ENDOPARASITES OF THE DIGESTIVE SYSTEMS OF FOUR SPECIES OF POCKET GOPHERS (GENUS: *GEOMYS*) IN TEXAS

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ABSTRACT

The 9 species of pocket gophers in the state of Texas are ecologically interesting in that their ranges overlap very little, leading to genetic and chromosomal variation in both pocket gopher hosts and their parasites. We examined 4 species of pocket gopher (*Geomys attwateri, G. bursarius, G. personatus*, and *G. texensis)* in Texas for helminth parasites of the digestive system. Both nematodes and cestodes were collected. Only 1 species of nematode was collected, and it was collected from all 4 pocket gopher species representing four new host records for the nematode *Protospirura ascaroidea.* Cestodes recovered were from two genera: *Monoecocestus* and *Hymenolepis*. There was no significant difference in prevalence or intensity of nematodes in pocket gopher hosts. Prevalence of cestode parasites varied significantly between *G. bursarius* and *G. texensis*. Intensity of cestode parasites did not differ significantly between species.

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INTRODUCTION

Pocket gophers, so called because of their fur-lined cheek pouches, are fossorial rodents that build extensive burrow systems in which they live, eat, and reproduce (Connior 2011). They live in isolated populations, and their ranges are oftentimes restricted because of available soil conditions that limit their ability to burrow. Highways, roads, and other man-made infrastructure, as well as rivers and other waterways, also serve as barriers to dispersal. Because of this, pocket gophers are more likely to experience speciation events than organisms with more uniform distributions and fewer barriers. Consequently, organisms that parasitize pocket gophers should live in isolated populations as well (Hafner and Page 1995). Pocket gophers also have very specific diets. Gophers feed on roots and tubers that protrude into their tunnels. Diet studies have not found any evidence that gophers feed on insects or other organisms that cohabitate gopher burrows. Limited dispersal and diet make pocket gophers a good organism for studying host-parasite relationships.

Previous studies have explored several aspects of pocket gopher-parasite relationships, including Gardner (1983) who studied both endoparasite and ectoparasite load and species diversity in eight species of pocket gopher from Colorado, Washington, Oregon, and Mexico. Other studies have examined the phylogenetic relationship of pocket gophers and their chewing lice and have found that pocket gophers and their ectoparasites exhibit similar speciation patterns (Light and Hafner 2007). Demastes and Hafner (1993) looked at the coevolution of chewing lice and pocket gophers in the genus *Geomys* in Texas and Louisiana. Timm and Price (1979) described a new species of *Geomydoecus*, a genus of

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chewing louse, in *Geomys personatus* from Texas, supporting the highly host-specific nature of pocket gopher ectoparasites. Other work on pocket gopher endoparasites, including nematodes, has been conducted outside Texas (Gardner 1983; Bartel and Gardner 2000). There is still very little known about the endoparasites of pocket gophers, especially in the state of Texas (Table 2). Of the nine species of pocket gopher in Texas, eight have documented parasite associates, most being ectoparasites (Table 1; Table 2). There have been no studies reporting parasites from *G. jugossicularis*. Endoparasites of *G. bursarius* in the state of Texas have not been well studied (Table 2). Only one nematode parasite has been reported from *G. attwateri* in Texas (Falcon-Ordaz et al. 2006). Endoparasites from *G. breviceps* were last reported in 1932 from Oklahoma (English 1932). No endoparasites have been reported from *G. personatus*, *G. streckeri*, *G. texensis*, or *G. tropicalis*, although ectoparasites have been documented from all four species (Price and Emerson 1971; Price and Hellenthal 1975; Wilkins and Houck 2001).

There are over 40 parasite species known to occur in pocket gophers in the genus *Geomys* (Table 1; Table 2). Of these, 46% are ectoparasites. Most ectoparasites are mites or lice, however, there is also evidence that pocket gophers are occasionally parasitized by ticks and fleas (Bartel and Gardner 2000; Wilkins and Houck 2001). Endoparasites known to occur in the genus *Geomys* include nematodes, cestodes, one acanthocephalan, and one documented protozoan parasite (Rissky 1962; Bartel and Gardner 2000). There are at least 11 unique species of cestode, *Anoplocephaloides infrequens*, *Anoplocephaloides variabilis*, *Andrya macrocephala*, *Hymenolepis weldensis*, *Aprostatandrya macrocephala*, *Paranoplocephala infrequens*, *Andrya translucida*, *Cittotaenia perplexa*, *Hymenolepis* *diminuta*, *Monoecocestus anoplocephalaloides*, and *Oochoristica* spp., found in the genus *Geomys*, all of which are documented from *G. bursarius* (Table 2).

Of the nine species of nematode found in the genus *Geomys*, six have been collected from *G. bursarius*: *Physaloptera limbata*, *Capillaria americana*, *Ransomus rodentorum*, *Capillaria hepatica*, *Litomosa filaria*, and *Mastophorus murus*. *Protospirura ascaroidea* has been collected from *G. breviceps* (English 1932); *Vexillata geomyos* has been documented from *G. attwateri* (Falcon-Ordaz et al. 2006); *Litomosoides westi* was collected from *Geomys* spp. (Pitts et al. 2000).

The acanthocephalan, *Moniliformis clarki*, has been collected from *G. bursarius* in Minnesota (Bartel and Gardner 2000). It is not known to occur in the genus *Geomys* in Texas. The protozoan *Monocercomonoides* spp. has been documented from *G. bursarius* in South Dakota (Rissky 1962). This parasite also has not been documented in Texas.

Several parasites documented in pocket gophers in Texas have life cycles that should not include pocket gophers. For example, many members of the genus *Physaloptera* infect carnivores. The carnivore host deposits eggs with the J_1 larval stage into the soil when it defecates. The eggs are then consumed by an arthropod, like a cricket, in which the parasite develops into the infective J_3 stage; the arthropod is usually consumed by an insectivore or generalist, like a shrew or mouse, serving as a paratenic host. The smaller secondary host is eaten by a carnivore like a dog or a cat, and the cycle is complete (Schell 1952). Pocket gophers do not prey on arthropods. It is possible that pocket gophers are accidentally consuming arthropods that have been infected with *Physaloptera* eggs, but pocket gophers have not been reported to consume arthropods, accidentally or otherwise. Little is known about the life cycle for *P. limbata* aside from the fact that it occurs in *G. bursarius*.

