PREVALENCE AND INTENSITY OF THE SINUS ROUNDWORM, *SKRJABINGYLUS CHITWOODORUM*, IN RABIES-NEGATIVE SKUNKS OF TEXAS

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ABSTRACT

The sinus roundworm, *Skrjabingylus chitwoodorum,* occurs in North American skunks (Mephitidae). Estimates of its distribution and prevalence have been largely based on the inspection of skunk skulls showing damage from *Skrjabingylus* infections. I examined 595 striped skunks (*Mephitis mephitis*) and 5 hog-nosed skunks (*Conepatus leuconotus*) that tested negative for rabies by the Texas Department of State Health Services between November 2010 and April 2015 to determine species of *Skrjabingylus*, prevalence and intensity of infection, and distribution in Texas by county. Prevalence of *S. chitwoodorum* in striped skunks was 48.7%. Mean intensity was 19.4 with a range from 1-181 nematodes. There was a left side bias in the host sinuses. Neither intensity nor prevalence of infections varied among ecoregions of Texas. The high prevalence of *Skrjabingylus* in rabies-negative skunks supports the hypothesis that behavioral changes due to skrjabingylosis may be responsible for the submission of many skunks for rabies testing.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS....................ii
ABSTRACT....................iv
TABLE OF CONTENTS....................v
LIST OF TABLES....................vi
LIST OF FIGURES....................vii
INTRODUCTION....................1
MATERIALS AND METHODS....................7
RESULTS....................10
DISCUSSION....................18
LITERATURE CITED....................22

LIST OF TABLES

Page

Table 1. Known prevalence of *Skrjabingylus chitwoodorum* in *Mephitis mephitis* from various regions of North America..3

LIST OF FIGURES

Page

Figure 1. Map of 12 ecoregions of Texas and counties sampled............................9

Figure 2. Intensity of infection ranged from 1-181 *Skrjabingylus chitwoodorum* per *Mephitis mephitis* host...11

Figure 3. A *Mephitis mephitis* skull infected with *Skrjabingylus chitwoodorum* and an uninfected *Mephitis mephitis* skull..12

Figure 4. Gubernaculum and copulatory bursa of *Skrjabingylus chitwoodorum*..13

Figure 5. Spicules of *Skrjabingylus chitwoodorum*..14

Figure 6. Copulatory bursa of *Skrjabingylus chitwoodorum*................................15

Figure 7. Prevalence of *Skrjabingylus chitwoodorum* in *Mephitis mephitis* by Texas ecoregion...17

INTRODUCTION

Skrjabingylus is a genus of nematode in the family Metastrongylidae that inhabits the sinus cavities of certain mustelids and mephitids as adults. Members of this genus have been described as "stout red nematodes" with transparent body walls (Lankester 1983). Four of the six species are known to occur in North America: *Skrjabingylus lutrae, S. santaceciliae, S. nasicola* and *S. chitwoodorum* (Lankester 1983; Carreno et al. 2005; Santi and Parker 2012). *Skrjabingylus lutrae* can be found in the river otter, *Lontra canadensis*; *S. nasicola* occurs in weasels, mink and other mustelids; and *S. santaceciliae* and *S. chitwoodorum* occur in skunks. *Skrjabingylus nasicola* was first described by Leuckart in 1842 in *Mustela vison* and is known to infect several species of *Mustela* (Santi and Parker 2012). *Skrjabingylus santaceciliae* was described by Carreno et al. in 2005 in the hooded skunk, *Mephitis macroura.* Hill (1939) first described *Skrjabingylus chitwoodorum* in striped (*Mephitis mephitis*) and spotted skunks (*Spilogale putorius*) from Oklahoma. Based on measurements and morphological features, *Skrjabingylus chitwoodorum* has since been reported from skunks in Ontario (Lankester 1983), Quebec (Webster 1965), California (Mead 1963), Illinois (Levine et al. 1962), Kansas (Ewing and Hibbs 1966), Minnesota (Fuller and Kuehn 1984), New York (Goble and Cook 1942), North Dakota (Dyer 1969) and Pennsylvania (Maldonado and Kirkland 1986). Two studies of endoparasites of skunks, the first in 1946 and the more recent in 2006, reported skull damage attributed to *S. chitwoodorum* in *M. mephitis* and *Spilogale gracilis* from Texas (Tiner 1946; Neiswenter et al. 2006).

Journal of Wildlife Disease

To my knowledge, no study using the actual presence of the worms has established how common skrjabingylosis is among Texas skunks, nor has anyone examined the nematodes closely to confirm the species of *Skrjabingylus* occurring here. Because the two species of roundworms resemble one another, they have often been misidentified or simply referred to as a "*Skrjabingylus* sp." in literature (Lankester 1983). *Skrjabingylus chitwoodorum* can be distinguished from the two other North American species by their longer bodies and the size and morphology of the copulatory spicules (males)—which may be up to four times longer than those of *S. nasicola* (Lankester 1983). The original species description of *S. chitwoodorum* (Hill 1939) from skunks (*M. mephitis* and *S. putorius*) in Oklahoma reported spicules ranging from 540-710 μm, whereas Lankester's 1983 species redescription from Canadian *M. mephitis* reported spicule lengths between 800-890 μm. Webster's (1965) description of *S. magnus* (placed in synonymy with *S. chitwoodorum* by Lankester 1983) from *M. mephitis* also from Canada reported spicule lengths ranging from 688-866 μm. A Minnesota study on *M. mephitis* reported spicule lengths of *S. chitwoodorum* ranging from 690-815 μm (Fuller and Kuehn 1984).

