Occurrence of the Sinus Nematode *Skrjabingylus* sp. (Nematoda: Metastrongyloidea) Inferred from Sinus Lesions in Arkansas Mustelidae and Mephitidae, with Review of Relevant Literature

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Running Title: Sinus Nematodes in Arkansas Mustelidae and Mephitidae

Abstract

Nasal nematodes of the genus *Skrjabingylus* occur in the mammalian families Mustelidae and Mephitidae, and in North America occur from Canada to Costa Rica. Ingestion of infected snails, frogs, snakes, or mice can infect mammalian hosts. Infection often causes pathology to bones in the sinus region, which may lead to discoloration, enlargement, and fenestrations of the bone. Examination of museum specimens for evidence of infection has been used to detect prior infection, but prevalence and intensity cannot be interpreted without actually recovering the parasite. We examined Mustelids and Mephitids in collections of mammals housed at Arkansas State University (ASU), Henderson State University (HSU), and the University of Arkansas at Little Rock (UALR) to evaluate the possible occurrence of nasal nematodes in Arkansas mammals. Evidence of infection was found in skulls of the Striped Skunk (*Mephitis mephitis*), Spotted Skunk (*Spilogale putorius*), Mink (*Neovison vison*), Long-tailed Weasel (*Mustela frenata*), and North American River Otter (*Lontra canadensis*) from Arkansas. We report for the first time evidence of the presence and distribution of *Skrjabingylus* sp. infecting mammals in Arkansas.

Introduction

The trematode *Troglotrema acutum* and species of the nematode *Skrjabingylus* can cause cranial lesions in skulls of certain mammals (Heddergott *et al*. 2015a), but *T. acutum* occurs only in Europe so does not affect New World mammals. In North and Central America, only nasal nematodes of the genus *Skrjabingylus* have been identified from several geographic locations and in several members of the mammalian families Mustelidae and Mephitidae. Records are known from as far north as the Northwest Territories of Canada (Dougherty and Hall 1955) to as far south as Costa Rica (Carreno *et al*. 2005). The infective third stage larvae of *Skrjabingylus* may be ingested when a host species consumes an intermediate host (such as snails) or consumes paratenic hosts such as frogs, snakes, or mice (Lankester and Anderson 1971; Hansson 1967; Gamble and Riewe 1982; Jennings *et al*. 1982; Weber and Mermod 1985).

Skrjabinglyus nasicola is the most widely distributed species of the genus (Santi *et al*. 2006), and is common and cosmopolitan. The definitive hosts of *S. nasicola* are members of the genus *Mustela* (then including mink, which is now in the genus *Neovison*) (Hawkins *et al.* 2010). Other species of *Skrjabingylus* appear also to have host specificity; *S. petrowi* of the genus *Martes* (Heddergott *et al*. 2015b), *S. chitwoodorum* of the skunks *Mephitis* and *Spilogale* (Hill 1939, Hobmaier 1941; Goble 1942), and *S. lutrae* of river otters (Lankester and Crichton 1972).

In mink, up to 63 worms were counted in an individual from Minnesota (Kinsey and Longley 1963), but the average infection was 4.1 worms per cavity in Ontario (Santi *et al*. 2006). Significant infection often causes bone pathologies of the sinus region, which may present as discoloration, swelling or enlargement, and fenestrations of bone in the region of the sinuses (Santi *et al*. 2006). Lesions, known as local rarefying osteomyelitis, result from a reaction to the worms in the sinuses, which eventually can result in damage to bone (Kierdorf *et al*. 2006). Significance of the infection, suggested by hypotheses of a relatively smaller braincase caused by swelling of the frontal sinsues in older, infected animals, was supported for striped skunks (*Mephitis mephitis*) (Maldonado and Kirkland 1986), older male mink (Bowman and Tamlin 2007), and also occurred with a bias toward males in river otters (Scherr and Bowman 2009).