Another nematode, *Protospirura*, has a direct life cycle more likely to affect *Geomys*. *Protospirura* eggs are deposited in the soil by the definitive host, where another definitive host will consume the eggs; the adult worm develops in this organism (Crook and Grundmann 1964). Because infection by *Protospirura* occurs when the host consumes eggs, pocket gophers that become infected with *Protospirura ascaroidea* must directly consume the eggs. It is possible that *Physaloptera* and *Protospirura* are infecting pocket gophers in a similar manner, probably by being washed into the soil where the pocket gopher consumes the eggs on a root or tuber.

The main objectives of this study were first, to determine which helminth parasites occur in the digestive tracts of pocket gophers (genus: *Geomys*) in the state of Texas. A second objective of this study was to determine whether the endoparasites of different species of pocket gopher vary in prevalence, intensity, or species richness. Because of the size and variation in ecological regions of Texas, pocket gophers live in a variety of different conditions and habitats. Regional differences in pocket gopher ranges could result in different parasites occurring in different species of *Geomys*. These same regional differences could also result in varying prevalence and intensity of infection. Prevalence and intensity of *Geomys* parasites were also examined in this study.

Table 1. — Ectoparasites reported in previous studies from pocket gophers in the genus *Geomys*.

Host Species	Parasite Species	State Collected	Source
G. attwateri	*Androlaelaps geomys*	Texas	Wilkins and Houck 2001
G. breviceps	"	"	"
G. knoxjonesi	"	"	"
G. personatus	"	"	"
G. streckeri	"	"	"
G. texensis	"	"	"
G. attwateri	*Geomylichus floridanus*	Texas	Wilkins and Houck 2001
G. breviceps	"	"	"
G. knoxjonesi	"	"	"
G. texensis	"	"	"
G. breviceps	*Echinonyssus geomydis*	Texas	Wilkins and Houck 2001
G. bursarius	"	"	"
G. knoxjonesi	"	"	"
G. streckeri	"	"	"
G. texensis	"	"	"
G. knoxjonesi	*Hyponeocula deserticola*	Texas	Wilkins and Houck 2001
G. texensis	"	"	"
G. texensis	*Parasecia gurneyi campestris*	Texas	Wilkins and Houck 2001
G. streckeri	"	"	"
Geomys arenarius	*Echinonyssus femuralis*	Texas	Wilkins and Houck 2001
Geomys attwateri	*Geomydoecus subgeomydis*	Texas	Timm and Price 1980
Geomys breviceps	*Trichodeates geomydis*	Oklahoma	English 1932
Geomys breviceps	*Geomydoecus ewingi*	Texas	Timm and Price 1980
Geomys breviceps	*Laelaps spp.*	Oklahoma	English 1932
Geomys bursarius	*Geomydoecus geomydis geomydis*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Opisocrostis bruneri*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Foxella ignota ignota*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Hiristionyssus geomydis*	Kansas	Ubelaker and Downhower 1965
Geomys bursarius	*Androlaelaps glasgowi*	Kansas	Ubelaker and Downhower 1965
Geomys bursarius	*Dermacentor variabilis (larvae)*	Minnesota	Bartel and Gardner 2000
Geomys personatus	*Geomydoecus texanus*	Texas	Price and Hellenthal 1975
Geomys personatus	*Geomydoecus dalgleishi*	Texas	Timm and Price 1979
Geomys streckeri	*Geomydoecus truncatus*	Texas	Price and Emerson 1971
Geomys texensis	*Pseudoschoengastia farneri*	Texas	Wilkins and Houck 2001
Geomys texensis	*Euschoengastoides sp.*	Texas	Wilkins and Houck 2001
Geomys texensis	*Pseudoschoengastia faneri*	Texas	Wilkins and Houck 2001

Table 2. — Endoparasites reported in previous studies from pocket gophers in the genus *Geomys*.

Host Species	Parasite Species	State Collected	Source
Geomys attwateri	*Vexillata geomyos*	Texas	Falcon-Ordaz et al. 2006
Geomys attwateri	*Monoecocestus centroovarium*	Texas	Dronen et al. 1994
Geomys breviceps	*Hymenolepis spp.*	Oklahoma	English 1932
Geomys breviceps	*Protospirura ascaroidea*	Oklahoma	English 1932
Geomys bursarius	*Moniliformis clarki*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Anoplocephaloides infrequens*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Anoplocephaloides variabilis*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Andrya macrocephala*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Hymenolepis weldensis*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Aprostatandrya macrocephala*	Kansas	Ubelaker and Downhower 1965
Geomys bursarius	*Paranoplocephala infrequens*	Kansas	Ubelaker and Downhower 1965
Geomys bursarius	*Andrya translucida*	Minnesota	Douthitt 1915
Geomys bursarius	*Cittotaenia perplexa*	Oklahoma	Burnham 1953
Geomys bursarius	*Hymenolepis diminuta*	Oklahoma	Burnham 1953
Geomys bursarius	*Monoecocestus anoplocephalaloides*	Oklahoma	Burnham 1953
Geomys bursarius	*Oochoristica spp.*	NA	Connier 2011
Geomys bursarius	*Physaloptera limbata*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Capillaria americana*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Ransomus rodentorum*	Minnesota	Bartel and Gardner 2000
Geomys bursarius	*Capillaria hepatica*	Kansas	Ubelaker and Downhower 1965
Geomys bursarius	*Litomosa filaria*	Oklahoma	Burnham 1953
Geomys bursarius	*Mastophorus muris*	Oklahoma	Burnham 1953
Geomys bursarius	*Monocercomonoides spp.*	South Dakota	Rissky 1962
Geomys bursarius	*Hymenolepis geomydis*	Colorado	Gardner and Schmidt 1987
Geomys bursarius	*Cittotaenia praecoquis*	Wyoming	Smith 1951
Geomys personatus	*Litomosoides westi*	Texas	Pitts et al. 2000

METHODS AND MATERIALS

Trapping:

