have a direct life cycle, and reside in the small intestines of their host. *Hassalstrongylus* spp. have only been reported in endemic Neotropical muroids inhabiting regions between southeastern North America and the

eastern part of South America (Kuhen et al., 2012; Pérez-Ponce de León et al., 2000). A few *Hassalstrongylus* spp. have been recovered in *Akodon, Calomys, Nectomys, Euryoryzomys, Oryzomys* and *Oligoryzomys* species inhabiting Brazil, yet none have been reported to be distributed as far west as Bolivia (Kuhen et al., 2012; Gomes et al. 2003). Since three individuals were infected in two separate localities (Tarija and Cochabamba), it is likely that these were not accidental infections and that *C. lepidus* can be added to the list of hosts for *Hassalstrongylus* spp.

Rictulariids:

Two unidentifiable rictulariids are the first of their family to be reported parasitizing *A. albiventer*. The opacity and sex of the specimens did not allow for any further taxonomic classifications. While numerous species of rictulariids parasitize a variety of marsupial and cricetid hosts throughout South America (Navone and Suriano, 1992), only one rictulariid species, *Pterigodermatites (Paucipectines) azarai* Sutton, 1984, has been reported from an *Akodon* host in Argentina (Miño et al., 2012). The two rictulariids reported in this study were independently infecting two *Akodon* hosts, and had co-occurred with *Syphacia* spp. Unlike *Syphacia* spp., rictulariids have indirect life cycles involving an arthropod intermediate host, and inhabit the small intestine of the definitive host.

Cestodes

This is the first record of *Raillietina* species recovered from *A. albiventer* in Bolivia. *Raillietina* species have been previously reported in wild rodents in North America and Neotropical primates and birds (Dunn, 1962; Smith, 1954). Old world *Raillietina* life cycles generally include a coleopteran intermediate host with birds typically serving as definitive hosts (Mino et al., 2012). Some species of New world *Raillietina* have been primarily found in rodents

and are therefore thought to naturally parasitize rodents (Sato et al., 1988; Dunn, 1962). *Raillietina* are classified in the Cyclophyllidea order, species of which have been recorded in *Akodon azarae* in Argentina (Mino et al., 2012). Because of considerable morphological variation, *Raillietina* species are not readily distinguished so it is possible that incorrect species have been unknowingly reported (Dunn, 1962; Smith, 1954).

Conclusion

The sympatric hosts *Akodon albiventer* and *Calomys lepidus* are parasitized by five taxa of gastrointestinal helminths that vary in prevalence. *Calomys lepidus* in this survey only hosted parasites with direct life cycles (*Syphacia* spp. and *Hassalstrongylus* sp.), whereas, the parasite fauna of *A. albiventer* comprised parasites with direct life cycles (*Syphacia* spp.) and indirect life cycles (*Raillietina* sp., *Protospirura* spp. and rictulariids). *Calomys* is one of few sigmodontine genera that is restricted to non-humid habitats, unlike *Akodon* (Almeida et al., 2007). The dry habitat of *C. lepidus* could affect its diet and encounter rates with arthropods, thus explaining why no helminth genus with arthropod intermediate hosts was recorded in *Calomys* hosts. Furthermore, *Calomys* are the only non-forest inhabiting genus of sigmodontines, excluding one species' distribution, reducing encounter and contact rates with other sigmodontines (Almeida et al., 2007). Though *A. albiventer* and *C. lepidus* are sympatric, their lack of shared parasites with indirect life cycles makes it evident that they do not share similar diets or exposure habits, and that they possibly differ in their microhabitat preferences (Pérez-Ponce de León et al., 2000).

However, interestingly, both hosts did share one parasite species with a direct life cycle, *Syphacia* spp, which had the highest prevalence and mean intensity compared to the other reported helminths. Miño et al. (2012) noted that some authors have detected a higher prevalence of *Syphacia* spp. when host populations were large. Host abundances can influence the

transmission success of parasites with direct life cycles, in that higher host densities increase the contact rate between the parasite and the new potential hosts. Other authors have stated that the transmission of *Syphacia* eggs is primarily through contact during grooming (Miño et al., 2012). The high prevalence of *Syphacia* spp. could suggest certain behavioral characteristics of both hosts, though the evidence gleaned from this survey is not sufficient to support or refute such hypotheses.

With the exception of *Syphacia* spp., the helminth composition of the *A. albiventer* and *C. lepidus* described in this survey lacks abundance and richness, with only a few helminth species present. The low prevalence of helminths other than *Syphacia* spp. could be attributed to a number of reasons. For instance, the requisite hosts may not be available for life cycle completion in some cases, and/or the number of hosts available in the area could be much lower than required for the parasite to be established in the host population (Robles, 2010). It is also possible that the low prevalence of these parasites could be a result of sampling error, given the fact that the survey was based on a random selection of hosts from a large, variable host sample. Because many parasites are thought to have been overlooked during collection at the field sites, the ones reported here may underestimate the parasite fauna of *A. albiventer* and *C. lepidus*. Based on the survey findings, it can be assumed that, like their rodent hosts, only a few parasite species were able to adapt to the environmental characteristics of the altiplano (Fuentes et al., 1997). All of these hypotheses and more await exploration and refinement in future studies.

REFERENCES

Dick T.A., Quentin J.C., Freeman R.S. 1973. Redescription of *Syphacia mesocriceti* (Nematoda: Oxyuroidea) parasite of golden hamster. Journal of Parasitology 59:256-259.

Dunn, F.L. 1962. *Raillietina (R.) trinitatae* (Cameron and Reesal, 1951), Baer and Sandars., 1956 (Cestoda) from a Peruvian Primate. Helminthological Society 29 (2): 148-152.

Fuentes, M.V., Coello, J.R., Bargues, M.D., Valero, M.A., Esteban, J.G., Fons, R., and Mas-Coma, S.1997. Small Mammals (Lagomorpha and Rodentia) and Fascioliasis Transmission in the Northern Bolivan Altiplano Endemic Zone. Asociacion de Parasitolgos Espafioles 57 (2): 115-121.

Gardner, S.L. 1996. Essential techniques for collection of parasites during surveys of mammals. In: Measuring and Monitoring Biological Diversity—Standard Methods for Mammals. Wilson, D.; Cole, R.; Nichols, J.D.; Rudran, R.; Foster, M., eds. Smithsonian Institution Press: Washington, DC, p. 291-298.

Gardner, S.L., Jiménez-Ruiz, F.A. 2009. Methods of endoparasite analysis. In: Kunz, T.; Parsons, S. (eds.). Ecological and Behavioral Methods for the Study of Bats. Johns Hopkins University Press, pp. 795-805.

Gardner, S. L., Perez-Ponce de León, G. 2002. *Yungasicola travassosi* gen. n., sp. n. (Digenea: Dicrocoeliidea: Eurytrematinae) from Two Species of Grass Mice of the Genus *Akodon* Meyen (Rodentia: Muridae) from the Yungas of Bolivia. Comparative Parasitology 69(1): 51-57.

Gomes, D.C., R. Pereira da Cruz, J.J. Vicente, and R.M. Pinto. 2003. Nematode parasites of marsupials and small rodents from the Brazilian Atlantic Forest in the State of Rio de Janeiro, Brazil. Revista Brasileira de Zoologia 20 (4):699-707.

Herrera, Elba J.R., M. H. Mino, J. Notarnicola, and M.R. Robles. 2011. A new species of *Syphacia* (Nematoda: Oxyuridae) from Calomys Laucha (Rodentia: Cricetidae) in an Agroecosystem of Central Argentina. Journal of Parasitology. 97 (4): 676-681.

Hugot, J. P. 1988. Les nematodes Syphaciinae parasites de rongeurs et de lagomorphs. Taxonomie, zoogeographie, evolution. Bulletin du Museum National d'Histoire Naturelle, Serie A 141: 1-153.

Kuhnen, V.V., Graipel, M.E., and Pinto, C.J.C. 2012. Differences in richness and composition of gastrointestinal parasites of small rodents (Cricetidae, Rodentia) in a continental and insular area of the Atlantic Forest in Santa Catarina state. Brazil Braz. J. Biol. 72 (3): 563-567.

Miño, M.H., E.J. Rojas Herrera, J. Notarnicola, M. del R. Robles and G.T. Navone. 2012. Diversity of the helminth community of the pampean grassland mouse (Akodon azarae) on poultry farms in central argentina. Journal of Helminthology 86: 46–53.

Navone, G.T., J. Notarnicola, S. Nava, M.R. Robles, C. Galliari, and M. Lareschi. 2009. Arthropods and helminths assemblage in sigmodontine rodents from wetlands of the Rio de Plata, Argentina. Mastozoologia Neotropical 16: 121-133.