Methods

Because no data exist about occurrence of

Skrjabingylus in Arkansas, we sought to determine which Arkansas members of the Mustelidae and Mephitidae might show evidence of infection. We examined skulls housed in collections of mammals at Arkansas State University (ASU), Henderson State University (HSU), and the University of Arkansas at Little Rock (UALR). We examined skulls of the Striped Skunk (*Mephitis mephitis*), Spotted Skunk (*Spilogale putorius*), Mink (*Neovison vison*), Long-tailed Weasel (*Mustela frenata*), and North American River Otter (*Lontra canadensis*), all of the members of the Mustelidae and Mephitidae in Arkansas with the exception of the American Badger (*Taxidea taxus*). We collected data regarding the county of origin of each specimen to examine distribution within Arkansas.

Results and Discussion

The apparent oldest skull from Arkansas exhibiting damage from infection with *Skrjabingylus* was a new subspecies of ermine named by Brown (1908) after collection from the Conard Fissure in Newton County. Originally named *Putorius cicognanii angustidens*, and now known as *Mustela erminea angustidens*, one specimen shown in plate XVII of Brown (1908) had a circular lesion on the left side just behind the postorbital process of the skull. The lesion is consistent with infection by *Skrjabingylus*.

In the modern species we examined, we detected lesions in all species. Skulls of smaller species appeared to be more likely to demonstrate lesions, and to have larger openings, likely due to the thinner bones constituting the frontal region. Bones of the North American River Otter are more substantive, and were deemed less likely to show lesions (Scherr and Bowman 2009).

Examination of museum specimens for bone pathologies in the sinuses often has been used to provide evidence of occurrence, prevalence, and intensity of infection. Stegeman (1939) found bone lesions in New York *Mephitis* and suggested infection by *Skrjabingylus*, and Tiner (1946) used occurrence of lesions to infer the presence of this nematode in Texas skunks. Reliance on observation of damage in museum specimens to determine prevalence and intensity of infection have added confusion, however, due to problems in inferring levels of parasite burden without actually recovering the parasite (Dougherty and Hall 1955).

Presence or absence of osteological damage may not be a good index of the incidence of these parasites. Goble and Cook (1942) examined the sinuses of 10 minks and 10 weasels and found infections in 4 of each species, but no external enlargement or abnormality was observed. On the other hand, they found no worms on opening the sinuses of a Bonaparte weasel that had an enlarged frontal region. Levine *et al*. (1962) also reported that these parasites were present in the brain cases of skunks that did not show the characteristic lesions. Thus, damage in the sinuses may indicate that infection has occurred, but not that it is current, and absence of damage does not mean no infection is present.

Considerable variation exists with respect to reported patterns of occurrence and manifestation of infection. Several studies report evidence that both prevalence and intensity of infection tend to be higher in older host animals (Dougherty and Hall 1955; Gamble and Riewe 1982; Fuller and Kuehn 1984), but other studies find this in juveniles (Santi *et al*. 2006). Both prevalence and intensity of infection were observed to be higher in male ermine (Dubay *et al*. 2014), and male otters were found to suffer greater damage to their skulls due to these parasites (Scherr and Bowman 2009). Skulls of male Least Weasels (*Mustela nivalis*) were more severely damaged in England (King 1977), as were skulls of male Long-tailed Weasels (*Mustela frenata*) in Manitoba (Gamble and Riewe 1982). In contrast, Debrot and Mermod (1980) reported no sex- or age-related differences in mustelids from Switzerland. Santi *et al*. 2006 summarized studies in which an infection bias to one sinus was believed to have caused asymmetrical damage to the sinus region, whereas other studies found no difference in damage between the sinuses (Hansson 1967; Lewis 1967).

Therefore, we present data only to demonstrate the inferred presence of the nematode in mustelids and mephitids from Arkansas, the distribution of such occurrences, and relevant literature to elucidate how our observations fit with other North American information.