Trapping locations for target species, *G. bursarius*, *G. texensis*, *G. personatus* and *G. attwateri*, were located using museum records, and mound sightings, as well as the known ranges for each species (Schmidly and Bradley 2016). A map (Fig. 1) was created in ArcGIS using GIS data for *Geomys arenarius*, *G. attwateri*, *G. breviceps*, *G. bursarius*, *G. personatus*, and *G. texensis* obtained from the USGS National Gap Analysis Program (ESRI 2013; Gergely and McKerrow 2013). The ranges for *G. jugossicularis*, *G. knoxjonesi*, and *G. streckeri* were georeferenced from a range map in Genoways et al. (2008), Davis and Schmidly (1994), and Chambers et al. (2009), respectively. *Geomys bursarius* occurs from the Texas panhandle southward into Tom Green County. Its distribution stretches eastward and into Dallas County. Pocket gophers from the southernmost part of their western distribution were trapped in and near Ballinger in Runnels County (Fig. 1). *Geomys texensis* occurs in a narrow range with its northern boundary spanning two counties, McCulloch and San Saba, and its southern boundary stretches into Zavala County. Pocket gophers from this range were trapped in Mason and McCulloch County (Fig. 1). East of the range for *G. texensis* is the range for *G. attwateri*. This range stretches to the coast with the northernmost boundary to Milam and Robertson counties. All *G. attwateri* specimens were trapped in Milam County (Fig 1). *Geomys personatus* occurs in the southern tip of Texas, and the range for this species extends northward to touch the southern boundaries of both *G. attwateri* and *G. texensis*. Specimens of *G. personatus* were trapped mainly in Kleberg County (Fig. 1). The range for *G. breviceps* lies directly north of *G. attwateri*, and east of that of *G. bursarius*.

Pocket gophers were usually trapped on public land such as land along highways, parks, and cemeteries, but some were also trapped on private land when permission from the landowner was granted.

Gophers were collected using a combination of Baker-Williams live traps and McAbee kill-traps, largely following methods in Witmer et al. (1999). I checked traps as soon as possible, but usually no sooner than 1 hour after being set. If there was a dead pocket gopher in a trap, it was immediately placed in a zip-close bag in order to prevent contamination or loss of external parasites (Bartel and Gardner 2000). The bags were marked, and the specimens were placed on ice (Bartel and Gardner 2000). On two occasions, specimens were not immediately placed on ice, but were frozen as soon as possible (within 2 hours of original capture). If a pocket gopher was still alive in a trap, I carefully removed it from the burrow. Once the pocket gopher was removed, it was either placed in a bucket with isoflurane until heart beat and breathing were no longer detected or thoracic pressure was applied until breathing had stopped and there was no heartbeat. Specimens were handled as little as possible to minimize distress to the animal. Once dead, the pocket gophers were placed in a zip-close bag, marked, and placed on ice (Bartel and Gardner 2000). Pocket gophers were frozen until they could be prepared and examined for parasites. Pocket gophers were prepared as soon as possible, and never refrozen as refreezing can form ice crystals that could damage softer-bodied parasites such as cestodes (Pence et al. 1988).

Trapping was conducted under Scientific Research Permit No. SPR-0390-029 issued to R. Dowler through Texas Parks and Wildlife Department. Research methods followed the American Society of Mammalogists guidelines for use of wild mammals in research (Sikes et al. 2016). This research was approved by the Angelo State University Institutional Animal Care and Use Committee under approval number 16-14.

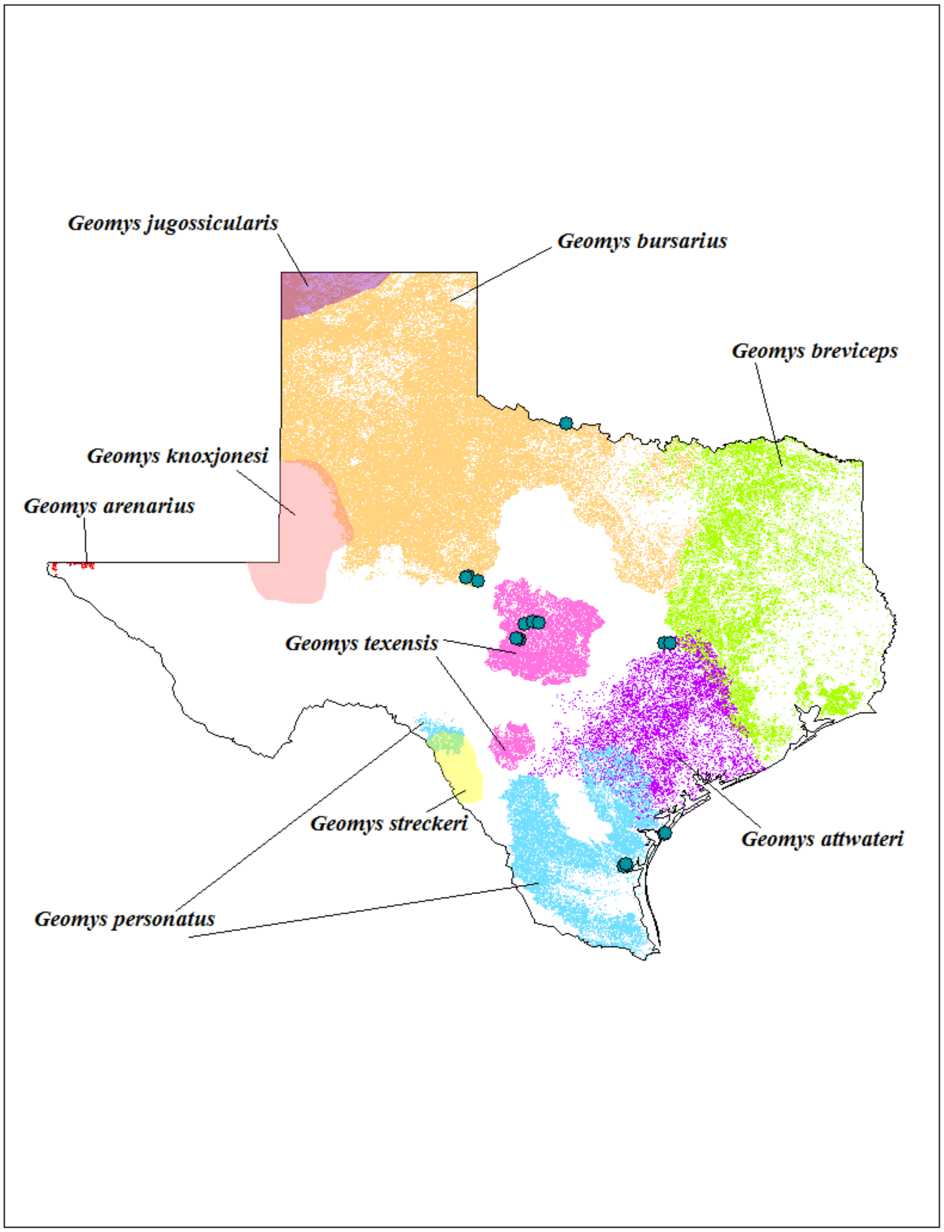

Fig. 1.–Ranges for all nine species of *Geomys* pocket gophers in Texas. Trapping locations are shown with dots.

