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## A NEW SPECIES OF *SKRJABINGYLUS* PETROV, 1927 (NEMATODA: METASTRONGYLOIDEA) FROM THE FRONTAL SINUSES OF THE HOODED SKUNK, *MEPHITIS MACROURA* (MUSTELIDAE)

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ABSTRACT: *Skrjabingylus santaceciliae* n. sp. is described based on specimens from the frontal sinuses of a hooded skunk, *Mephitis macroura*, collected from the Area de Conservación Guanacaste, Costa Rica. *Skrjabingylus santaceciliae* n. sp. differs from the other 5 species in the genus in having pointed spicule tips that lack a rounded or lobed formation and by lacking a prominent distal projection at the tail tip. Morphometric comparisons show that *S. santaceciliae* n. sp. is much smaller than the only other valid species from *Mephitis, Skrjabingylus chitwoodorum* Hill, 1939. Likewise, morphometric comparisons also distinguish *S. santaceciliae* n. sp. from other described *Skrjabingylus* species.

The metastrongyloid *Skrjabingylus* Petrov, 1927 includes 5 described species, the adults of which parasitize the frontal sinuses of mustelids (Lankester, 1983). Species for which life cycles have been studied use gastropod intermediate hosts; paratenic hosts including rodents, reptiles, and amphibians have also been implicated (Anderson, 2000). *Skrjabingylus* species can be pathogenic in their definitive hosts, causing cranial lesions in species of *Mustela*, *Lutra*, *Martes*, and *Mephitis* (Hansson, 1968; Addison et al., 1988).

During May 2003, as part of an ongoing biodiversity inventory of eukaryotic parasites of vertebrates inhabiting the Area de Conservación Guanacaste in northwestern Costa Rica, an undescribed species of *Skrjabingylus* was discovered in the frontal sinuses of a hooded skunk, *Mephitis macroura*. This species is described herein.

### MATERIALS AND METHODS

A hooded skunk (Mephitis macroura Lichtenstein, 1832) was found dead by the side of a road near Santa Rosa National Park, Area de Conservación Guanacaste, Costa Rica (11°02'53"N, 85°37'38"W) (details on this host record are found on the Web site at http://brooksweb. zoo.utoronto.ca/index.html). Nematodes were collected from the frontal sinuses and preserved in ethanol. Specimens for light microscopy were cleared in Berlese's medium (Pritchard and Kruse, 1982) and observed using a Nikon E600 microscope using both bright field and differential interference contrast microscopy. Specimens for scanning electron microscopy were fixed in 5% formalin, dehydrated in a graded ethanol series, and critical point dried using an Autosamdri 795 Supercritical Point Dryer (Tousimis, Rockville, Maryland). Specimens were mounted on aluminum stubs, sputter coated with gold using an SPI-Module Sputter Coater (SPI Supplies, West Chester, Pennsylvania), and observed using a Leo 435 VP scanning electron microscope at an average accelerating voltage of 20 kV.

Description and measurements of *Skrjabingylus santaceciliae* n. sp. are based on 6 males and 7 females, including the holotype and allotype. These specimens include intact worms and anterior or posterior pieces of both sexes. Measurements for specimens are reported in micrometers unless specified otherwise. The range of each measurement is given first, followed in parentheses by the mean value and its standard deviation. Geographic coordinates are given in Lambert units.

For comparative purposes, specimens of *Skrjabingylus chitwoodorum* Hill, 1939 were obtained from the frontal sinuses of a striped skunk that was collected (February 2001) in Santa Clara County, California. These specimens were used for measurements and comparative morphology, and a voucher specimen from the same host was deposited in the U.S. National Parasite Collection (accession number 92614).

### DESCRIPTION

## *Skrjabingylus santaceciliae* sp. nov. (Figs. 1–12)

*Diagnosis:* Metastrongyloidea Lane, 1917; Skrjabingylidae Kontrimavichus et al., 1976; *Skrjabingylus* Petrov, 1927. Reddish brown nematodes. Cuticle smooth or with several annulations along the body. Prominent tegumental sheath absent. Buccal capsule with heavily cuticularized wall. Stoma hexagonal, surrounded by inner circle of 6 papillae and outer circle of 6 pairs of closely spaced papillae, each pair posterior to an inner papilla. Amphid openings in shape of elliptical pores, situated posterior to oral papillae. Esophagus cylindrical, with nerve ring situated in anterior half, and excretory pore slightly posterior to midregion of esophagus. Male bursa consisting of 2 thick lobes, each of which extends from 1 side of the posterolateral end of tail. Short mucron extending from end of female tail. Vulva located in midregion of female. Amphidelphic uteri containing eggs and first-stage larvae.