Navone, G.T., Suriano, D.M. 1992. Pterigodermatites (Paucipectines) spinicaudatis n. sp. (Nematoda: Rictularidae) From Dromiciops australis (Marsupilia: microbiotheriidae) in Bariloche, Rio Negro, Argentina, Biogeographical Distribution and Host-Parasite Relationships. Memórias do Instituto Oswaldo Cruz 87(4): 533-538.

Notarnicola, J.F., A. Jimenez, and S.L. Gardner. 2012. A New Species of *Molina* (Nematoda: Onchocercidae) in Bolivian Rodents and Emended Description of *Litomosoides Esslingeri* Bain, Petit. and Diagne, 1989. Journal of Parasitology 98:1200-1208.

Pérez-Ponce de León, G.L., Gardner S.L., and Falcón-Ordáz J. 2000. Phylogenetic relationships among species of Stilestrongylus Freitas, Lent and Almeida, 1937 (Trichostrongyloidea: Heligmonellidae: Nippostrongylinae), parasites of myomorph rodents (Rodentia: Muridae) in the Neotropics. Journal of Parasitology 86(6):1326-1335.

Pinto, R.M., Knoff, M., Gomes, C.D. & Noronha, D. 2011. Nematodes from mammals in Brazil: an updating. Neotropical Helminthology 5 (2): 139-183.

Poulin, R. 2014. Parasite biodiversity revisited: frontiers and constraints. International Journal for Parasitology 44: 581–589.

Quentin, J.C. 1971. Morphologie comparee des structures cephaliques et genitales des Oxyures du genre *Syphacia*. Annales de Parasitologie 46: 15-60.

Robles, M. R. 2010. La importancia de los nematodes Syphaciini (Syphaciinae-Oxyuridae) como marcadores especifícos de sus hospedadores. Mastozoology neotropical 17 (2): 305-315.

Robles, M.R. and Navone, G.T. 2007a. A new species of Syphaci (Nematoda: Oxyuridae) from Akodon Azarae (Rodentia: Cricetidae) in Argentina. Journal of Parasitology. 93(2): 383-391.

Robles, M.R. and Navone, G.T. 2007b. A new species of *Syphacia* (Nematoda: Oxyuridae) from *Oligoyzomys nigripes* (Rodentia: Cricetidae) in Argentina. Parasitology Research. 101: 1069-1075.

Robles, M.R. and Navone, G.T. 2010. Redescription of *Syphacia venteli* Travassos 1937 (Nematoda: Oxyuridae) from *Nectomys squamipes* in Argentina and Brazil and description of a new species of *Syphacia* from *Melanomys caliginosus* in Columbia. Parasitology Research. 106: 1117-1126.

Simões, R.O., Souza, J.G.R., Maldonado-Junior, A. and Luque. 2011. Variation in the helminth community structure of three sympatric sigmodontine rodents from the coastal Atlantic Forest of Rio de Janeiro, Brazil. Journal of Helminthology 85 (2):171-178.

Simões, R.O. Maldonado-Júnior, and A, Luque, J.L. 2012. Helminth communities in three sympatric rodents from the Brazilian Atlantic Forest: contrasting biomass and numerical abundance. Brazilian Journal of Biology 72 (4): 909-914.

Smales, L.R., Harries P.D., and Behnke, J.M. 2009. redescription of Protospirura muricola Gedoelst, 1916 (Nematoda: Spiruridae), a parasite of murid rodents. Systematic Parasitology 72:15–26.

Smith, C.F. 1954. Four New Species of Cestodes of Rodents from the High Plains, Central and Southern Rockies and Notes on Catenotaenia dendritica. The Journal of Parasitology 40 (3): 245-254.

Sutton, C. 1989. Contribution to the knowledge of Argentina's parasitological fauna. XVII Spirurida (Nematoda) from neotropical Cricetideae: *Physaloptera calneunsis* n sp and *Protospirura numidica criceticola* Quentin, Karimi and Rodriguez de Almeida. Bulletin du Muséum National D'Histoire Naturelle, Paris 4° Série 11, Sección A 1:61-67.

Vitone, N. D., S. Altizer, and C. L. Nunn. 2004. Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates. Evolutionary Ecology Research 6:183-199.

CHAPTER 3: Analysis of Morphological Characters of Oxyurid Parasites recorded in White-Bellied Grass Mice (Akodon albiventer) and Andean Vesper Mice (Calomys lepidus) of the Bolivian Altiplano.

Summary

In a study of oxyurid parasites recovered from *Akodon albiventer* and *Calomys lepidus* collected in the Bolivian altiplano, many morphological measurements and features were found to overlap and resemble two or more species, causing many specimens to be unidentifiable. To evaluate the accuracy of manual identification techniques through the examination of morphological relationships between species, statistical analyses including one-way ANOVA, post hoc test using Tukey HSD method, and principal component analysis were performed on male and female oxyurid specimens (*Syphacia* spp.) separated into three species groups. The analyses showed evidence that two species groups from the host, *Akodon albiventer*, shared more morphologic measurement similarities than with the species group from *Calomys lepidus*. Though further analysis is necessary, it is possible that the species group derived from *Calomys lepidus* is a new *Syphacia* species.

Introduction

Methods of helminth parasite identification have expanded to include an array of techniques including genetic and molecular analyses using protein and DNA-based sequencing procedures (Parel et al., 2008; McManus and Bowles, 1996). Though the advancement and popularity of these approaches is increasing, the high cost of equipment, lack of resources, and the occasional requirement of morphologic descriptions make it necessary for most helminth parasite identifications to be performed by manual examination of the parasite's morphology. Manual parasite identifications are executed through meticulous microscopic examinations and

measurements of diagnostic morphological characters of adult worms and eggs followed by a comparison of those measurements and any morphologic features to the literature and/or dichotomous keys for genus and species classification. This approach is not without problem. Even if the parasite was recovered intact, not all parasites can be identified manually. Many species' paratype and holotype measurements overlap, and some diagnostic morphological features resemble two or more species. Some classifications are unable to be determined due inconspicuous morphologies (Parel et al., 2008). These problems and others allow for misidentifications, discrepancies, and lack of classifications in the manual identification processes (see: Robles and Navone, 2010).

The previously established parasite records of *Akodon albiventer* and *Calomys lepidus* collected in Bolivia were based on manual measurements of diagnostic morphologies. These parasite records include oxyurid parasites from the genus *Syphacia*, which were categorized in three *Syphacia* species groups. Only one *Syphacia* group was identified to species, while the other two groups were determined to be potentially new *Syphacia* species. To understand differences between the species groups and confirm the accuracy of manual identification processes, I used statistical analyses including one-way ANOVA, post hoc test using Tukey HSD method, and principal component analysis (PCA) to investigate the relationships of the diagnostic characters of the *Syphacia* specimens that comprise the three *Syphacia* species groups. The results lend insight into the relationships between *Syphacia* species and their morphologies, as well as potentially improve the identification of these species.

Methods

Morphological measurements were taken from 70 oxyurid specimens of the *Syphacia* genus recovered from *Akodon albiventer* and *Calomys lepidus* collected in the Bolivian altiplano

during 1984-1993. The 70 oxyurid specimens included 19 males and 51 females randomly chosen from a large group of 680 oxyurids. Since male oxyurids die after mating, they are not typically recovered from host necropsies (Parel et al., 2008), which is why the male sample size is small. The specimens were organized into three *Syphacia* species groups: species groups A, B, and C. Species group A represents a *Syphacia* sp. collected from *Akodon albiventer*, species group B represents a *Syphacia* sp. collected from *Calomys lepidus,* and species group C is *Syphacia carlitosi* collected from *Akodon albiventer*.

Because *Syphacia* species are sexually dimorphic, the male and female specimens were analyzed separately. The morphological characters present in both sexes of *Syphacia* included nine variables used in the analyses: body length (Body_L) and width (Body_W), buccal length (Buc_L), esophageal bulb length (EsoBulb_L) and width (EsoBulb_W), esophagus length (Eso_L), tail length (Tail), the distance from the anterior end to the excretory pore (DFAE_Ex), and the distance from the anterior end to the nerve ring (DFAE_Nerve). A total of 18 characters were used for male analyses, with nine additional characters being primarily reproductive characters. The additional nine male characters were anterior mamelon length (Antm_L), middle mamelon length (Midm_L), posterior mamelon length (Posm_L), spicule length (Spicule), gubernaculum length (Gub_L), tail tip length (Tail_Tip), the distance from the anterior end to the anterior mamelon (DFAE_Antm), the distance from the anterior end to the middle mamelon (DFAE_Midm), and the distance from the anterior end to the posterior mamelon (DFAE_Posm). A total of 10 measured characters were used for female analyses; in addition to the nine shared characters above, the female specimens had one additional character, the distance from the anterior end to the vulva (DFAE_Vulva). Another dimorphic character in these nematodes is the shape of the tail at the posterior end. In males, the tail extends into a slimmer tip that varies in

length depending on species, whereas in females, the tail tapers gradually towards the end of the body to a pinpoint, hence the common name "pinworm". There are other diagnostic characters, like the presence and absence of cervical and lateral alae, that were not used in the analyses because of the limitations of the statistical designs. All characters were measured in micrometers using a Carl Zeiss Axioplan 2 imaging digital microscope with an Axiocamera accompanied with AxioVision LE64 software, and a Nikon compound microscope using an Amscope Toupview camera.