Much of today's knowledge about the distribution of *Skrjabingylus* in skunks in North America comes from research conducted using cleaned and dried skulls (Table 1). For weasels, Dougherty and Hall (1955) concluded *S. nasicola* was widespread across North America based on skull damage they observed in weasel specimens, and Lankester (1983) suggested *S. nasicola* has a "worldwide" distribution. Kirkland and Kirkland (1983) examined the crania of 3,055 striped skunks, *Mephitis mephitis*, from 42 museum collections across the United States and Canada. Based on the presence of bone damage, degradation or other signs of nematode infections, they concluded the range of *Skrjabingylus* spans most, if

Table 1. Known prevalence of *Skrjabingylus chitwoodorum* in *Mephitis mephitis* in North America.

Location	Prevalence of *S. chitwoodorum* in *M. mephitis*	Average Intensity	Sample Size of *Mephitis*	Method of Determination	Study
Mexico	73%	-	67	Examined cleaned, dried crania	Kirkland and Maldonado (1988)
United States					
California	20%	-	15	Nematode presence	Mead (1963)
Illinois	1.1%	4	184	Nematode presence	Levine et al. (1962)
	65.9%	-	164		Verts (1967)
Kansas	32%	21	117	Nematode presence	Ewing and Hibbs (1966)
Minnesota	75%	-	88	Examined cleaned, dried crania	Fuller and Kuehn (1984)
	85.7%	131.83	7	Nematode presence (subset of 7)	
New York	72%	-	25	Nematode presence	Goble and Cook (1942)
North Dakota	12%	19	25	Nematode presence	Dyer (1969)
Oklahoma	100%	-	2	Nematode presence	Hill (1939)
Pennsylvania	82.7%	-	127	Examined cleaned, dried crania	Kirkland (1975)
	50%	12.25	16	Nematode presence	Maldonado and Kirkland (1986)
Texas	48.7%	19.4	595	Nematode presence	This study

not all, of North America. They estimated 72.2% of the 619 *M. mephitis* from the Great Plains region of their study (which included Texas) had lesions attributable to skrjabingylosis. A study on museum specimens from Mexico suggested a high percentage (73%) of *M. mephitis* were infected with *Skrjabingylus* (Kirkland and Maldonado 1988).

In addition to resembling one another, *S. nasicola* and *S. chitwoodorum* follow similar routes to the frontal sinuses of their hosts (Lankester and Anderson 1971). Definitive hosts become infected upon ingesting invertebrates or small vertebrates housing infective third-stage larvae (Santi and Parker 2012). Several species of terrestrial gastropods serve as intermediate hosts and a range of small vertebrate species (e.g. mice, shrews, frogs) are viable paratenic hosts (Lankester and Anderson 1971). First-stage larvae are shed in the infected animal's feces and then penetrate into terrestrial gastropods. They then undergo two molts to reach their infective (third-stage) larval form (Hansson 1967).

Upon ingestion, the larvae penetrate the wall of the host’s stomach and intestine, undergo two additional molts in rapid succession, and the now fifth-larval stage worms can migrate to the peritoneal cavity as soon as four days post-infection (Lankester and Anderson 1971). Next, they perforate the muscles of the abdominal wall to reach and follow the perineurium of localized nerves in the vertebral column of the host to the sinuses. They can reach the sinuses as soon as six days post-infection (Santi and Parker 2012).

The migration and presence of the worm can cause noticeable deformations (e.g. bulges and lesions) and/or discoloration in the sinuses, but these are usually only observed postmortem. Dyer (1969) reported two skunks with skrjabingylosis had noticeable protrusions over the frontal sinuses ante mortem. The presence of the nematodes has been shown to cause the frontal sinuses to expand in striped skunks, which could cause the roof of the braincase to exert pressure on the brain (Maldonado and Kirkland 1986). It has been hypothesized that hosts may exhibit abnormal behavior caused by the added pressure to the brain (Maldonado and Kirkland 1986; Bowman and Tamlin 2007). The worms' migration might also result in neurological problems for their host, especially if errors are made during migration (Santi and Parker 2012).

The effects of the parasite infection on the brain of skunks and their behavior are still not understood completely. Two records exist of *S. chitwoodorum* "found on the brain surface of skunks"; both were part of rabies research projects (Levine et al. 1962). One of these reports stated that the "antemortem behavior" suggested the individual was rabid, but it was found to be negative after testing for presence of antibodies to the rabies virus. (Ewing and Hibbs 1966). Based on the few descriptions of behavior of infected individuals, skrjabingylosis symptoms are thought to be similar to those of mercury poisoning, which have been recorded in mink (Ewing and Hibbs 1966; Wobeser 1976). Experimentally infected skunks showed signs of lethargy, lack of motor coordination, and suffered brain hemorrhaging (Lankester 1970). Lankester and Anderson (1971) suggested the migration of *S. chitwoodorum* can cause neurological diseases in their hosts and in an experiment in which striped skunks were given infective larvae of *S. chitwoodorum*, three out of six animals showed neurological disturbance and one experienced partial paralysis 22 days after infection.