Processing, Identification, and Analysis:

Prior to preparation as museum specimens, I brushed pocket gophers for any external parasites over a sheet of paper (Bartel and Gardner 2000). Any external parasites were placed in 70% ethanol. Pocket gophers were prepared using standard museum specimen preparation methods. Skins and skeletal material were deposited into the mammal collection of the Angelo State Natural History Collections. Heart, kidney, and liver tissue were deposited into the tissue collection of the Angelo State Natural History Collections. Nobuto strips were used to collect blood samples on some individuals.

The digestive tract of pocket gophers was removed from the esophagus to the large intestine and examined under a dissection microscope for nematode and cestode parasites. I carefully extracted any nematode parasites and placed them in a vial containing 70% ethanol for storage until identification could be confirmed (Gardner 1996). Cestode parasites were carefully removed with forceps. Special consideration was given to keeping the scolex and as much of the worm intact as possible. If the cestode was still alive, it was first killed and relaxed in hot water, then transferred to either 70% or 90% ethanol or 10% formalin for preservation. A few individuals were mounted on slides. These individuals were first stained using Semichon’s acetocarmine or hematoxylin. Nematodes were cleared using lactophenol and examined under a compound light microscope at 40x and 100x magnifications. Identification of nematode and cestode parasites was accomplished using key structures for individual families, genera, and species. For nematodes, the key in Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea by Hall (1916) was used. For cestodes, individuals were first keyed to family using a general key. The key for the family Anoplocephalidae by Douthitt (1915) was used to identify individuals further.

Parasites were examined and identified to genus. Nematodes were identified to species. Nematode parasites from each pocket gopher species were compared. Parasite load was recorded and frequency of parasitic infections among species was calculated. I conducted statistical analyses comparing the significance of any differences in parasite prevalence among sex, distribution, or species using a logistic regression, Tukey HSD was performed as a post-hoc test if differences were found. Differences in mean intensity were investigated using ANOVA. No P-value adjustments were used. All statistical analysis was performed in the open-source program R, version 3.1.2. (R Development Core Team 2016).

RESULTS

From November of 2015 through March of 2017, 4 species of pocket gophers were collected from 8 counties. *Geomys attwateri* was collected from Milam County. *Geomys bursarius* was collected from Runnels County and Wichita County. *Geomys personatus* was collected from Aransas County, Kleberg County, and Nueces County. *Geomys texensis* was collected from Mason County and McCulloch County. A total of 85 pocket gophers were collected and examined for analysis of parasites (Appendix I).

Species Richness:

I keyed nematode parasites obtained from pocket gophers to *Protospirura ascaroidea* using Hall (1916). Nematodes were found in the small intestine of all species of *Geomys* sampled.

I also found cestodes in all species of pocket gopher sampled. Cestode parasites were keyed to the families Anoplocephalidae and Hymenolepidae using Khalil et. al. (1994). Some cestodes were keyed to the genus *Monoecocestus* (Figs 2, 3) using Douthitt (1915). Key features used in identifying cestodes were the position of the ovary and testes in mature proglottids, the pattern of cirrus pouch alternation, the presence or absence of a neck, as well as size. *Monoecocestus* sp. was found in *G. attwateri*, *G. personatus,* and *G. texensis*.

Other cestodes were identified as *Hymenolepis* (Fig. 4).Two cestodes, both from the genus *Hymenolepis*, were collected from 1 *G. bursarius* specimen and 1 *G. attwateri* specimen. The 2 *Hymenolepis* sp. individuals are currently thought to be different species. A third *Hymenolepis* sp. specimen was mounted and identified by Dr. S. L. Gardner of the

University of Nebraska to be another member of the genus *Hymenolepis*. During this project, I found members of the genus *Hymenolepis* in *G. attwateri*, *G. bursarius*, and *G. texensis*.

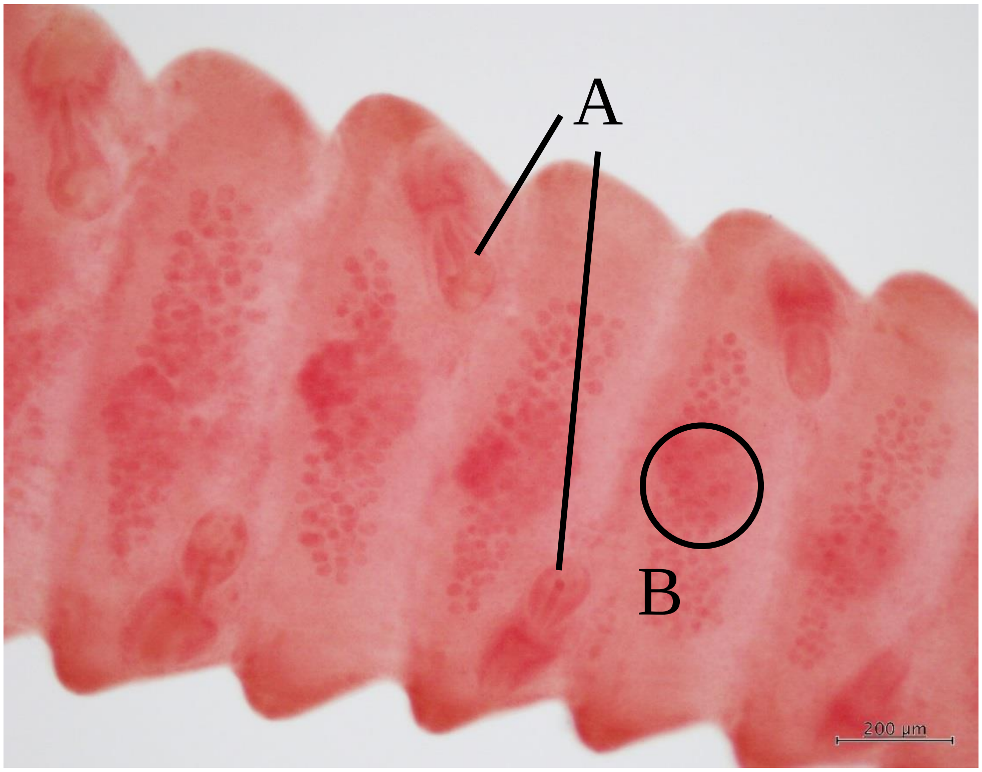

Fig. 2.—Mature proglottids of *Monoecocestus*. Alternating cirrus pouches are shown (A) partially crossing the excretory canals. Ovaries are more or less central (B) with many testes on both sides of the ovary.