The male and female measurement data matrices of the 70 specimens studied were used as the input data for the statistical analyses. One-way ANOVAs with Tukey HSD post hoc tests, and principal component analyses (PCA) were performed using R version 3.2.0 (R core team, 2015). The analyses were used to assess the potential for statistical differentiation between the three species groups. The one-way ANOVA was performed first to test for significant differences in the characters among species, followed by Tukey HSD analyses for further corroboration and a more focused comparison among the species groups. Principal component analysis examined possible correlations among characters through variations among measurements.

Results

Female Character Distributions and One-way ANOVA

Beeswarm box plots (Figure 3.1) were created to illustrate the measurement distributions of each character for the three *Syphacia* species groups. Because of the low sample sizes, specimen dispersions (data points) were applied for further descriptive richness. Though the character plots of each species group demonstrate both similar and varying distributions, species group A and C seem to be most similar in respect to measurement means and ranges.

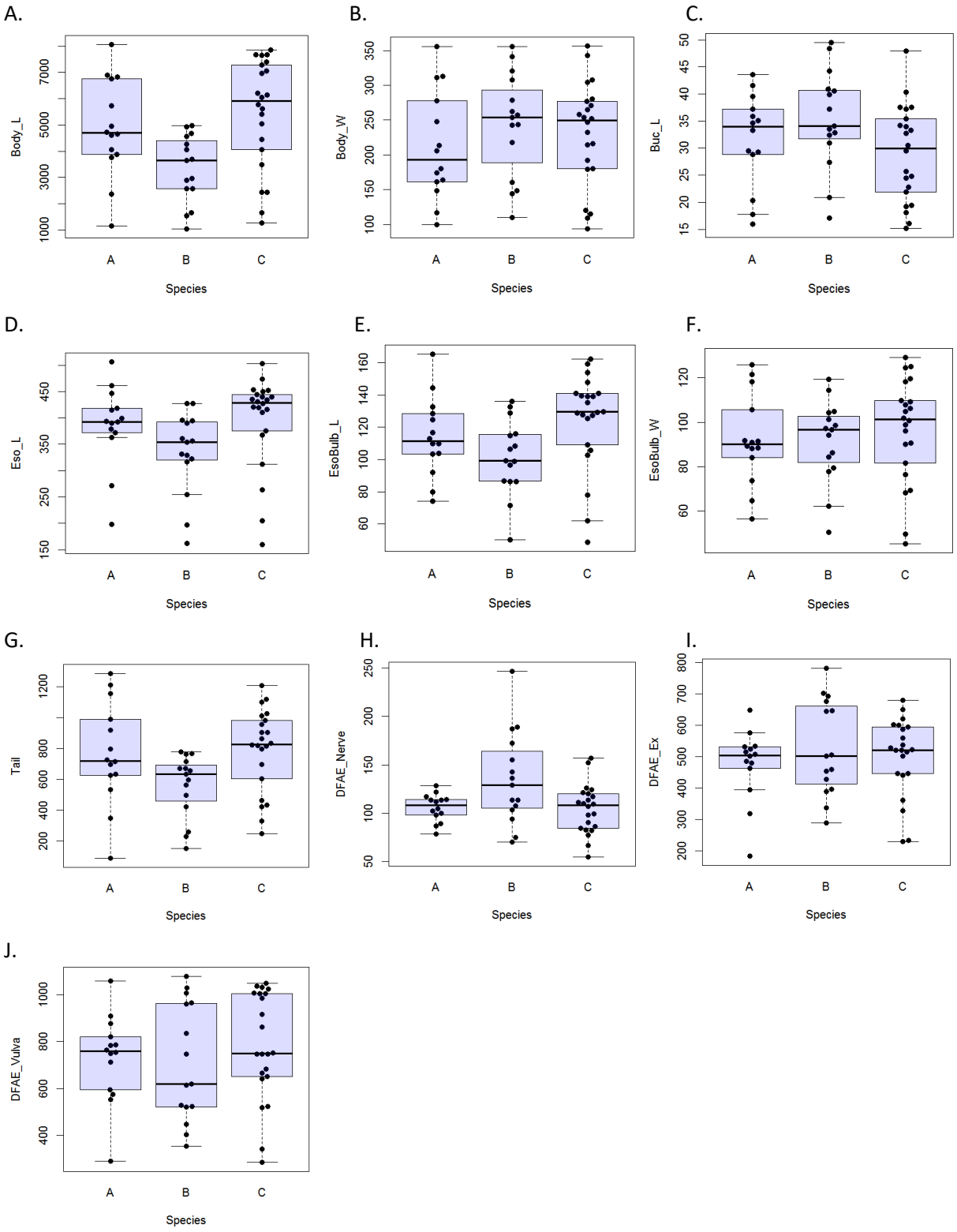


Figure 3.1: Female Beeswarm Box Plots. Plots A.- J. illustrate the distribution of each morphologic character of the female specimens across the three *Syphacia* species groups A, B, and C. The dots represent individual specimens.

The one-way ANOVA (Table 3.1) indicated the presence of significant differentiation among four characters from female specimens of the three *Syphacia* species groups. The characters, Body_L, EsoBulb_L, Tail, and DFAE_Nerve were significantly different between at least two species groups, as indicated by the low p-values ($\alpha < 0.05$; in bold). To determine which species groups had significantly differing measurements of these four significant characters, the Tukey HSD post hoc test was performed (Table 3.2).

Table 3.1: One-way ANOVA of Female Specimens. $F_{0.05}(2, 48) = 3.19$

Character	Species	Mean	SD	F-statistic	DF	P-value
Body_L	A	4889.455	1867.355	6.064	2, 48	**0.005**
	B	3335.894	1276.631			
	C	5434.222	2081.954			
Body_W	A	212.237	78.140	0.608	2, 48	0.549
	B	242.965	74.745			
	C	230.437	74.026			
Buc_L	A	31.597	8.614	2.279	2, 48	0.113
	B	35.330	9.176			
	C	28.990	8.817			
Eso_L	A	386.019	76.040	2.765	2, 48	0.073
	B	334.636	77.795			
	C	396.832	86.713			
EsoBulb_L	A	114.091	24.300	3.348	2, 48	**0.044**
	B	101.311	23.257			
	C	124.181	29.473			
EsoBulb_W	A	92.162	20.241	0.318	2, 48	0.729
	B	91.391	18.540			
	C	96.504	23.290			
Tail	A	766.251	331.635	3.587	2, 48	**0.035**
	B	558.171	204.609			
	C	789.110	267.849			
DFAE_Nerve	A	105.730	14.3072	5.067	2, 48	**0.010**
	B	135.703	47.917			
	C	104.120	25.227			
DFAE_Ex	A	476.200	114.070	0.558	2, 48	0.576
	B	527.143	152.091			
	C	501.476	122.758			
DFAE_Vulva	A	729.638	185.0517	0.535	2, 48	0.589
	B	708.107	251.012			
	C	782.338	230.180			

The post hoc tests using the Tukey HSD method further established where the significant difference of the four character measurements lies among the species groups (Table 3.2). This method tested the pairwise comparisons between the species group means and indicated specifically which species had significantly differing character measurements compared to its counterpart and how much these character measurement means differed. In looking at the mean differences for the four characters, species group C had larger mean lengths for Body_L, EsoBulb_L, and Tail compared to species group B. However, for the character, DFAE_Nerve, species group B had a larger mean length compared to species groups C and A. The adjusted p-values and mean differences suggest that species groups C and A are morphologically similar relative to species group B.

Table 3.2: Post Hoc Test using the Tukey HSD Method on Female Character Measurements

Tukey HSD					
Characters	Species	Mean Difference	Lower CI	Upper CI	Adjusted P-value
Body_L	B-A	-1553.56	-3190.18	83.0585	0.066
	C-A	544.767	-960.919	2050.453	0.659
	C-B	**2098.329**	**623.633**	**3573.024**	**0.003**
EsoBulb_L	B-A	-12.780	-36.519	10.960	0.401
	C-A	10.090	-11.750	31.931	0.508
	C-B	**22.870**	**1.479**	**44.260**	**0.034**
Tail	B-A	-208.079	-451.545	35.386	0.108
	C-A	22.860	-201.128	246.847	0.967
	C-B	**230.939**	**11.562**	**450.316**	**0.037**
DFAE_Nerve	**B-A**	**29.973**	**1.502**	**58.444**	**0.037**
	C-A	-1.609	-27.803	24.584	0.988
	C-B	**-31.583**	**-57.237**	**-5.929**	**0.012**

Female PCA

The PCA analysis examining the character relationships revealed differences among all three groups in the amount of variance contributed by each character. These differences were

indicated by the principal components. The first two principal components accounted for 81.3% of the variance (Table 3.3). Since principal components 1 and 2 explain a large proportion of the variance and the ten subsequent principal components each explain less than 6.5% of the variance, this suggested that the focus should be primarily on principal components 1 and 2.