Studies of *Mephitis* skulls from museum collections have shown greater damage tended to occur on the left side of the cranium (Kirkland and Kirkland 1983). Age has been shown to be an important factor on host infection status; older skunks had more severe lesions and higher frequencies of cranium damage than younger individuals in multiple studies (Kirkland 1975; Kirkland and Kirkland 1983; Fuller and Kuehn 1984; Kirkland and Maldonado 1988). Older animals would have higher likelihoods of being exposed to infected intermediate or paratenic hosts and thus are much more likely to become a host than a younger individual (Kirkland 1975).

Examination of museum specimens has proven to be a useful resource for determining whether an individual was infected with *Skrjabingylus* or not, but cranial damage does not reveal the species of *Skrjabingylus*. Maldonado and Kirkland (1986) used museum and fresh specimens to conclude inspecting cleaned and dried crania is a reliable method for determining infection status. Goble and Cook (1942) re-examined cleaned skulls of mink from their study, and none of the four infected individuals had enlarged or damaged sinuses while some of the uninfected individuals did have bulging sinuses. Thus, there is some disagreement as to the reliability of using museum specimens for determining prevalence of *Skrjabingylus* infections, but many studies have use one or both methods (Table 1).

Because a large number of skunks are submitted annually for rabies testing in Texas, rabies-negative skunk heads were salvaged from the Texas Department of State Health Services between November 2010 and April 2015 and examined for *Skrjabingylus*. I undertook this study to determine the incidence, intensity, and geographic distribution of *Skrjabingylus* in the state. In addition, I hoped to verify the identity of species infecting skunks in Texas.

MATERIALS AND METHODS

Frozen heads of six hundred skunks determined to be negative for the rabies virus were received from the Texas Department of State Health Services after rabies testing. Each head had the braincase excised and the brain removed. Access to sinuses was available because of the partial removal of the top portion of the cranium. Both portions of the cranium were examined for nematodes in this study. Data on hosts included collection date and location (county). During examination of each individual, nematodes were extracted and preserved in either formalin, ethanol and/or were frozen. Of these, 595 were striped skunks, *M. mephitis*, and 5 were hog-nosed skunks, *C. leuconotus*. Host vouchers were deposited in the Angelo State Natural History Collection of Mammals after being cleaned in a dermestid beetle colony.

I use the terms prevalence (the percentage of hosts infected with that species/total number of hosts examined) and intensity (number of nematodes/host) in accordance with definitions outlined by Bush et al. (1997). For 215 specimens, I recorded the intensity of infection, and for 167 specimens, I recorded the number of nematodes extracted from each sinus side. Early specimens in the study were just scored as to presence or absence of *Skrjabingylus*.

To test the accuracy of determining nematode infestation based on dried crania, I reexamined 80 skulls from this study and attempted to identify the infected individuals based on degradation and bone damage. Determinations were made without knowledge of *Skrjabingylus* infections. Following the procedure outlined by Kirkland and Kirkland (1983) to assign age classes, I reexamined 80 skulls after they had been cleaned and dried and assigned each specimen to one of eight relative age classes. Based on the presence of lesions, discoloration, and bulging, I attempted to independently ascertain whether an individual had been infected with *Skrjabingylus* or not.

Species of *Skrjabingylus* were identified using spicule measurements and images from Lankester (1983). Nematodes from each county in Texas were cleared using lactophenol and examined using an Olympus microscope (Olympus America Corporation, Center Valley, Pennsylvania, USA) equipped with an OMAX camera (OMAX Corporation, Bucheon-si, Gyeonggi-do, Korea). Copulatory bursa, gubernaculum, and/or spicules for some adult males from each county were photographed; when possible, measurements of spicules or the gubernaculum were obtained using the image-processing program ImageJ (2012, version 1.49; Wayne Rasband, National Institute of Health, USA). I was able to measure the complete spicules of 49 nematodes from Texas.

Griffith et al. (2007) recognized twelve ecoregions of Texas (Fig. 1). Because our collection locality data were limited to county, and because some counties could be divided into multiple ecoregions, I assigned 16 additional ecoregion names for split counties, producing a total of 28 ecoregions. Of these, 7 ecoregions had a sample size less than 5 and had to be removed for statistical analysis, resulting in 21 ecoregions.

Statistical analyses were performed using R (2014 edition, version 0.98.1091; RStudio, Inc., Boston, Massachusetts, USA). A paired t-test was used to determine if a bias existed among sinus sides. I performed randomization tests using generalized linear models to test the effects of age and ecoregion on infection status (prevalence). A permutational ANOVA with 1,000 iterations was performed to compare intensity across ecoregions.