Spotted Skunk (*Spilogale putorius*) - Hill's (1939) description of a new species of sinus nematode, *Skrjabingylus chitwoodorum*, was based partly on specimens collected from *Spilogale interrupta* collected in Oklahoma. In California, *S. chitwoodorum* was reported to occur frequently in spotted skunks taken from the Davis area (Mead 1963). Lesions assumed to have been cause by an infestation by *S. chitwoodorum* were found in skulls of 3 *Spilogale* from Texas, wherein damage varied from bulging and osteitis to holes in the frontal sinuses (Tiner 1946).

Spotted Skunks are not well represented in collections in Arkansas. We examined 5 specimens, originating from Franklin, Izard, Pulaski, and Sebastian Counties. Those from Franklin, Pulaski and Sebastian Counties, showed evidence of infection (Fig. 1). The Pulaski Co. specimen had a small hole in the right sinus, the Sebastian Co. specimen had a large hole on the left sinus, and the Franklin Co. specimen had 2 holes in the right sinus (see Fig. 1).

Figure 1. Skulls of the Spotted Skunk (*Spilogale putorius*) from Arkansas, showing significant sinus lesions creating large holes due to infestation by *Skrjabingylus* sp. Top image shows two large holes in the right sinus, and bottom image is a large hole in the left sinus of a different specimen.

Striped Skunk (*Mephitis mephitis*) – Stegeman (1939) noted skull lesions attributable to *Skrjabingylus* parasitism in skunks from New York, and Hill (1939) described *Skrjabingylus chitwoodorum*, based on specimens collected from skunks (*Mephitis* and *Spilogale*) in Oklahoma. Goble (1942) reported *S. chitwoodorum* from *Mephitis* near Schenectady, NY, Tiner (1946) reported lesions attributed to this parasite in 3 *Mephitis* skulls from Texas, Levine *et al*. (1962) reported them in Illinois, and Bailey (1971) recorded them in Ohio. Dyer (1969) listed records of *S. chitwoodorum* in *Mephitis* from Maryland, Illinois, California, Kansas, and Quebec. Carreno *et al*. (2005) described *Skrjabingylus santaceciliae* based on specimens from a hooded skunk, *Mephitis macroura* from Costa Rica.

We examined 94 skulls of Striped Skunks, of which 5 (5.3%) showed lesions. Manifestations of infection included swollen discolored sinuses, a swollen bubbly appearance with tiny perforations, numerous small perforations, and a remodeled forehead with a high rise ridge (Fig. 2). Specimens originated from 26 counties, and 5 counties were represented by lesioned skulls (Fig. 5).

Figure 2. Skulls of the Striped Skunk (*Mephitis mephitis*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp. Top, the bubbly appearance resulting from swelling and tiny perforation; middle, numerous small perforations; bottom; swelling with considerable bone restructuring.

American Mink (*Neovison vison*) – The American Mink is a common mustelid of North America. Heavily infected specimens often exhibit inflated, discolored, and perforated sinuses (Sealander 1943, Kinsey and Longley 1963), though infections may occur with no visible manifestations (Goble and Cook 1942). In Minnesota, up to 63 worms were counted in an individual mink (Kinsey and Longley 1963).

Our mink sample originated from 15 counties, and specimens with damage caused by *Skrjabingylus* came from 8 counties (Fig. 6). Of 94 specimens we examined, 51 (54.3%) showed evidence of infection (Fig. 3). Prevalence of infection by *S. nasicola* in mink appears to be generally high throughout North America. Sealander (1943) found *Skrjabingylus* to be the most common parasite of mink in Michigan, occurring in 94% of specimens. Goble and Cook (1942) found 40% infection in New York, Dorney and Lauerman (1969) found 85% infection in Wisconsin, and Kinsey and Longley 1963) reported 75% infection in Minnesota. Santi *et al*. (2006) reported that 80.5% of mink were infected in Ontario, whereas Schulte- Hostedde and Elsasser (2011) reported infection in 43.6% of male mink in Ontario.