Table 3.3: Importance of the First Four Principal Components Summary

	PC1	PC2	PC3	PC4
Standard deviation	2.646	1.063	0.794	0.675
Proportion of Variance	0.700	0.113	0.063	0.046
Cumulative Proportion	0.700	0.813	0.876	0.922

Based on the component loadings of principal component 1, it was determined that the loading value of 0.32 was of importance, and characters that had components larger than this value were important contributors to the principal components (Table 3.4). The level of the loading value was determined by characters with component loadings that exceeded the value of what the component loading would be, based on the sum of the squares, if all characters contributed equally to the first principal component. The characters with larger loadings (whether negative or positive; the signs simply imply positional contrasts in relation to other characters) contribute more variability, or weight, than the other characters and thus, have stronger effects on the principal component. Therefore, those characters with larger loadings were deemed significant.

The loading value indicated that the first principal component was most strongly correlated with six characters: EsoBulb_W, Eso_L, Body_W , Tail, Body_L, and EsoBulb_L . The similarity in component loading values and sign of these characters within the first principal component indicated that these characters were equally weighted and contributed similar

variability. In other words, the first principal component did not suggest what character measurements differentiated the species groups, since it was an average of the essential characters that describe all three species.

Table 3.4: Principal Component Analysis of the Morphological Characters of Female *Syphacia*

Morphological Characters	Component Loadings	
	PC1	PC2
Body_L	**-0.335**	**-0.350**
Body_W	**-0.338**	0.157
Buc_L	-0.261	**0.469**
Eso_L	**-0.350**	-0.201
EsoBulb_L	**-0.325**	-0.263
EsoBulb_W	**-0.354**	-0.0287
Tail	**-0.336**	-0.267
DFAE_Nerve	-0.212	**0.640**
DFAE_Ex	-0.314	0.206
DFAE_Vulva	-0.311	-0.011

The second principal component was most strongly correlated with three characters: DFAE_Nerve, Buc_L, and Body_L. The large component loadings of DFAE_Nerve and Buc_L in proportion to Body_L indicate that these characters contribute more variance to the second principal component than to the other characters. The character, DFAE_Nerve, had the largest component loading, suggesting that its measurements in relation to the two other characters may be more diagnostic among the species groups. The values of these characters for the second principal component in conjunction with the Tukey HSD output describe the important character measurement composition for species group B as opposed to species groups A and C.

In examining the relationship among the characters on a distance biplot, characters formed clusters and were strongly correlated with one another within the space of the first two principal components (Figure 3.2). Based on PC1, the distance biplot reflects 70% of character

length differences; however, as stated previously, PC1 was not informative about the species groups' differences. The small angles between the character arrows indicated that the characters were correlated, whereas right angles indicated which characters were uncorrelated. The characters DFAE_Vulva and Bulb_W were the most strongly correlated, as were Tail and EsoBulb_L characters. The DFAE_Nerve was the character most weakly correlated with Body_L, but the contrast of the DFAE_Nerve and Body_L characters does signify an important relationship in species differentiation within PC2, as supported by the Tukey HSD output. Of all the characters, the DFAE_Vulva character showed the least amount of variance. The remainder of the variables indicated no other significant amounts of variance.

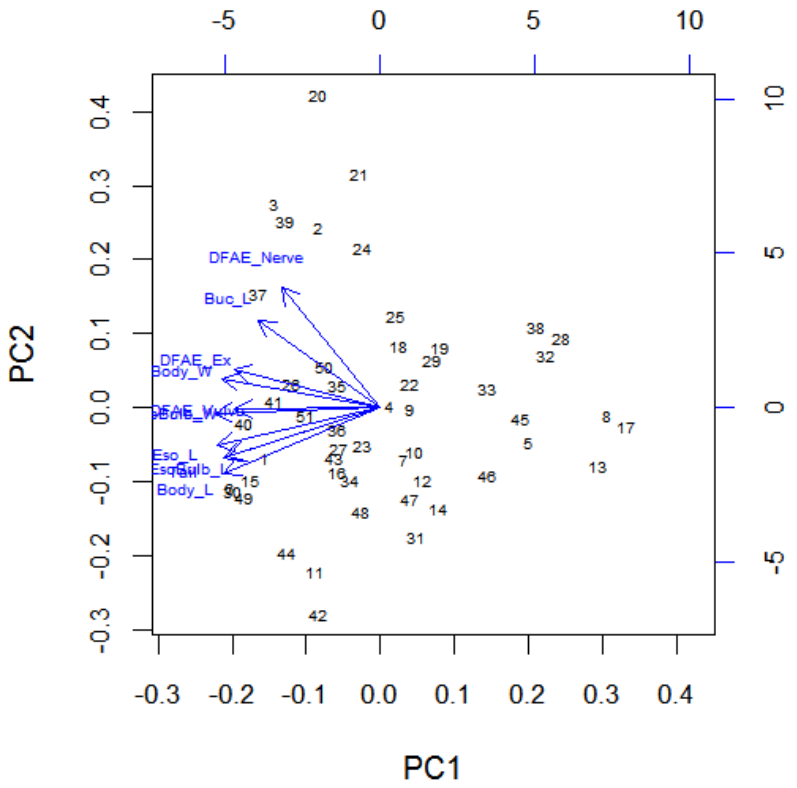


Figure 3.2: Distance Biplot of the First Two Principal Components of Female *Syphacia* Specimens. The black numbers represent individual specimens. The length of the character arrows is proportional to the total variance. The cosine of the angle between two character arrows equals the correlation of the two characters. The specimens that are close together correspond to character measurements that have similar loading scores on the components.

Applying the two principal components to the individual specimens yielded some suspected results (Figure 3.3). Based on the first principal component, the specimens of all three species were somewhat evenly distributed as a result of character measurements contributing equally to the variance, further signifying that PC1 is not of importance when differentiating species groups. In examining the Y-axis of PC2, species group B formed a defined clustered grouping, where as species groups A and C specimens were intermingled into one grouping. The separation of species group B from species groups A and C further corroborates the one-way ANOVA and Tukey HSD outputs. Some character measurements were larger in species group C compared to species group B, and the separation of species group B from species groups A and C is driven by the relationship of three character measurements, DFAE_Nerve and Buc_L in contrast to Body_L

.