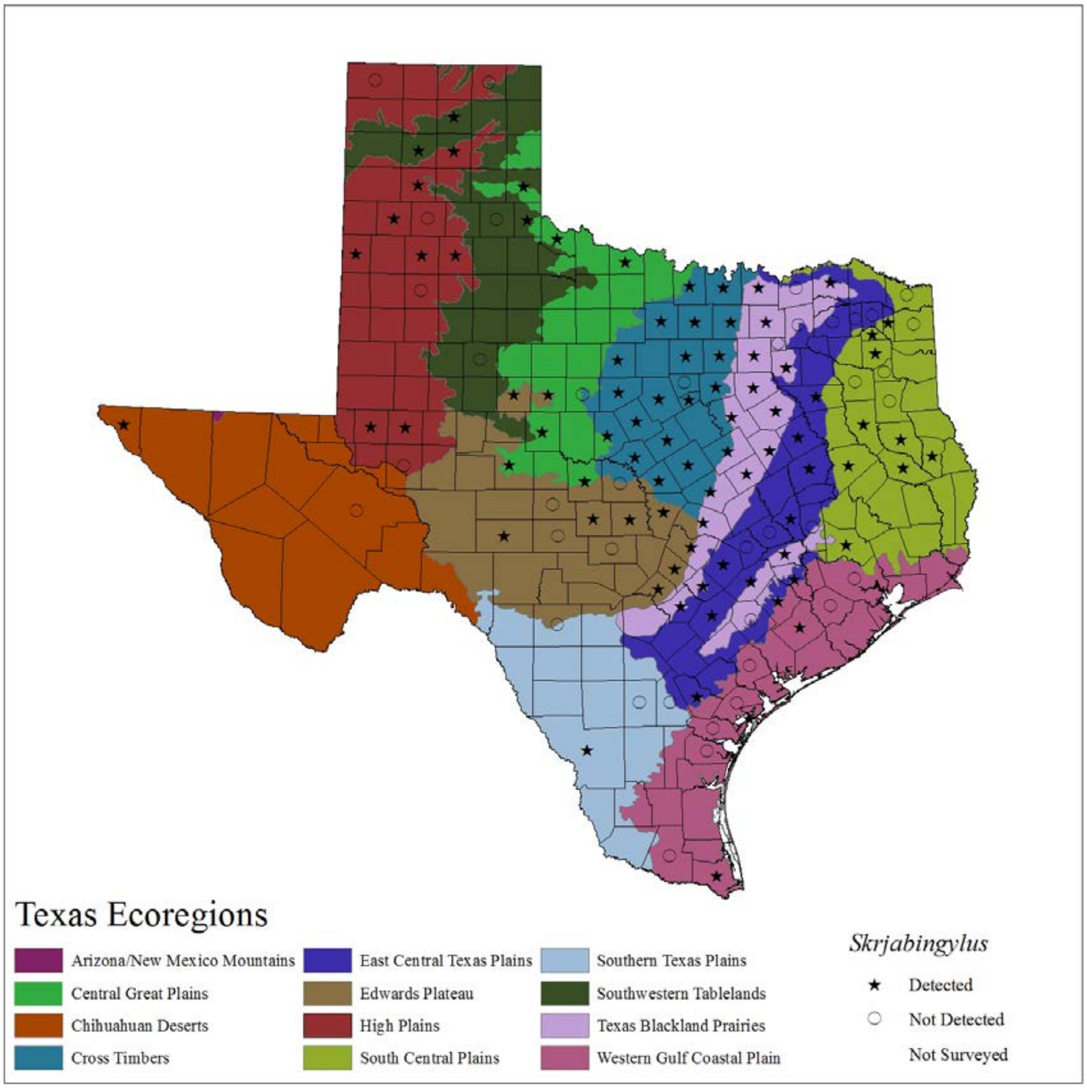

Figure 1. Map of ecoregions of Texas modified from Griffith et al. (2007). Counties sampled in this study are displayed by stars (*Skrjabingylus chitwoodorum* detected) or open circles (*S. chitwoodorum* not detected).

RESULTS

Two hundred and ninety of the 595 *M. mephitis* from Texas, 48.7%, were infected with *S. chitwoodorum*. None of the five *C. leuconotus* inspected had *Skrjabingylus* infections. Eighty-four of the 124 counties sampled in Texas had skunks positive for *Skrjabingylus* (Fig.1).

After tissues had been removed, an infected individual could usually be distinguished by discoloration of the skull. Infections were often apparent even before removing the skull cap; *Skrjabingylus* can often be seen through the bone. Typically, the higher the intensity of infection, the darker the mass under the bone appeared. The intensity varied from one to one hundred eighty-one nematodes per host ($\bar{X}$=19.64, SE=1.79) (Fig. 2). Individual nematodes were sometimes recovered outside the sinus cavities. Some were found in the trachea and mouth and in one case, *Skjrabingylus* was recovered from the eye. One skunk had a lesion with a mass of *Skrjabingylus* in the diastema between the canine and first premolar. These could represent the movement of nematodes after death of the host.