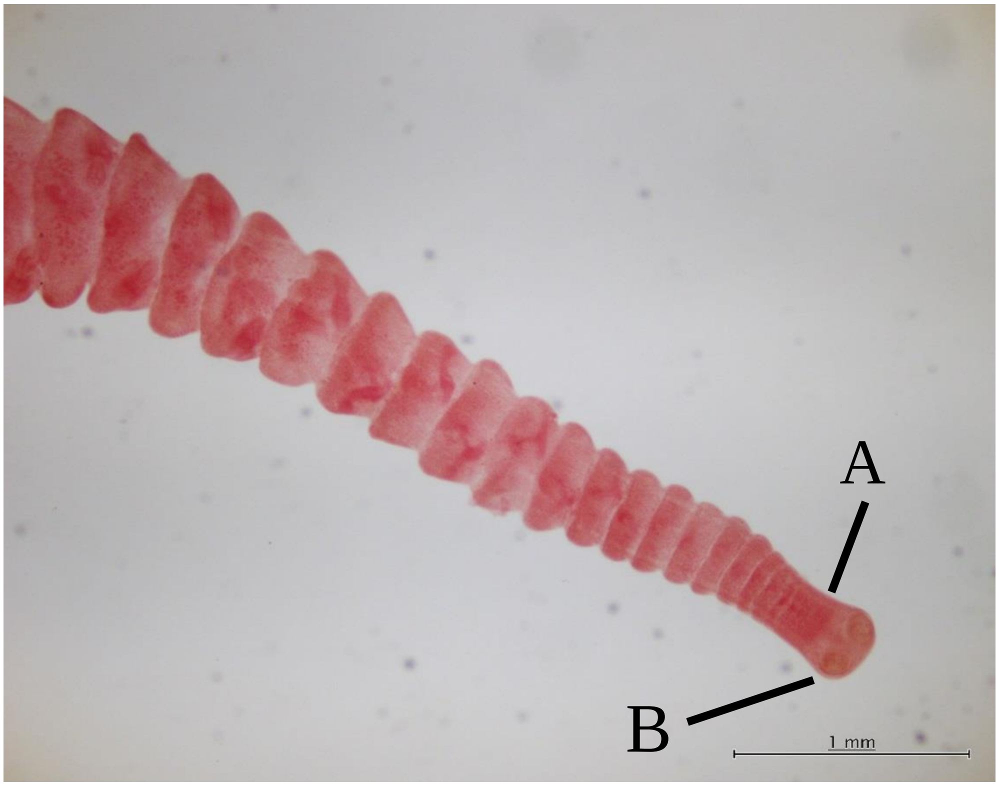

Fig. 3.— Scolex and immature proglottids of *Monoecocestus* sp. There is no neck with proglottids beginning immediately after the scolex (A). The scolex is acetabular, with well-defined suckers (B). There is no rostrum.

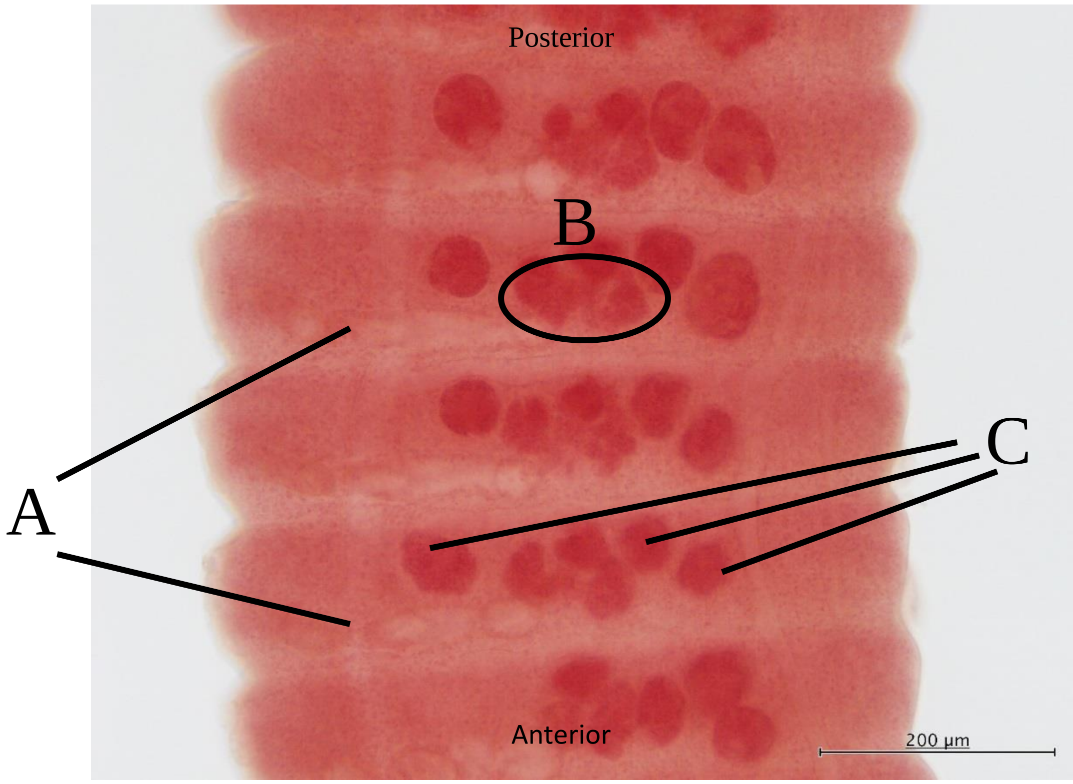

Fig. 4.— Mature proglottids of *Hymenolepis* sp. Cirrus pouches are on one side of the cestode (A). The ovary is central, and lobed (B). Testes are large (C), with two testes on one side of the ovary, and one testis on the opposite side of the ovary.