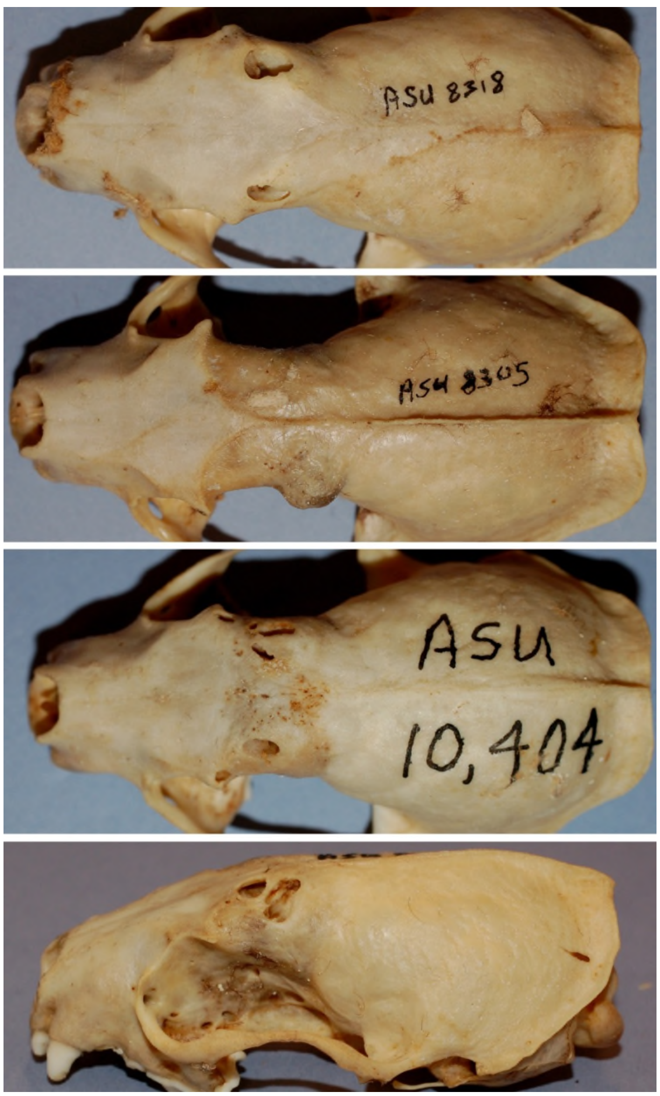

Figure 3. Skulls of American Mink (*Neovison vison*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp. Top, bilateral presentation of large holes: second from top, bulge in left sinus; third, bubbly appearance with bilateral large holes; and bottom, left sinus with 2 large holes.

The higher numbers of infected specimens allowed us to examine occurrence in more detail than for other species examined. Infections were represented bilaterally (in both sinuses) in 78.4% of the specimens. Santi *et al*. (2006) reported that a higher proportion of mink (58.2%) from Ontario were infected in both sinus cavities concurrently (double sinus infection), but frequency or intensity of infection did not differ significantly between left and right sinus cavities.

We ranked nature of infection for each side of each specimen according to the degree of damage, classifying based on the highest level of damage. Evidence of infection ranged from discoloration only (14.7% of sinuses), to a bubbly appearance with tiny holes (18.9%), small holes (< 2 mm; 33.7%), medium holes (2-4 mm; 15.8%), and large holes (> 4 mm, or 2 or more medium-sized holes; 16.8%). Sinuses with holes could also have a bubbly texture, and be discolored (Fig. 3). Our infected specimens originated from 8 counties (Fig. 6).

High frequencies of infection may have a yet unknown effect on behavior. Bowman and Tamlin (2007) found that infection with *Skrjabingylus* in older male mink caused down-warping of the braincase, resulting in reduced braincase volume caused by swelling of the frontal sinuses.

Long-tailed Weasel (*Mustela frenata*) – Goble and Cook (1942) found *Skrjabingylu*s infections in 4 weasels, though they had caused no external enlargement or abnormalities in the skull. Clapp (1952) reported *S. nasicola* in Long-tailed Weasels from Oregon, where up to 14 individuals were taken from 1 specimen.