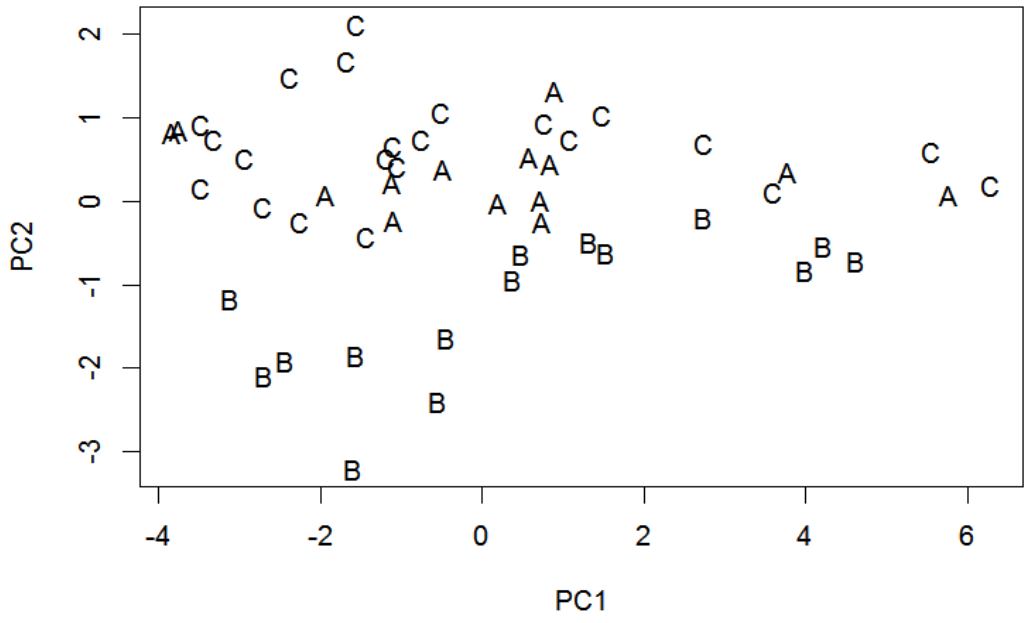
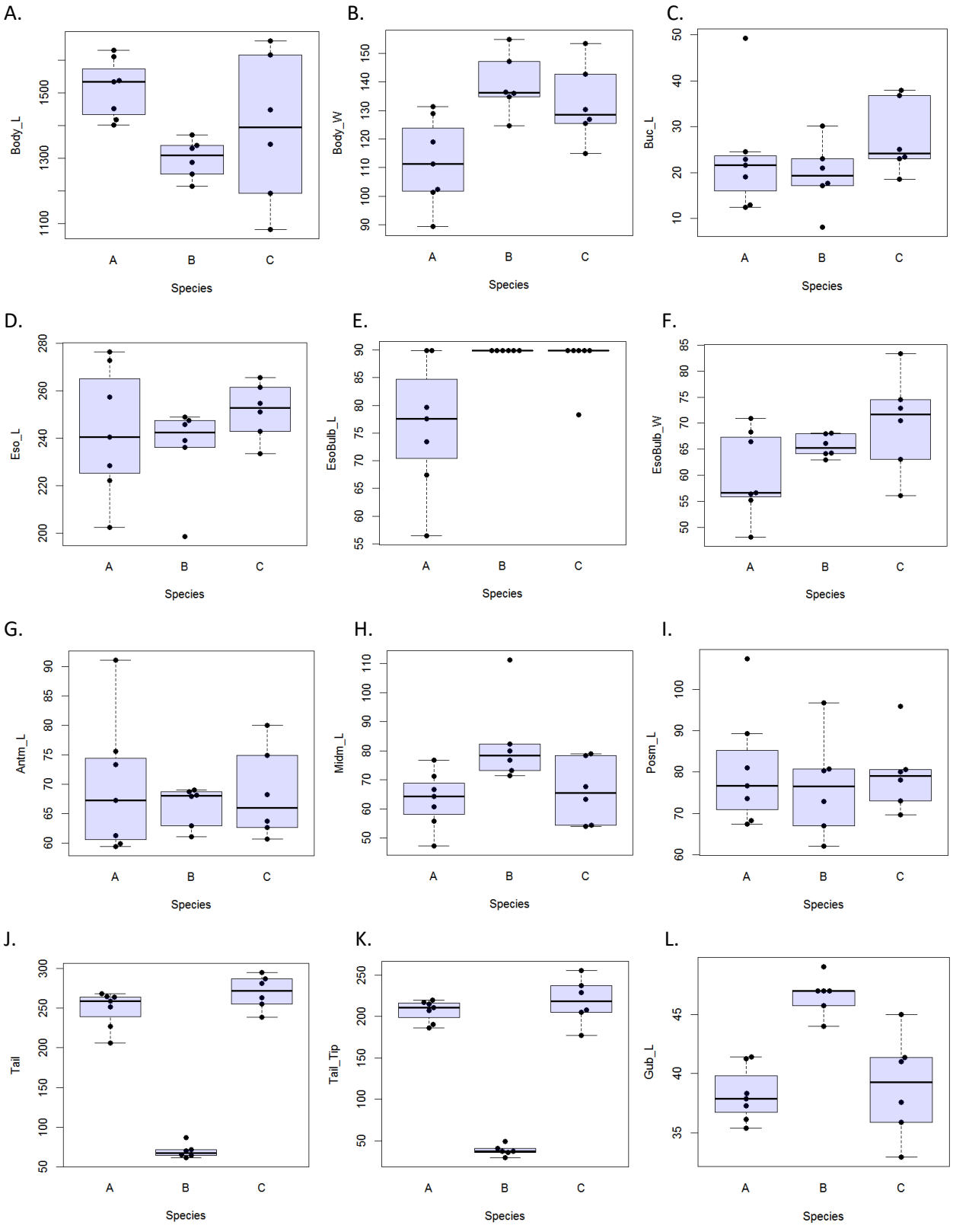


Figure 3.3: Scores Plot of Female *Syphacia* Specimens within the First Two Principal Components. The A group represents an unknown *Syphacia* species collected from *Akodon albiventer*, B represents an unknown *Syphacia* species collected from *Calomys lepidus*, and C represents *Syphacia carlitosi* collected from *Akodon albiventer*.

Male Character Distributions and One-way ANOVA

The beeswarm box plots of the male specimens illustrate the measurement distributions of each character from the three *Syphacia* species groups (Figure 3.4). The box plots for each character demonstrate varying differences in specimen distributions, means, medians and skewness. Though the sample size of the male specimens was extremely low (n=19), species groups A and C seem to be more similar in measurement distributions than either is to species group B.

The one-way ANOVA (Table 3.5) indicated the presence of significant differentiation among the measurements of eleven characters of the male specimens from the three *Syphacia* species groups. The characters, Body_L, Body_W, EsoBulb_L, Tail, Tail_Tip, DFAE_Ex, Midm_L, Spicule, Gub_L, DFAE_Antm and DFAE_Midm were significantly different between at least two species groups, as indicated by the low p-values (in bold). A post hoc test using the Tukey HSD method was then performed to determine which species groups differed significantly for each of the eleven character measurements.


A.
Body_L
1100
1300
1500
A
B
C
Species
B.
Body_W
90
100
110
120
130
140
150
A
B
C
Species
C.
Buc_L
10
20
30
40
50
A
B
C
Species
D.
Eso_L
200
220
240
260
280
A
B
C
Species
E.
EsoBulb_L
55
60
65
70
75
80
85
90
A
B
C
Species
F.
EsoBulb_W
50
55
60
65
70
75
80
85
A
B
C
Species
G.
Antm_L
60
65
70
75
80
85
90
A
B
C
Species
H.
Midm_L
50
60
70
80
90
100
110
A
B
C
Species
I.
Posm_L
60
70
80
90
100
A
B
C
Species
J.
Tail
50
100
150
200
250
300
A
B
C
Species
K.
Tail_Tip
50
100
150
200
250
A
B
C
Species
L.
Gub_L
35
40
45
A
B
C
Species

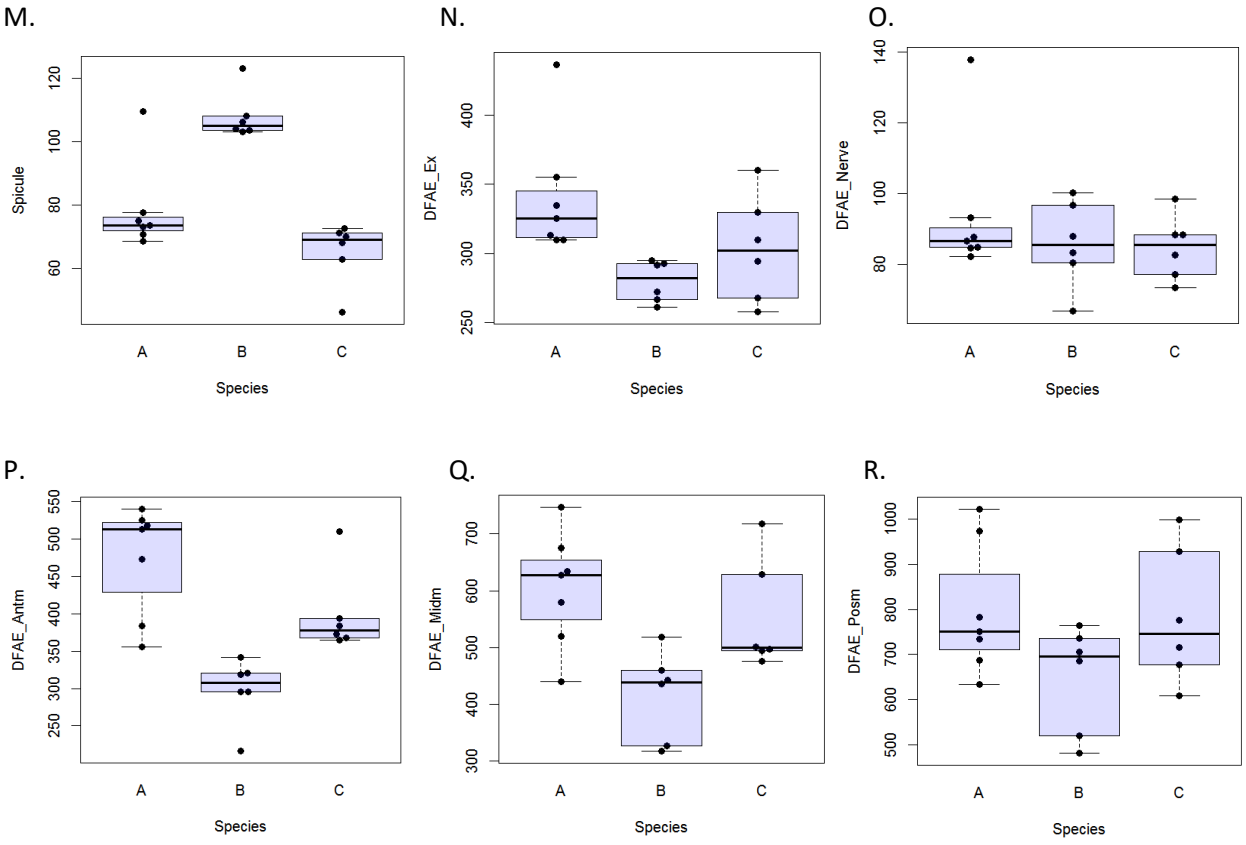


Figure 3.4: Male Beeswarm Box Plots. Beeswarm box plots A.- R. illustrate the distribution of each morphologic character of male specimens across the three *Syphacia* species groups A, B, and C. The dots represent individual specimens.