Prevalence:

I found nematode parasites in all species of *Geomys* sampled. The highest prevalence of nematodes was in *G. personatus* and the lowest was *G. bursarius.* Infections occurred in 4 of 25 (16.6%) *G. attwateri* individuals sampled, one of 20 (5%) *G. bursarius* individuals sampled, four of 19 (21.05%) *G. personatus* individuals sampled, and two of 21 (9.52%) *G. texensis* individuals sampled (Fig. 5). Using logistic regression, nematode prevalence was compared using a model to include species and sex of the host. Nematode prevalence was not statistically different among species, (Likelihood ratio= 4.482, $P>0.05$).

Cestode parasites were also found in all species. Cestode parasites were found in nine of 25 (36%) *G. attwateri* individuals, ten of 20 (50%) *G. bursarius* individuals, seven of 19 (36.8%) *G. personatus* individuals, and two of 21 *G. texensis* individuals (9.52%) (Table 3). Using logistic regression, cestode prevalence was compared using a model to include species and sex of the host. A statistical difference among species was detected, (Likelihood Ratio= 9.4744, $P=0.02361$). Using a pairwise logistic regression with a Bonferroni, it was determined that cestode prevalence was statistically different between *G. bursarius* and *G. texensis*, *P* adj.= 0.0378, but no other statistical difference was detected. Very few individuals were infected by both nematodes and cestodes. Total prevalence (nematodes and cestodes) was 44.33% for *G. attwateri*, 55% for *G. bursarius*, 42.1% for *G. personatus*, and 14.28% for *G. texensis*.

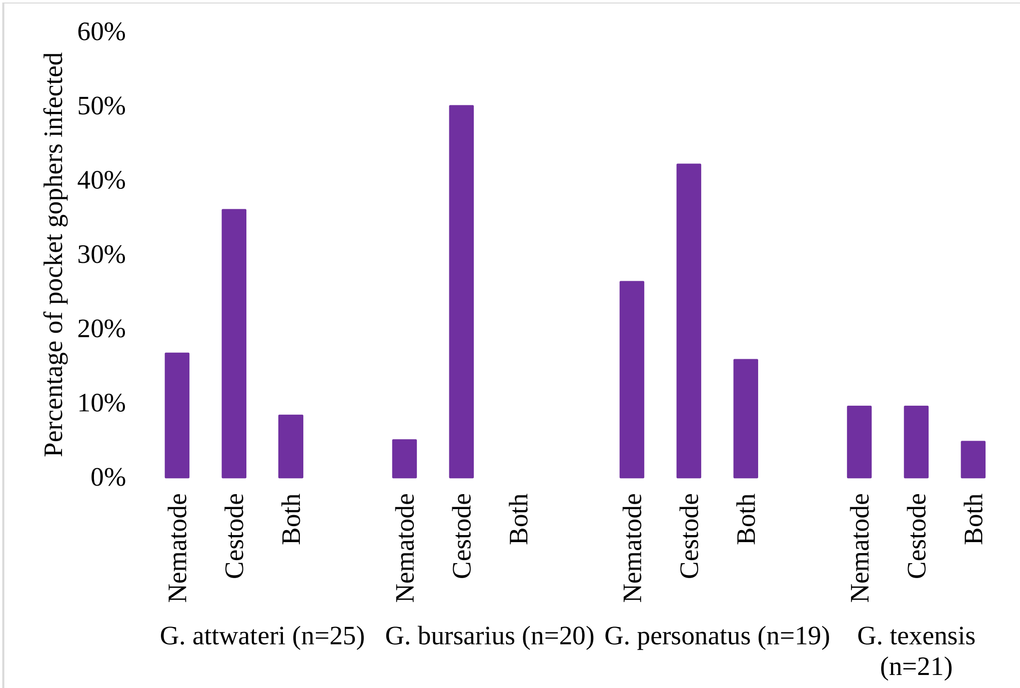

Fig. 5.— Prevalence data for nematodes and cestodes for all four species of *Geomys* pocket gophers sampled (n=85). Nematode and cestode prevalence is defined as the percentage of pocket gophers of a given species infected with a nematode or cestode parasite. A third bar, 'Both', is used to display the percentage of pocket gophers of a given species infected with both nematode and cestode parasites.

Intensity:

Mean intensity, or the average number of individuals collected in infected hosts, was calculated for nematodes. The mean intensity was highest in *G. texensis* (44, n=2), followed by *G. personatus* (8.25, n=4). Mean intensity for *G. attwateri* was 4.75 (n=4). Mean intensity was the lowest for *G. bursarius* (1, n=1). Mean intensity for all female gophers was 7.57 (n=58). For all male gophers, mean intensity was 22 (n=21). Using an ANOVA, it was determined that mean intensity did differ significantly between male (n= 4) and female (n= 7) gophers, (P<0.05, df_1, df_2), with males having a higher parasite load than females when all species were combined. Intensity was also compared among species using an ANOVA. Nematode intensity did not differ among species, (P>0.05, df_1, df_2).

DISCUSSION

Species Richness:

For the 4 species of pocket gopher sampled, I identified 1 species of nematode and 2 genera of cestode. The nematodes, *P. ascaroidea*, had not been previously reported in any pocket gopher species from Texas. Hall (1916) described *P. ascaroidea* from *G. breviceps* collected in Oklahoma, but *P. ascaroidea* in *G. personatus*, *G. texensis*, *G. attwateri*, and *G. bursarius* represent four new host records. *Protospirura ascaroidea* has been reported, however, in the East Texas cotton rat, *Sigmodon hispidus* (Chandler and Suttles 1922). My study involved surveying pocket gophers from as far west as Runnels County. While the life cycle for *P. ascaroidea* has not been well studied, life cycles for other *Protospirura* species, such as *P. numidica*, require the eggs to be directly ingested by the host (Crook and Grundmann 1964). Pocket gophers could become infected by *P. ascaroidea* by ingesting the eggs while digging or grooming.