After cleaning, I reexamined 80 specimens to try to determine infection status based on skull damage or discoloration (Fig. 3). None of the infected individuals were thought to be negative during this assessment, but many uninfected individuals appeared to have signs of skrjabingylosis. My success rate was 41% (33 were correctly scored).

Spicule length (Fig. 4) for the 49 males sampled averaged 677.5 ± 84 µm (range = 299-871 µm). Gubernaculum measurements and copulatory bursa morphology were also indicative of *Skjrabingylus chitwoodorum* (Figs. 4 and 5). A paired t-test revealed a bias toward the left sinus side (t = 2.1666, df = 166, P-value=0.03169; means: 11.16>10.16).

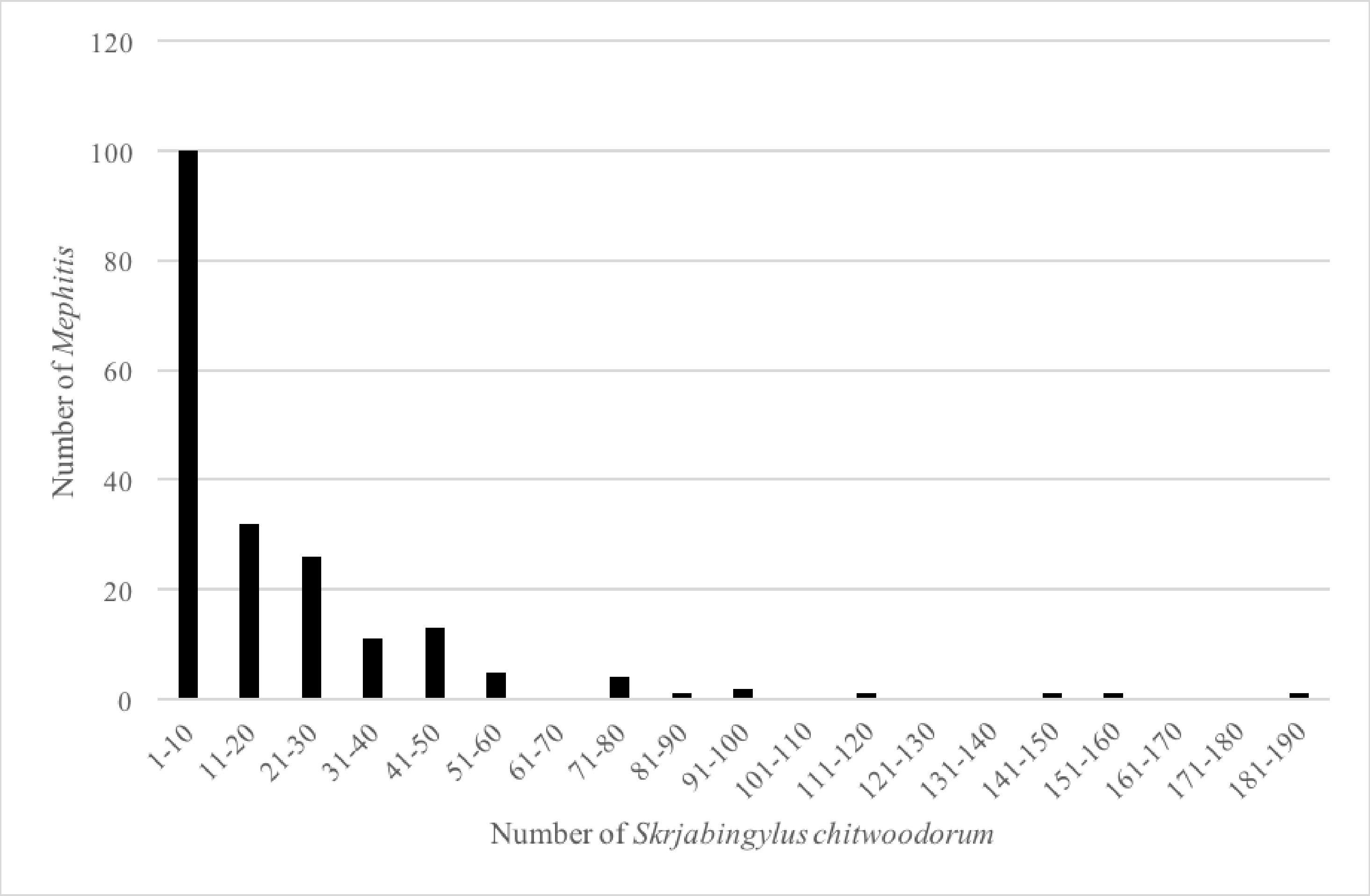

Figure 2. Intensity of infection ranged from 1-181 *Skrjabingylus chitwoodorum* per *Mephitis mephitis* host with a mean of 19.43 nematodes per host. The majority of infections were of lower intensities with only a few infections occurring at higher intensities.

Figure 3. Skull of *Mephitis mephitis* from Bastrop county, Texas (left), infected with 149 *Skrjabingylus chitwoodorum*. Small lesions and bulging of the right sinus side are noticeable. Skull from Travis County, Texas (right), was uninfected and has no noticeable lesions or bulging of the sinuses.