Table 3.5: One-way ANOVA of Male Specimens. $F_{0.05}(2, 16) = 3.63$

Character	Species	Mean	SD	F-statistic	DF	P-value
Body_L	A	1512.651	91.216	3.632	2, 16	**0.050**
	B	1299.248	58.991			
	C	1390.705	229.273			
Body_W	A	111.973	15.426	7.169	2, 16	**0.006**
	B	139	10.522			
	C	132.332	13.691			
Buc_L	A	23.241	12.375	1.002	2, 16	0.389
	B	19.535	7.311			
	C	27.460	8.007			
Eso_L	A	242.827	27.364	0.836	2, 16	0.4515
	B	235.912	18.941			
	C	251.485	11.898			
EsoBulb_L	A	76.321	11.979	5.846	2, 16	**0.012**
	B	89.870	0			
	C	87.937	4.736			
EsoBulb_W	A	60.311	8.328	2.809	2, 16	0.090
	B	65.610	2.161			
	C	70.087	9.455			
Tail	A	248.414	23.312	197.2	2,16	**5.34E-12**
	B	70.036	9.068			
	C	269.760	21.397			
Tail_Tip	A	206.501	13.287	196.9	2, 16	**5.406E-12**
	B	38.433	6.398			
	C	218.414	27.496			
DFAE_Nerve	A	93.801	19.651	0.743	2, 16	0.492
	B	85.895	12.030			
	C	84.790	9.016			
DFAE_Ex	A	340.763	45.235	4.73	2, 16	**0.024**
	B	279.962	14.927			
	C	303.470	38.475			
Antm_L	A	69.660	11.415	0.264	2, 16	0.772
	B	66.276	3.415			
	C	68.339	7.612			
Midm_L	A	63.249	9.794	4.778	2, 16	**0.024**
	B	82.489	14.600			
	C	66.115	11.043			

Table 3.5: Continued...

Characters	Species	Mean	SD	F statistic	DF	P-value
Posm_L	A	80.536	14.018	0.179	2, 16	0.838
	B	76.624	12.268			
	C	79.568	9.046			
Spicule	A	78.221	14.050	23.69	2, 16	**1.648E-5**
	B	107.928	7.615			
	C	65.078	9.877			
Gub_L	A	38.250	2.335	15.27	2, 16	**1.950E-4**
	B	46.623	1.658			
	C	38.968	4.324			
DFAE_Antm	A	472.764	73.492	13.76	2, 16	**3.341E-4**
	B	298.144	43.513			
	C	398.852	55.715			
DFAE_Midm	A	602.977	101.138	6.655	2, 16	**0.008**
	B	416.980	78.760			
	C	552.623	98.168			
DFAE_Posm	A	797.756	145.355	2.158	2, 16	0.148
	B	649.128	118.159			
	C	784.100	150.968			

The bolded adjusted p-values of the Tukey HSD tests (Table 3.6) indicated significantly different character measurements among species groups A, B, and C. Species groups B and A had ten significantly different character measurements, groups B and C had five (four of which were the same characters as B and A), and groups C and A had two significantly different character measurements. Compared to species group B, group A had on average significantly larger mean measurement lengths for Body_L, Tail, Tail_Tip, DFAE_Ex, DFAE_Antm, and DFAE_Midm, while group C had significantly larger mean lengths for Tail, Tail_Tip, and DFAE_Antm. Species group C also had significantly larger mean lengths for Body_W and EsoBulb_W than group A. However, for the characters, Body_W, EsoBulb_L, Midm_L, Spicule, and Gub_L, species group B had a significantly larger mean lengths compared to species groups A and C. The characters, Tail and Tail_Tip, seem to be key characters in

distinguishing between groups A and C from group B due to their zero p-value. Similar to the female specimens, the adjusted p-values and mean differences of the male specimens suggest that species groups C and A are distinctly different in comparison to species group B.

Table 3.6: Post Hoc Test using the Tukey HSD Method of Male Character Measurements

Characters	Species	Mean Difference	Lower CI	Upper CI	Adjusted P-value
Body_L	**B-A**	**-213.403**	**-419.618**	**-7.188**	**0.042**
	C-A	-121.947	-328.162	84.268	0.306
	C-B	91.456	-122.543	305.456	0.526
Body_W	**B-A**	**27.027**	**7.639**	**46.416**	**0.006**
	C-A	**30.359**	**0.970**	**39.747**	**0.039**
	C-B	-6.669	-26.789	13.452	0.675
EsoBulb_L	**B-A**	**13.548**	**2.353**	**24.744**	**0.017**
	C-A	**11.615**	**0.420**	**22.810**	**0.042**
	C-B	-1.933	-13.551	9.684	0.901
Tail	**B-A**	**-178.378**	**-206.086**	**-150.669**	**0**
	C-A	21.346	-6.363	49.055	0.147
	C-B	**199.724**	**170.969**	**228.479**	**0**
Tail_Tip	**B-A**	**-168.069**	**-193.558**	**-142.580**	**0**
	C-A	11.913	-13.577	37.402	0.467
	C-B	**179.981**	**153.530**	**206.433**	**0**
DFAE_Ex	**B-A**	**-60.800**	**-112.551**	**-9.050**	**0.021**
	C-A	-37.293	-89.044	14.458	0.183
	C-B	23.508	-30.197	77.212	0.511
Midm_L	**B-A**	**19.241**	**2.213**	**36.268**	**0.026**
	C-A	2.866	-14.161	19.894	0.902
	C-B	-16.374	-34.045	1.296	0.072
Spicule	**B-A**	**29.707**	**13.809**	**45.605**	**5.212E-4**
	C-A	-13.143	-29.041	2.755	0.114
	C-B	**-42.850**	**-59.348**	**-26.352**	**1.44E-5**
Gub_L	**B-A**	**8.373**	**4.128**	**12.619**	**3.051E-4**
	C-A	0.718	-3.527	4.964	0.901
	C-B	**-7.655**	**-12.061**	**-3.249**	**0.001**
DFAE_Antm	**B-A**	**-174.620**	**-260.600**	**-88.641**	**2.260E-4**
	C-A	-73.913	-159.892	12.067	0.0984
	C-B	**100.708**	**11.483**	**189.933**	**0.026**
DFAE_Midm	**B-A**	**-185.997**	**-320.557**	**-51.438**	**0.007**
	C-A	-50.354	-184.913	84.206	0.608
	C-B	135.64338	-3.996	275.282	0.0576

Male PCA

The first two principal components account for only 58.7% of the variance, whereas the first five components comprise 84.4% of the variance (Table 3.7). Since at least 80% of variance is necessary to describe any significance of the principal components, the first five principal components will be the focus, although a greater emphasis will be placed on the first two principal components because they hold the largest percentage of the variability. The loading value of 0.23 was of importance, and those characters that had components larger than this value were important contributors to its principal components (Table 3.8).

Table 3.7: Importance of the First Five Principal Components Summary

	PC1	PC2	PC3	PC4	PC5
Standard deviation	2.629	1.913	1.373	1.218	1.122
Proportion of Variance	0.384	0.203	0.105	0.083	0.070
Cumulative Proportion	0.384	0.587	0.692	0.774	0.844

Based on the loading value, the first principal component was most correlated with 12 characters: DFAE_Antm, DFAE_Midm, Tail, Tail_Tip, Spicule, Gub_L, Body_L, Body_W, EsoBulb_L, Midm_L, DFAE_Posm, and DFAE_Ex (Table 3.8). The majority of the 12 characters had similar loading values meaning they were equally weighted in variability. Of these 12 characters, seven characters had negative component loadings while the other five characters had positive component loadings. Though the minus and plus signs do not mean negative and positive, the signs do explain the placements and relationships of the characters in the multi-dimensional space of the principal components. Despite the similar loading values, the relationship among the seven negatively loaded characters in contrast to the five other characters contributes more to the variance of the first principal component than the other individual characters do. When these characters are viewed in conjunction with the Tukey output, it is

evident that the character's relationships explain the measurement differences that mainly drive the species differentiation of species groups A and C from species group B.