I identified cestodes from the genus *Hymenolepis* in *G. attwateri*, *G. bursarius*, and *G. texensis*. Prior to this study, only 1 species of cestode, *Monoecocestus centroovarium,* had been reported in *Geomys attwateri,* making *G. attwateri* a new host record for the genus *Hymenolepis*. *Geomys texensis* also represents a new host record for *Hymenolepis*. The second genus of cestode I identified, *Monoecocestus*, has previously been reported in *G. bursarius*, but not in *G. texensis*, or *G. personatus*, making both pocket gopher species new host records for this parasite species (English 1932; Burnham 1953; Bartel and Gardner 2000). The genus *Hymenolepis* has previously been reported in *G. bursarius*, but only in Minnesota (Bartel and Gardner 2000).

Hymenolepis diminuta, a well-studied member of the genus *Hymenolepis*, has a life cycle that could include pocket gophers. Adults of *H. diminuta* are found in the small intestine of rodents. Eggs are shed in feces and consumed by an arthropod intermediate host, usually a beetle. Intermediate stages develop in arthropod hosts, until the arthropod intermediate host is consumed by a rodent; inside this host, the cestode develops into a sexually mature adult (Bush et al. 2001). It is likely that pocket gophers are becoming infected by accidentally consuming arthropods.

The intermediate host for *Monoecocestus* and many other Anoplocephalids is a mite. Intermediate stages develop in the mite, and the mite is consumed by the definitive host, in this case, a pocket gopher. The mite intermediate host for *Monoecocestus* is usually in the family Oribatulidae or Galumnatidae, or the soil mites (Melvin 1952). It is likely that pocket gophers are becoming infected with *Monoecocestus* when they accidentally consume mites in the soil.

Prevalence and Intensity:

Gardner (1985) studied endoparasites of the geomyid, *Thomomys bulbivorus*, and reported significant differences between two study sites in prevalence of cestodes and nematodes with 80% of 25 pocket gophers being infected with a helminth at one site and 20% of 48 being infected at another site. In my study, prevalence for *Protospirura ascaroidea* varied from 5% to 21.05% among four species of *Geomys*. For all helminth parasites in my study, total prevalence ranged from 14.28% to 55% among pocket gopher species. In this study, nematode prevalence and intensity did not differ significantly between species. Nematode intensity seemed extremely high for *G. texensis* (44), but that was because one male was infected with 84 *P. ascaroidea* individuals. This particular male had the highest number of nematode parasites of any host in this study. Only 2 of 21 individuals of the species *G. texensis* were infected with nematode parasites. The second infected individual was a female with only 4 nematode parasites. Many hosts were not infected with a high number of nematode parasites. Only one nematode parasite was collected in each of 2 *G. attwateri* hosts, 1 *G. personatus* host, and 1 *G. bursarius* host. These 4 individuals represented 36.36% of infected pocket gophers collected. Without a larger sample size, it is difficult to attribute the high number of worms in the infected male *G. texensis* to any one factor.

The difference in intensity between male and female gophers, not taking species into consideration, was statistically significant. This could be due to a small sample size and male gophers being underrepresented in this study. For example, Williams and Cameron (1990) reported equal sex ratio of males and females (n=406) in a study of *G. attwateri*. In my study, 76% of *G. attwateri* individuals were female (n=25). Pitts et al. (2005) reported a female biased *G. bursarius* population, at 60% female (n=691). In this study, 85% of *G. bursarius* individuals were female (n=20). Sex ratios have not been previously reported or *G. texensis* or *G. personatus*. In this study, 71% of *G. texensis* individuals collected were female (n=21), and 68% of *G. personatus* individuals collected were female (n=19).

Limitations:

This study had several limitations. First, it was difficult to regularly trap pocket gophers. Pocket gophers were collected during different parts of the year from October-March and over a period of two years. It was also difficult to necropsy pocket gopher hosts in a timely manner, so pocket gophers were frozen until they could be processed. If possible, parasites should be extracted soon after the host has expired, while the parasites are still alive, as freezing may damage parasites and make key structures difficult to identify (Shoop et al. 1987; Gardner 1996). However, Pence et al. (1988) suggested that the use of frozen hosts does not adversely affect parasite identification in many cases. Many hosts in this study were prepared within several months of initial collection. Some hosts, however, were frozen for an extended period of time, allowing for the degradation of more fragile parasites, especially cestodes.

The number of hosts sampled was also a limitation for this study. Many other studies involving pocket gophers include several hundred host specimens (Williams and Cameron 1990; Pitts et al. 2005). One reason sample size was low was the difficulty and cost to effectively sample across the range of the 4 species. In any given area, only one species in the genus *Geomys* can be encountered and collected. For these reasons, I attempted to collect 20 pocket gophers per species. In all cases except one, that sample size was met or surpassed. Only 19 *G. personatus* individuals were able to be collected and prepared.

Future Research:

Only 4 of the 8 species of *Geomys* that can be found in Texas were sampled. In the future, all 9 species should be sampled. This research could even be expanded to include all twelve species of pocket gopher in the genus *Geomys*. Additional individuals could be caught and necropsied to improve the sample size and power of the dataset, and more thorough parasite identification could be done. With a larger sample size, habitat influence on prevalence and intensity could also be addressed. Lastly, genetic research seems to be at the forefront of modern parasite-host relationship studies. It seems pocket gophers are becoming infected with parasites that generally utilize some sort of arthropod as an intermediate host. In order to better understand pocket gopher involvement in parasitic life cycles, molecular diet studies could be used to determine whether pocket gophers are actually ingesting arthropods. Additionally, despite recent studies of geomyid phylogeny, it would be informative to continue to sequence pocket gopher hosts and their nematode and cestode parasites in order to better understand patterns in both host and parasite phylogenies, and how they might relate to each other (Chambers et al. 2009). While all nematodes collected in my study are thought to be of the same species, there is a possibility that there are subtle genetic differences in the parasites that align with those differences in their pocket gopher hosts. With the emergence of new sequencing techniques and databases for genetic sequences, it would be helpful to future research endeavors to expand efforts to sequence parasite DNA. Having this information available would not only help in identification, but also help to answer questions regarding additional host-parasite relationships.

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APPENDIX I

Pocket gopher specimens from four species, *Geomys attwateri*, *G. bursarius*, *G. personatus*, and *G. texensis*, deposited in the Angelo State Natural History Collection and used in analysis of endoparasites.