Long-tailed weasels are not collected often in Arkansas, and our sample consisted of only 2 specimens, from Bradley and Craighead Counties. The weasel collected in Craighead Co. had a small hole resulting from infection of the right sinus.

North American River Otter (*Lontra canadensis*) – Lankester and Crichton (1972) described *Skrjabingylus lutrae* from river otters in Ontario. We examined 188 skulls of river otters from Arkansas, of which 16 (8.5%) showed lesions. Because skulls of otters are more robust than those of other hosts, it is less likely that they will show lesions. Most of our affected specimens presented discoloration, swelling, and small openings, but 1 specimen had a relatively large hole (4 mm) on the left sinus (Fig. 4). Our sample originated from 31 counties, of which 10 produced specimens infected by *Skrjabingylus* (Fig. 7).

Otters may harbor many *S. lutrae* and have major skull lesions without showing obvious clinical disease (Addison *et al*. 1988), but other infected otters have nasal discharge and neurologic signs (Petrini 1992). However, Scherr and Bowman (2009) found that skulls of male otters lesioned by infection also had reduced braincase volume, and to a lesser extent, female otters were similarly affected. This might affect behavior and survival of otters.

Conclusions

Through sinus lesions attributed to infection by the nasal nematode *Skrjabingylus*, we document the inferred occurrence of this parasite in the Mustelidae and Mephitidae of Arkansas. If host specificity is absolute, we have *S. nasicola* in Long-tailed Weasels and American Mink, *S. chitwoodorum* in Striped and Spotted Skunks, and *S. lutrae* in North American River Otters. We believe these parasites occur throughout the distribution of their hosts in Arkansas.

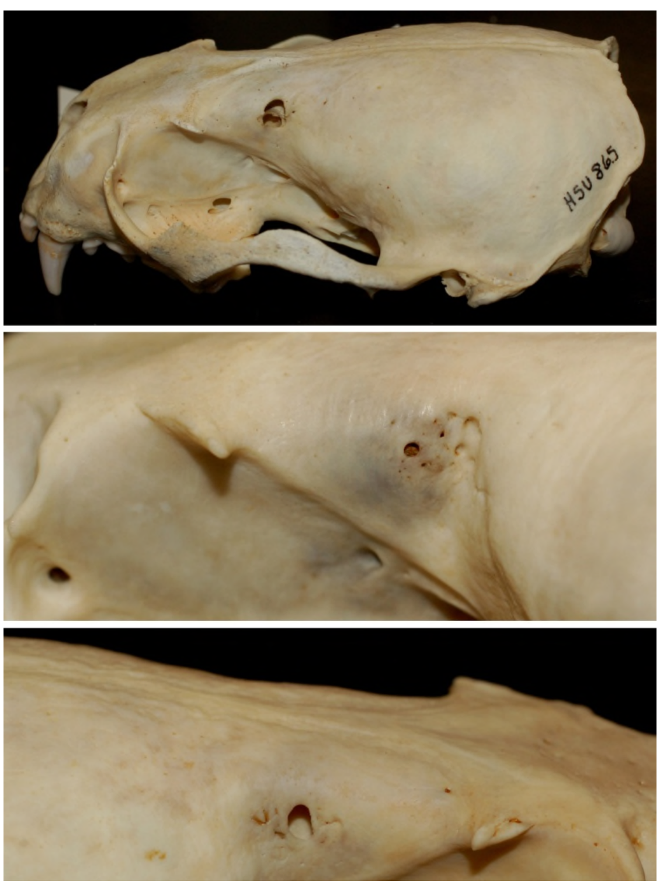

Figure 4. Skulls of North American River Otter (*Lontra canadensis*) from Arkansas, showing significant sinus lesions due to infestation by *Skrjabingylus* sp