*Male:* Length 8.98–11.6 mm (9.98  $\pm$  1.04); maximum width 429-506 (457  $\pm$  30.0). Inner diameter of stoma 29-47 (39.3)  $\pm$  7.76). Esophagus length 598–731 (656  $\pm$  56.5); nerve ring 211–282 (248  $\pm$  31.0) from anterior end; excretory pore 253– 377 (327  $\pm$  53.0) from anterior end. Spicules equal, brown, with 2 lateral alae, one dorsal, arising near proximal end of the spicule, the other ventral, arising more distally along proximal part of spicule column. Alae with prominent trabeculae. Spicule tips tapered and ending in pointed tip, without extensions of shaft or alae. Spicule length 385–428 (400  $\pm$  23.3). Gubernaculum slightly broader at distal end; gubernaculum length 75-90 (81.94  $\pm$  5.837). Each lobe of bursa containing 6 pedunculate papillae. Single precloacal papilla present, consisting of circular extension from cuticle where papilla protrudes. Single, bilobed (or closely spaced pair) papillae immediately posterior to phasmids. Spikelike appendage on tail tip absent.

*Female:* Length 16.29–22.87 mm (18.84  $\pm$  2.623); maximum width 671–777 (728  $\pm$  5.37). Inner diameter of stoma 32–41 (35.7  $\pm$  4.73). Esophagus length 662–794 (749  $\pm$  56.0); nerve ring 244–385 (314  $\pm$  64.3) from anterior end; excretory pore 326–424 (367  $\pm$  35.9) from anterior end. Vulva 8.74–12.7 mm (10.58  $\pm$  1.65) from anterior end. Ovejector consisting of 2 sphincters equally positioned at either end of vestibule. Anus 90–102 (95.6  $\pm$  4.72) from posterior end. Two small cuticular projections present ventrally at posterior end. Egg length (n = 23) 52–104 (76.48  $\pm$  17.12); width (n = 23) 28–65 (45.48  $\pm$ 

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FIGURES 1–2. *Skrjabingylus santaceciliae* n. sp., cervical region. Bars in micrometers. **1.** Anterior cervical region of a male showing relative positions of esophagus, nerve ring, and excretory pore. **2.** Cephalic extremity, en face view.

FIGURES 3–5. *Skrjabingylus santaceciliae* n. sp., female tail with mucron, vulva, and ovejector. Bars in micrometer. **3.** Tail, ventral view. **4.** Tail, lateral view. **5.** Ovejector and vulva, lateral view.



FIGURES 6–7. *Skrjabingylus santaceciliae* n. sp., male posterior extremity showing relative positions of bursa, spicules, gubernaculum, and cloaca. Bars in micrometers. **6.** Ventral view. **7.** Lateral view.



FIGURES 8–10. Skrjabingylus santaceciliae n. sp., scanning electron micrographs. 8. Cephalic extremity, en face, slightly tilted view, showing 1 of the amphids (a), inner papillae (i), and outer papillae (o). Bar =  $25 \mu m$ . 9. Male, ventral view of caudal extremity showing relative positions of bursal lobes. Bar =  $50 \mu m$ . 10. Male, ventral view of tail region showing precloacal papilla (pr), phasmids (ph), and papillae posterior to the phasmids (po). Bar =  $10 \mu m$ .