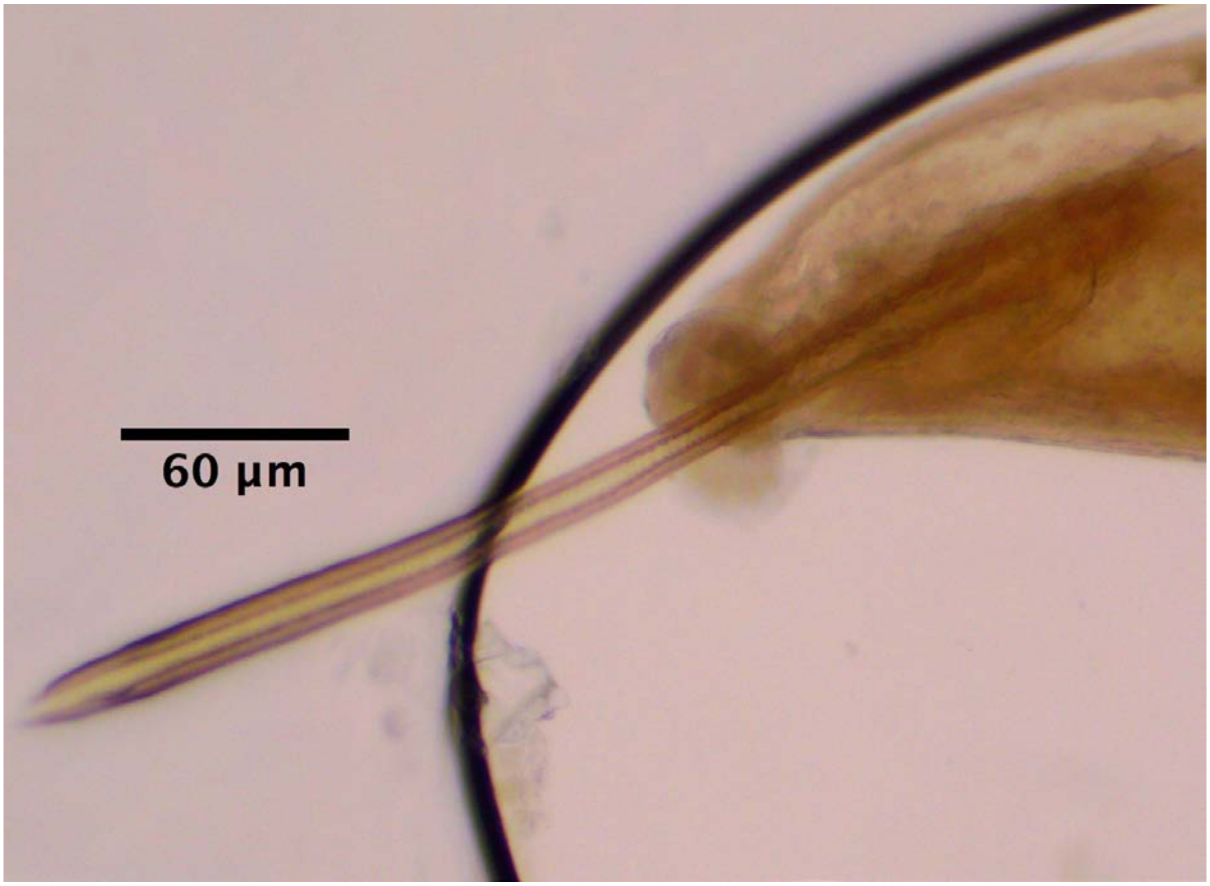

Figure 4. Spicules of a male *Skrjabingylus chitwoodorum* from a *Mephitis mephitis* host from Bell County, Texas.

Figure 5. Gubernaculum (indicated by the arrow) and copulatory bursa of a *Skrjabingylus chitwoodorum* isolated from a *Mephitis mephitis* host from Parker County, Texas.

Figure 6. Copulatory bursa of a male *Skrjabingylus chitwoodorum* isolated from a *Mephitis mephitis* host from Comanche County., Texas.

Randomization tests confirmed that age is a significant variable of infection status ($P<0.001$), and an odds ratio analysis showed that with each year of age the chances of an *S. chitwoodorum* infection increased by a factor of 1.375.

Prevalence of *S. chitwoodorum* by ecoregion varied from 9.1% to 74.1% (Fig. 5). The initial randomization test for ecoregions also showed variation in prevalence among ecoregions existed ($P<0.001$), but pairwise logistic regression (1,000 iterations) with Holm adjustment showed there were no significant differences among ecoregions ($P_{adj}>0.1$ for all comparisons). Because percentages of ecoregions ranged on a continuum, they could not be conveniently grouped using logistic regression for further comparison. I also performed a logistic regression using latitude and longitude coordinates for each county; these tests revealed that neither latitude nor longitude were significant predictors of prevalence (P-values=0.95 and 0.31, respectively). The permutational ANOVA showed intensity did not vary among ecoregions (Fligner-Killeen: P-value < 0.05; Permutational ANOVA: P-value=0.23).