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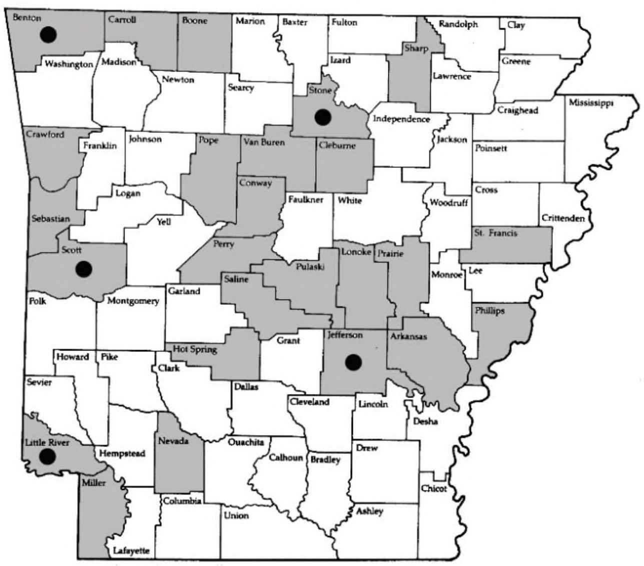

Figure 5. Distribution of Striped Skunks (*Mephitis mephitis*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected Striped Skunks included Benton, Jefferson, Little River, Scott, and Stone.

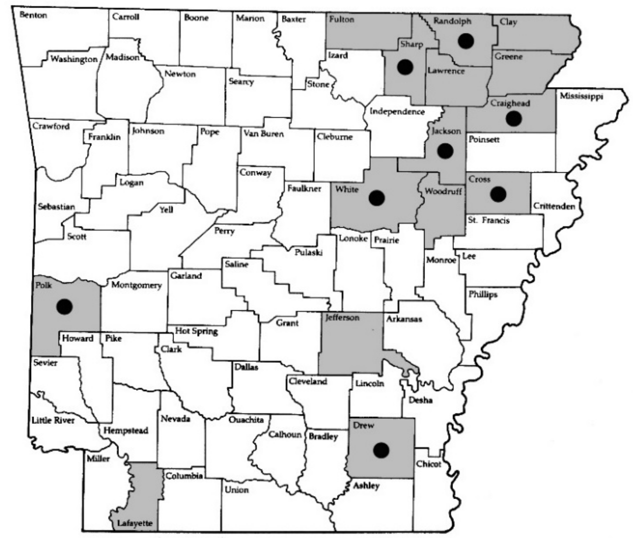

Figure 6. Distribution of Mink (*Neovison vison*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected Mink included Cross, Craighead, Drew, Jackson, Polk, Randolph, Sharp, and White.

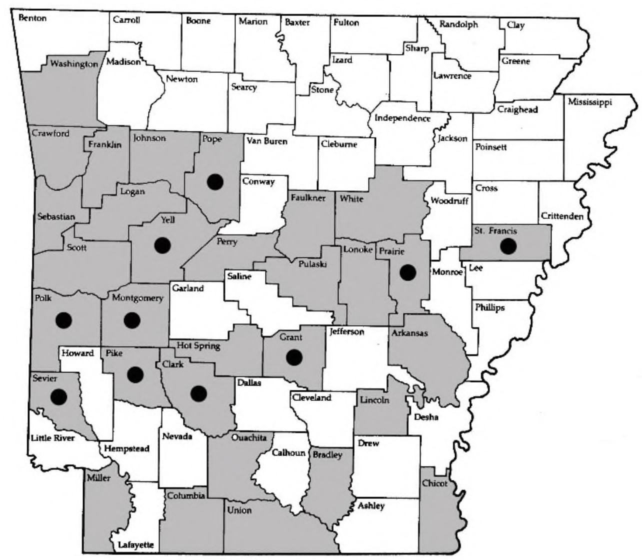

Figure 7. Distribution of River Otters (*Lontra canadensis*) in the sample (gray-shaded counties) and occurrence of lesions caused by *Skrjabingylus* sp. (dots). Counties with infected otters included Clark, Grant, Montgomery, Pike, Polk, Pope, Prairie, Sevier, St. Francis, and Yell.