The second principal component was correlated with Buc_L, EsoBulb_W, Eso_L, Tail, Posm_L, EsoBulb_L, Body_W and Tail_Tip. The similarity in component loading sign and values of these characters indicated that the combination of these characters was equally weighted and contributed similar variability. There were a few characters, Buc_L, Eso_L and EsoBulb_W, that did have larger component loadings, but were not found to be significant in the one-way ANOVA or Tukey HSD test. The second principal component did not add any more information on the character relationships that differentiated the three species groups.

Table 3.8: Principal Component Analysis of the Morphological Characters of Male *Syphacia*.

Morphological Characters	Component Loadings				
	PC1	PC2	PC3	PC4	PC5
Body_L	**-0.269**	-0.114	**0.245**	-0.024	**0.346**
Body_W	**0.267**	**0.247**	0.015	0.224	-0.025
Buc_L	0.022	**0.457**	0.082	**-0.272**	0.008
Eso_L	-0.058	**0.356**	0.047	**0.395**	**0.338**
EsoBulb_L	**0.259**	**0.254**	0.002	**0.250**	0.135
EsoBulb_W	0.104	**0.399**	-0.217	**0.332**	0.041
Antm_L	-0.035	0.064	**0.583**	0.213	-0.154
Midm_L	**0.255**	-0.050	**0.328**	-0.014	-0.052
Posm_L	0.050	**0.255**	**0.449**	**-0.424**	-0.076
Spicule	**0.279**	-0.213	0.095	-0.037	0.222
Gub_L	**0.268**	-0.154	0.102	0.088	**0.367**
Tail	**-0.313**	**0.256**	-0.025	-0.011	**-0.231**
Tail_Tip	**-0.310**	**0.239**	-0.041	-0.011	**-0.232**
DFAE_Nerve	-0.012	**0.292**	0.064	**-0.402**	**0.485**
DFAE_Ex	**-0.239**	-0.001	**-0.265**	-0.152	**0.373**
DFAE_Antm	**-0.360**	-0.050	-0.012	0.078	0.179
DFAE_Midm	**-0.333**	-0.062	0.175	0.189	0.093
DFAE_Posm	**-0.255**	-0.109	**0.328**	**0.293**	0.088

In examining the relationships among the characters, characters formed roughly three "datasets" and expressed correlations within the space of the first two principal components (Figure 3.5). The biplot depicts three clustered datasets, with PC1 reflecting only 38% of character differences due to measurement lengths. The first dataset comprises the seven characters with negative component loadings from the PC1 output. The second dataset includes Eso_L, DFAE_Nerve, Buc_L, Posm_L, and EsoBulb_W. The third dataset is made up of the five characters with positive component loadings also from the PC1 output. The first and third data sets illustrate the relationships or rather, the contrasts, of the significant character measurements that mainly describe species groups A and C in comparison to species group B within PC1, further corroborating the Tukey HSD output. In particular, PC1 indicates that the characters, Tail and Tail_tip, and Gub_L and Spicule, are inversely correlated; this relationship is a heavy contributor to the variation between the species groups. The second dataset represents PC2, which mainly describes what is driving PC2 rather than the character measurements underlying the differentiation of the species groups. Several characters correlated strongly with one another including Body_L and DFAE_Posm, DFAE_Midm and DFAE_Antm, Body_W and EsoBulb_L, and Tail and Tail_Tip. The Antm_L character showed the least amount of variance, followed by Posm_L, DFAE_Nerve, and DFAE_Ex.

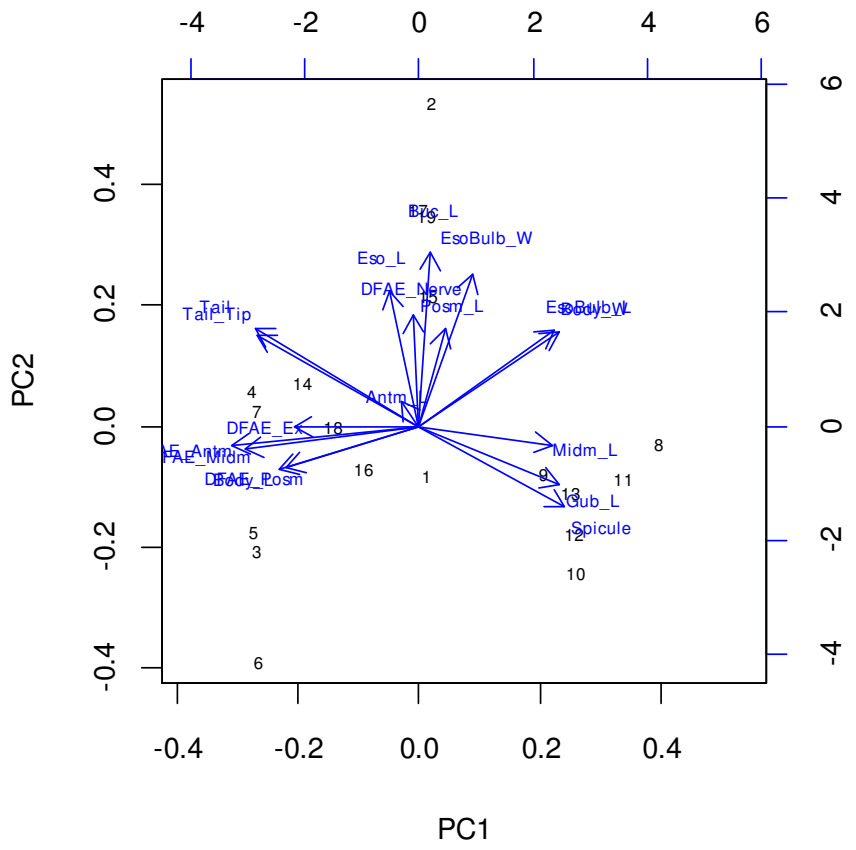


Figure 3.5: Distance Biplot of the First Two Principal Components of Male *Syphacia* Specimens. The black numbers represent individual specimens. The length of the blue arrows for each character is proportional to the total variance. The cosine of the angle between two character arrows equals the correlation of the two characters. The numbers/specimens that are close together correspond to character measurements that have similar loading scores on the components.

When the individual male specimens were applied to the first two principal components, the importance of the PC1 results were further corroborated (Figure 3.6). Based on the first principal component, the specimens were clustered in their respective species groups, with groups A and B in more defined clusters compared to group C, where specimens from the other two groups were present. The contrasting ratio of the 12 significant character measurements of PC1 do support the measurement compositions that separate three species groups, primarily species group A from species groups B. The ratio that describes the measurement composition of

species group A includes larger mean measurement lengths of Body_L, Tail, Tail_Tip, DFAE_Ex, DFAE_Antm. DFAE_Midm, and DFAE_Posm, in proportion to smaller mean lengths of the Spicule, Gub_L, Body_W, EsoBulb_L, and Midm_L, whereas the opposite holds true for species group B. In examining the Y-axis of PC2, no defined species groups were illustrated. The specimens of all three species were similarly distributed because the significant characters defined by PC2 contributed equally to the variance, further signifying that PC2 is not of importance when differentiating species groups.

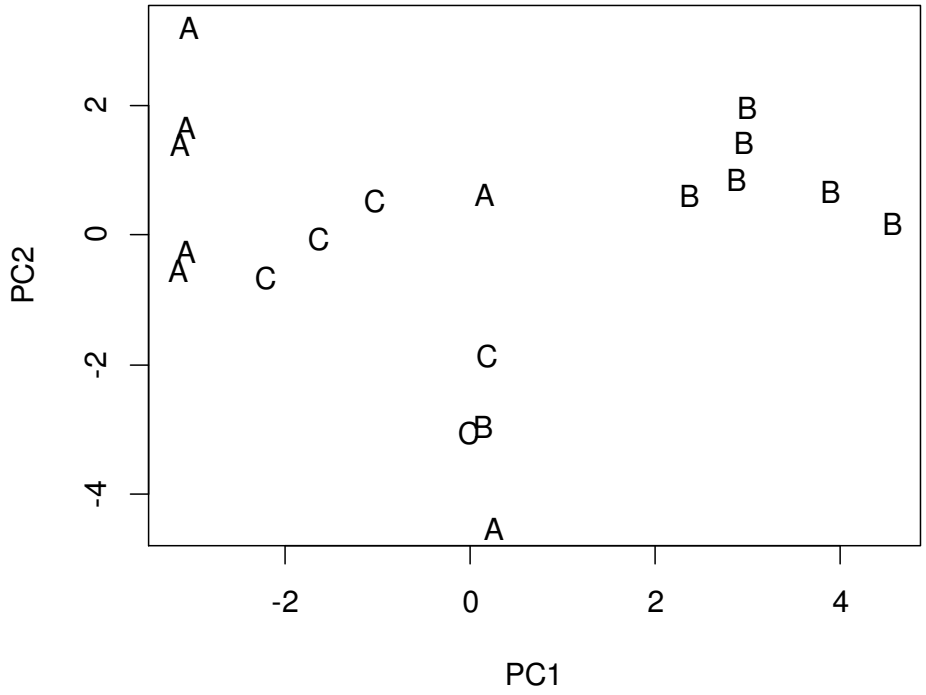


Figure 3.6: Scores Plot of the First Two Principal Components of the Male *Syphacia* Specimens. The A group represents an unknown *Syphacia* species collected from *Akodon albiventer*. The B group represents an unknown *Syphacia* species collected from *Calomys lepidus*. The C group represents *Syphacia carlitosi* collected from *Akodon albiventer*.