Geomys attwateri (n=25) TEXAS, Milam County; 1 miles west, 0.8 miles south of Minerva, Texas, 5 A Ranch, 30.746531°, -97.003137°: ASNHC: 18009, 18010, 18011, 18012, 18015, 18016, 18017, 18018, 18019, 18022, 18024, 18025, 18026, 18027, 18286, 18287, 18288, 18289, 18290, 18291, 18292, 18293, 18294, 18295, 18296.

Geomys bursarius (n=20) TEXAS, Runnels County; about 2 miles southwest of Ballinger on Hwy 67, 31.756944°, -100.171388°: ASNHC 18298, 18299, 18304; 31.779444°, -100.120833°: ASNHC 18301, 18302, 18305, 18306; 31.71032°, -99.96924°: ASNHC 18307; 31.71063°, -99.96905°: ASNHC 18303; 31.70017°, -99.98635°: ASNHC 18300; 31.708282°, -99.970946°: ASNHC 18297; TEXAS, Wichita County; northwest of Burkburnett, 3326 Bohner Road, The Flying Horseman Ranch, 34.13867°, -98.61729°: ASNHC 18308, 18309, 18310, 18311, 18312, 18313, 18314, 18315, 18316.

Geomys personatus (n=19) TEXAS, Nueces County; Palmilla Beach Golf Course near Junction of Access Road and Highway 361, 27.796671°, -97.089461°: ASNHC 18317, 18319, 18320; Palmilla Beach Golf Course by road on Hwy 361, 27.801914°, -97.683992°: ASNHC 18318; Port Aransas, 27.799512°, -97.088331°: ASNHC 18332; Port Aransas, 27.802443°, -97.085910°: ASNHC 18333, 18334; Port Aransas, 27.802443°, -97.085910°: ASNHC 18335; Hwy 771, 0.1 mile west of junction of 2510, 27.29599°, -97.73193°: ASNHC 18326; Riviera, F.M. Road 771: ASNHC 18323, 18331; Riviera RV Park, 0.05

miles from the junction of Hwy 771, 27.28884°, -97.67751°: ASNHC 18328; Riviera Beach RV and Mobile Home Park, 27.288754°, -97.677755°: ASNHC 18321; Hwy 771 at Coral Lane, 27.320518°, -97.685941°: ASNHC 18324, 18325, 18327; Riviera, 0.25 miles east of the junction of C. R. 1120 on Hwy 771, 27.29515°, -97.72347°: ASNHC 18329; Riviera, Junction of C. R. 1105 on Hwy 771, 27.2500°, 97.76124°: ASNHC 18322, 18330.

Geomys texensis (n=21) TEXAS, Mason County; Junction of Route 29 and Red Lane, 30.82498°, 99.38603°: ASNHC 18339; Route 29, 30.81189°, -99.36397°: ASNHC 18004, 18006, 18020, 18021; Route 29 and Turkey Springs, 30.80261°, -99.34010°: ASNHC 18007, 18023, 18336, 18337; Union Bane Cemetery, Hwy 71, 31.038611°, -99.256111°: ASNHC 18338, 18340, 18341; TEXAS, McCulloch County; TX 71, 31. 085555°, -99.132222°: ASNHC 18008, 18013, 18014, 18342, 18343, 18344, 18345, 18346, 18347.

APPENDIX II

Parasite material deposited in the University of Nebraska State Museum Systematics Research Collections under accession P-2017-019 and P-2017-059. HWML numbers refer to the parasite specimen number in the University of Nebraska State Museum Systematics Research Collections. ASK numbers refer to the host specimens deposited in the Angelo State Natural History Collection. ASNHC numbers refer to that hosts catalog number in the Angelo State Natural History Collection.

	HWML	ASK	ASNHC	Host species	State	County
Accession# P-2017-019						
Cestodes						
	99780	12567	18289	*G. attwateri*	Texas	Milam
	99781	12568	18290	*G. attwateri*	Texas	Milam
	99784	12630	18306	*G. bursarius*	Texas	Runnels
	99785	12653	18315	*G. bursarius*	Texas	Wichita
	99786	12659	18319	*G. personatus*	Texas	Aransas
	99787	12686	18335	*G. personatus*	Texas	Aransas
Nematodes						
	99777	12519	18012	*G. attwateri*	Texas	Milam
	99779	12528	18021	*G. texensis*	Texas	Mason
	99782	12572	18330	*G. personatus*	Texas	Kleburg
	99783	12579	18300	*G. bursarius*	Texas	Runnels
Accession# P-2017-059						
Cestodes						
	110110	12532	18025	*G. attwateri*	Texas	Milam
	110111	12567	18289	*G. attwateri*	Texas	Milam
	110112	12570	18292	*G. attwateri*	Texas	Milam
	110113	12571	18322	*G. personatus*	Texas	Kleberg
	110114	12573	18331	*G. personatus*	Texas	Kleberg
	110115	12574	18293	*G. attwateri*	Texas	Milam
	110116	12578	18299	*G. bursarius*	Texas	Runnels
	110117	12632	18340	*G. texensis*	Texas	Mason
	110118	12634	18325	*G. personatus*	Texas	Kleberg
	110119	12636	18309	*G. bursarius*	Texas	Wichita
	110120	12638	18311	*G. bursarius*	Texas	Wichita
	110121	12639	18312	*G. bursarius*	Texas	Wichita

	110122	12643	18329	*G. personatus*	Texas	Kleberg
	110123	12654	18317	*G. personatus*	Texas	Aransas
	110124	12684	18295	*G. attwateri*	Texas	Milam
Nematodes						
	110126	12524	18017	*G. attwateri*	Texas	Milam
	110127	12567	18289	*G. attwateri*	Texas	Milam
	110128	12641	19327	*G. personatus*	Texas	Kleberg
	110129	12643	18329	*G. personatus*	Texas	Kleberg
	110130	12686	18335	*G. personatus*	Texas	Aransas

APPENDIX III

ANGELO STATE UNIVERSITY

College of Graduate Studies & Research

Institutional Animal Care & Use Committee

30 November 2017

Dr. Robert C. Dowler/Kaitlynn LeBrasseur
Department of Biology
Angelo State University
San Angelo, TX 76909

Dear Dr. Dowler and Ms. LeBrasseur:

This letter is to confirm that your proposed project titled, "Endoparasite comparisons among Texas pocket gopher species, (genus *Geomys*)." was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 1 January 2016 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on 15 August of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 16-14. Please include this number in the subject line of all future communications with the IACUC regarding the protocol.

Sincerely,

Steven T. Brewer, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee

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