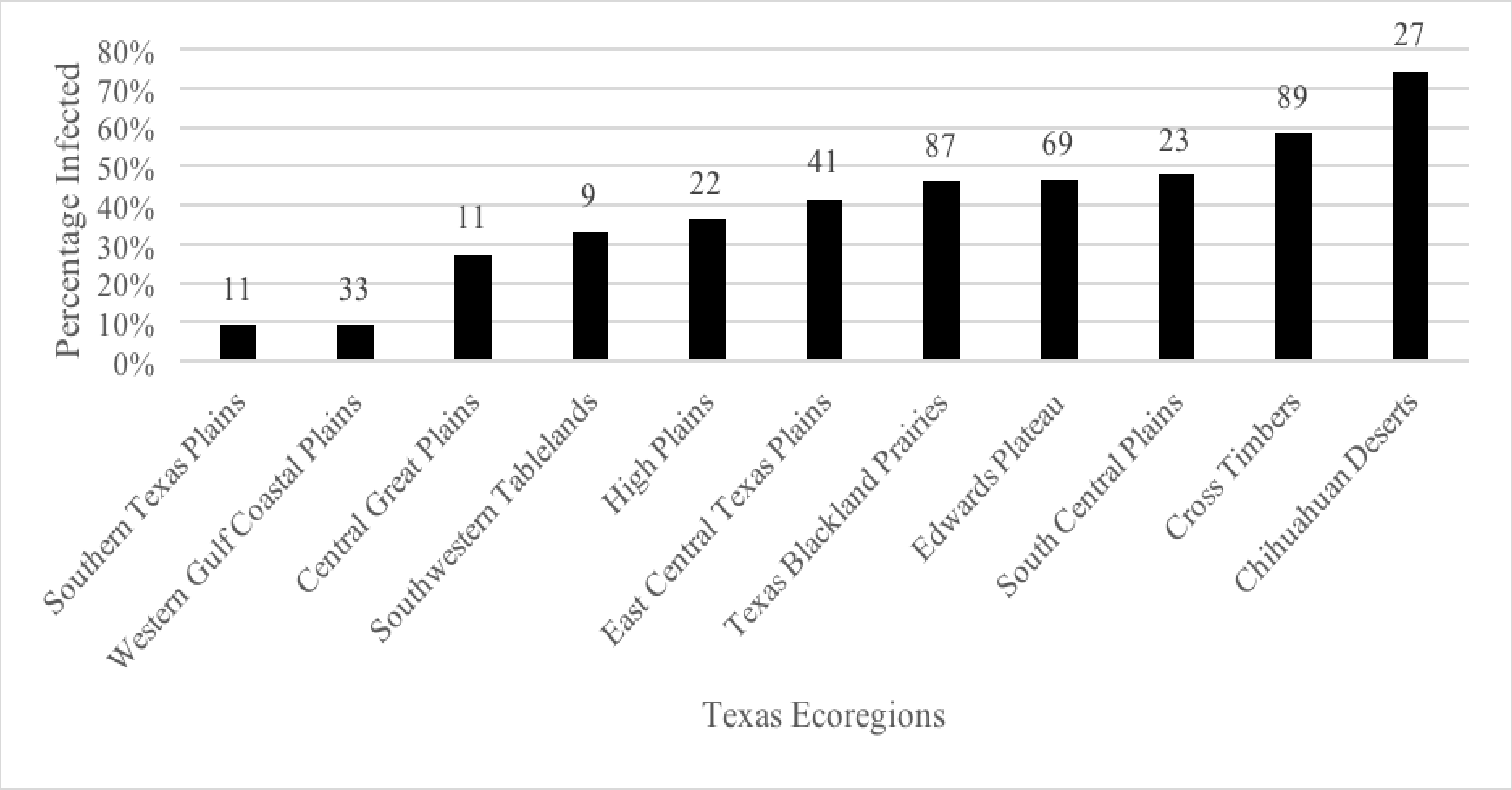

Figure 7. Prevalence of *Skrjabingylus chitwoodorum* in *Mephitis mephitis* by Texas ecoregion (Griffith et al. 2007). Numbers displayed on the graph represent sample sizes for that ecoregion.

DISCUSSION

Spicule measurements from our study were not consistent with those from Lankester's (1983) species redescription, but were in agreement with the measurements from the original species description for *S. chitwoodorum* from Oklahoma (Hill 1939) and those from the studies in Minnesota (Fuller and Kuehn 1984) and Canada (Webster 1965). Copulatory bursa of the Texas nematodes closely resemble those in the figures provided by Lankester (1983) for *S. chitwoodorum*. The difference in spicule lengths could be attributable to geographic variation or possibly phenotypic plasticity existing among *S. chitwoodorum* from different geographic regions.

It is interesting that both this study that done by Kirkland and Kirkland (1983) on museum specimens of *M. mephitis* found a bias toward the left sinus side. A study on *S. nasicola* in Sweden also revealed a left sinus side bias in *S. nasicola* from *Mustela ermina* while *M. nivalis* and *M. putorius* had right sinus side biases (Hansson 1968). Hansson (1968) suggested that these biases might be due to "irregularities in the structure of the crania" based on a study done by Rosen and Sarnat (1954) on sinus maxillaris of dogs.