Discussion

The output of the three statistical analyses, one-way ANOVA, post hoc test using Tukey HSD method, and PCA indicated the presence of significant differentiation among the character measurements of both female and male *Syphacia* specimens. The tests indicated that male specimens had twelve significant characters and the female specimens had three important characters; whose relationships influenced the differentiation between the three *Syphacia* species groups. Such a difference between the sexes in the number of significant characters is expected. Male specimens not only have more diagnostic characters than females, their species-specific character lengths, specifically the reproductive, mating and tail morphologies, make better indicators for determining the *Syphacia* species than those of females, which tend to be morphologically similar across species (see Figure 3.1; Parel et al., 2008). Instead of the number of significant characters, the extent of the character relationships' influence on species differentiation comes into question.

For the female specimens, character measurements were more variable between the species groups than within the species groups. Initially, the Tukey HSD output determined that four characters, Body_L, EsoBulb_L, DFAE_Nerve, and Tail, exhibited important measurement differences among the species groups, primarily between species group C and B. In the PCA, the relationship among only three characters, DFAE_Nerve, Buc_L, and Body_L, were deemed significant and became the primary focus of the differentiation of species groups A and C in contrast to species group B. Though EsoBulb_L and Tail were deemed significant in PC1, the extent of their contribution to the variance in relation to the other PC1 characters was not significant in differentiating the species groups. Interestingly, Buc_L was not deemed significant

in the one-way ANOVA or Tukey HSD test but was considered an important contributor to the contrasting relationships among the species groups in the PCA.

The other characters that did not express any significance could be the result of overlapping or similar measurements among the three species groups. Furthermore, it also possible that some female pinworms had not fully matured to adulthood, and thus, their size was not typical of their species group. Though measurement overlap is a classic obstacle when identifying parasites, the similarity of female specimen measurement ranges indicate that these basic morphologies were not reliable enough to determine concrete species classifications of the female *Syphacia*. These results also supported the notion that the presence and absence of other characters that could not be used in the analyses, such as cervical alae, dierids and eggs, are necessary in the diagnosis of species. Based on the test outputs, the characters with significant mean differences and the relationship of the three significant characters did influence the categorization the female *Syphacia* specimens into the three species groups, but there was no indication of any major distinctions among all species groups.

In regard to the male specimens, the Tukey HSD test found eleven characters with important measurement differences that distinguished species groups A and C from species group B. Principal component analysis then determined that the relationship among the twelve character measurements, seven characters in relation to five other characters (Table 3.8), were significant. This specific character ratio described the measurement composition of species group A, where unlike group B, the seven characters have larger mean measurement lengths compared to the five different characters with smaller mean lengths. Compared to species group B, group C had a ratio of three characters with larger mean measurements, like species group A, to two characters with smaller mean measurements. Though these male character ratios are supported

by the PCA output, the output describes less than 40 percent of the variability of these ratios. Principal component analysis cannot explain why the male principal components variation is a low percentage, but it does suggest that there is a possibility of some discrepancies in the data. The unequal sample sizes of the species groups and low number of male specimens create low power in the post hoc Tukey results. The low power limits the importance of the character relationships, causing the results to be only weakly supported. Because of this, these male results cannot be adequately generalized.

Though some of the results were inconclusive, it is reasonable to note a reoccurring trend in each of the analyses: distinct differences were established between species groups A and C versus group B. Both male and female results indicated that species groups A and C differed from species group B. The female results emphasized differences between groups C and B, while the male results indicated more differentiation between groups A and B. The female results did not yield any significant differences between species groups A and C, but the male Tukey HSD results did express two characters that had smaller mean differences in group A compared to groups B and C. However, these two characters were the only significant characters resulting from one test that separated species group A from species group C. Based on the overall results, it could be hypothesized that species groups A and C are the same *Syphacia* species, especially since they share the same host, *Akodon albiventer*. Though this study does not provide enough evidence to discredit this statement, there is also not enough evidence to support it.

One conclusion that was apparent from the male and female analyses was that the specimens that constituted species group B were unique and may possibly be an undocumented *Syphacia* species of *Calomys lepidus*. The host, *Calomys lepidus*, currently does not have any recorded *Syphacia* species and the specimen morphologies of species group B do not match any

other documented *Syphacia* species. These factors suggest that these specimens are a new species of *Syphacia.*

Conclusion

While disparities were identified among the species groups, there was not enough evidence to confirm that the male and female specimens were comprised three species of *Syphacia.* The results did, however, lend insight into the relationships among *Syphacia* species, specifically as they compared species group B. The outputs supported the notion that species group B differed from the other two species groups in terms of character measurements and relationships, but were inconclusive about the species A and C groupings. The inconclusive results may be partially due to the statistical design, which used subjective criteria and was not all-inclusive, as well as the small and unequal sample sizes, which diminished the support of the analyses results. While this study was conducted to evaluate the accuracy of manual identification of these *Syphacia* specimens, it did shed light on the impacts and implications of using morphological variations for species classifications.

The practicality of morphometrics as a diagnostic tool is undeniable, but as the sole basis of diagnosis, it is unreliable. The morphology of species' can be variable and influenced by multiple factors. For example, genetic polymorphisms in parasites can occur due to host and environmental influences (McManus and Bowles, 1996). Some morphologic measurements can also be affected by growth, age, or the organism's state of nutrition (Sneath and Sokal, 1973). On the other hand, there might be no detectable morphological differences between species leading one to believe they are closely related, if not the same (Parel et al., 2008). While generalized methods employed in morphological analyses may not yield conclusive results, its utilization is necessary in understanding the physical characteristics of the organism of interest (Giese et al.,

2005; Jenner, 2003; Rohlf, 1990). Morphometric concepts address the multiple applications and interpretations of numerical taxonomy, which set the foundation for taxonomic study and remain extremely useful (see: Aurahs et al., 2011; Geise et al., 2005; Shoshani and McKenna, 1998; Sneath and Sokal, 1973). Despite the increasing efficiency and convenience of molecular approaches to parasite identification, this field cannot exist alone. Morphometrics provide the foundation for hypothesizing cladistic relationships and will remain an essential tool in identification processes of parasites.

REFERENCES

Aurahs, R., Treis, Y., Darling, K., and Kucera, M. 2011. A revised taxonomic and phylogenetic concept for the planktonic foraminifer species Globigerinoides ruber based on molecular and morphometric evidence. Marine Micropaleontology 79: 1–14.

Geise, L., Moraes, D. A., Silva, H.S. 2005. Morphometric Differentiation and Distributional of Three Species of Akodon (Muridae, Sigmodontinae, Akodontini) in the Atlantic Coastal Area of Brazil. Arquivos do Muséum National, Rio de Janeiro 63 (1): 63-74.

Jenner, R. 2003. Unleashing the Force of Cladistics? Metazoan Phylogenetics and Hypothesis Testing. Integrative Comparative Biology 43:207–218.

McManus, D.P. and Bowles, J. 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. International Journal of Parasitology. 26 (7): 687-704.

Parel, J.D.C., Galula, J.U. and Ooi, H.K.2008. Characterization of rDNA sequences from Syphacia obvelata, Syphacia muris, and Aspiculuris tetraptera and development of a PCR-based method for identification. Veterinary Parasitology. 153: 379–383.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL http://www.R-project.org/.

Robles, M.R. and Navone, G.T. 2010. Redescription of *Syphacia venteli* Travassos 1937 (Nematoda: Oxyuridae) from *Nectomys squamipes* in Argentina and Brazil and description of a new species of *Syphacia* from *Melanomys caliginosus* in Columbia. Parasitology Research. 106: 1117-1126.

Shoshani, J. and McKenna, M. C. 1998. Higher Taxonomic Relationships among Extant Mammals Based on Morphology, with Selected Comparisons of Results from Molecular Data. Molecular Phylogenetics and Evolution 9 (3): 572–584.

Sneath, P.H.A. and Sokal, R. R. 1973. Numerical Taxonomy. W. H. Freeman and Company, San Francisco.