All counties I report as negative for *Skrjabingylus* had relatively small sample sizes of 1-3 skunks, Hidalgo County was an exception with 14 specimens examined. I believe *S. chitwoodorum* is found in skunks throughout all of Texas based on the high prevalence observed. The percentage of *M. mephitis* with skrjabingylosis I reported is lower than the estimated prevalences for this region from Kirkland and Kirkland (1983) and this is likely because the methodology I used is more accurate than examining museum specimens. My results suggested that examination of skulls alone will overestimate the prevalence of *Skrjabingylus*. Goble and Cook (1942) also concluded lesions or damage to sinuses of specimens may not be an effective method for determining infection status. An alternative explanation for our lower reported prevalence for *Skrjabingylus* is that skunks may be capable of clearing the nematode infection but still show skull damage from a previous infection. To my knowledge, this has not been reported for infections of this nematode.

The prevalence of *S. chitwoodorum* in Texas skunks is, however, slightly higher than that of infected skunks from California, Kansas and North Dakota (Mead 1963; Ewing and Hibbs 1966; Dyer 1969). Those studies also used nematode presence (instead of skull damage) to determine prevalence (Table 1).

Hansson (1974) found moisture and temperature influence the survival of the first-stage larvae of *S. nasicola*; low humidities and high temperatures reduce survival of the larvae in feces. *Skrjabingylus nasicola* larvae can survive freezing for some time in dry conditions, but are highly sensitive to desiccation (Hansson 1974). Kirkland and Kirkland (1983) found a positive correlation between precipitation and frequency of lesions in *M. mephitis* skulls from various regions of the United States. Because of their findings and what is known about favorable conditions for larvae of *S. nasicola*, I suspected prevalence and/or intensity of *S. chitwoodorum* infections might also vary among ecoregions of Texas due to differences in precipitation. Kirkland and Maldonado (1988) also found no significant relationship between precipitation and cranial damage in *M. mephitis* from Mexico. My inability to identify any lack of variation in prevalence or intensity of *S. chitwoodorum* infections among the different ecoregions in Texas could be attributed to mild winters and sufficient rainfall throughout the state or perhaps the wide availability of intermediate and paratenic hosts of the parasite.

Tiner (1946) reported none of the skunks in their study from the Trans-Pecos region, which corresponds to the Chihuahuan Desert ecoregion in this report, had lesions. Patton (1974) reported all of the *Spilogale* from the Trans-Pecos region in his study had lesions. Twenty of the 26 *M. mephitis* I examined from this area were positive for *S. chitwoodorum*. All skunks from this ecoregion were collected within El Paso city limits, meaning water was readily available versus more rural areas of the Chihuahuan Desert where water is far less available. Tiner did find *S. chitwoodorum* in skunks from the neighboring Edwards Plateau region; however, it is possible that since the time of his study the range of *S. chitwoodorum* has spread westward.

None of the hog-nosed skunks in our study showed any signs of *Skrjabingylus* which is consistent with previous work. Tiner (1946) examined the skulls of 170 museum specimens of *Spilogale*, *Mephitis,* and *Conepatus* from Texas and reported 3 *Spilogale* and 3 *Mephitis* skulls had lesions caused by *S. chitwoodorum*; none of the *Conepatus* skulls had lesions or damaged crania. Neiswenter et al. (2006) examined 8 *Conepatus* skulls from Texas, all of which were free of lesions and deformations. Kirkland and Kirkland (1983) reported that none of the 83 *Conepatus* examined in a study on North American museum specimens had damage caused by *Skrjabingylus.* Patton (1974) reported *S. chitwoodorum* in *C. leuconotus* based on osteitis and bulging in the frontal sinuses of some individuals from the Trans-Pecos region of Texas, but no actual sinus worms were recovered in his study or any others on *Conepatus* to my knowledge. *Conepatus* could be more resistant to *Skrjabingylus* infections or the differences in diet amongst skunk species may explain why *Conepatus* appear to be unaffected by *Skrjabingylus*. *Conepatus leuconotus* is “more insectivorous than other skunks”, but when insects are not available it will opportunistically feed on small vertebrates (Dragoo and Sheffield 2009). A more likely explanation is that the frontal sinuses found in *Mephitis* and *Spilogale* are completely absent in *Conepatus* (Van de Graaff 1969). Kirkland and Kirkland (1983) also speculated that *Conepatus* avoid infections due to the lack of frontal sinuses.

The reason individual skunks were submitted for testing could not be determined; however, because all skunks in this study were rabies negative, I believe some may have been behaving abnormally. This hypothesis could be biased, however, because individuals from this study were submitted for rabies testing. Ewing and Hibbs (1966) also suggested rabies-like behavior in skunks could be caused by *Skrjabingylus* infections. Based on what is known about the effects of skrjabingylosis on host behavior (Lankester and Anderson 1971) and the high prevalence of infected Texas skunks, it is likely that many skunks submitted for testing have aberrant behavior from *Skrjabingylus* infections rather than from rabies.

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