	S. santaceciliae n. sp.	S. chitwoodorum Hill, 1939	<i>S. nasicola</i> (Leuckart, 1842)	<i>S. lutrae</i> Lankester and Crichton, 1972	<i>S. ryjikovi</i> Kontrimavichus, 1961	S. petrowi Bageanow and Petrov, 1941
Males						
Body length (mm) Fearhaons length (nm)	8.98–11.6 598–731	16-23*; 13-17†; 17-24‡ 900-1 310*: 840-968†	6–15*; 7.12–10.23§ 750–765*• 528–6168	9–12   700–980	17.34§ 1.0158	11.0–15.6§ 1/12 hody length8
Spicule length (µm)	385-428	800-890*; 540-710†; 688-866‡	180–232*; 226–236§	239–275	1,296§	449-5708
Gubernaculum length (µm)	75–90	83–100*; 72–88†	39-62*; 49-52§	42-52	63\$	4384§
Distance of nerve ring from anterior end (u.m)	211–282	$310-460*; 236-340\dagger; 280-400\ddagger$	240–260*	210–390	348§	NA#
Distance of excretory pore from anterior end (µm)	253–377	460–680*; 375–493†; 432–600‡	365–380*	290–575	631§	437–892§
Females						
Body length (mm)	16.29–22.87	37-53*; 23-29†; 34-56‡	10-32*; 18.48-22.47	22–26	26.25§	28.0–39.5§
Esophagus length (µm)	662–794	$1,130-1,460*; 1,040-1,520\ddagger$	780-820*; 776-826§	800–925	1,250§	1,400-1,550\$
Distance of nerve ring from anterior end (11m)	244–385	310-360*; 270-330†; 360-552‡	190–230*	225-325	472§	NA
Distance of excretory pore from anterior end	326-424	420–690*; 405–450†; 520–848‡	270–320*	310–675	812§	437–892§
(mm)						
Distance of vulva from anterior end (mm)	8.74–12.7	18–26*	5-17*; 12.38§	11 - 13	13.65§	13.7–17.0§
Distance of anus from posterior end (µm)	90-102	130–170*; 75†; 120–160‡	145-180*; 141-150\$	200–260	265§	139–174§
<ul> <li>* Lankester (1983).</li> <li>† Hill (1939).</li> <li>‡ Webster (1965).</li> <li>§ Kontrimavichus et al. (1985).</li> <li># NA, not available.</li> </ul>						

TABLE I. Morphometric measurements of Skrjabingylus spp. Measurements of Skrjabingylus suntaceciliae are original, and others were tabulated from the sources given in the footnotes.

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FIGURES 11–12. Skrjabingylus santaceciliae n. sp., spicule tips. The spicules were dissected from an adult male. The gubernaculum is also visible. Bar = 15  $\mu$ m. **12.** Skrjabingylus chitwoodorum, spicules with "egg-shaped" tips protruding through the cloaca. Bar = 15  $\mu$ m.

12.48). Length of first-stage larvae (n = 27) 400–485 (446  $\pm$  23.3).

#### **Taxonomic summary**

Type host: Mephitis macroura Lichtenstein, 1832.

*Type locality:* Area de Conservación Guanacaste, Costa Rica (11°02′53″N, 85°37′38″W).

Site of infection: Frontal sinuses.

*Type specimens:* Holotype male, USNPC no. 94493; allotype female, USNPC no. 94494; paratypes, 4 intact females, 1 female anterior fragment, 1 female posterior fragment, 6 intact males USNPC no. 94495.

*Etymology: Skrjabingylus santaceciliae* n. sp. is named after the town of Santa Cecilia, Guanacaste, Costa Rica.

### Remarks

Three species of Skrjabingylus have previously been described from hosts in North America (Lankester, 1983). The only species previously described from Mephitis, S. chitwoodorum from M. mephitis, differs from S. santaceciliae in having different spicule tips (Skrjabingylus magnus Webster, 1965 was also described from *M. mephitis* but was synonymized with *S.* chitwoodorum by Lankester, 1983). Spicule tips of S. chitwoodorum are "egg shaped" (Lankester, 1983 and Fig. 12), whereas those of S. santaceciliae are sharply pointed with no expansion. This spicule character also distinguishes S. santaceciliae from the other 2 species reported in North America, Skrjabingylus nasicola (Leuckart, 1842) and Skrjabingylus lutrae Lankester and Crichton, 1972. The spicule tip of S. lutrae is rounded or "globe shaped," whereas that of S. nasicola is "boot shaped" (Lankester and Crichton, 1972; Lankester, 1983). The 2 Palearctic species of Skrjabingylus that have been described, Skrjabingylus petrowi Bageanow and Petrov, 1941 from Martes spp. and Skrjabingylus ryjikovi Kontrimavichus, 1961 from Martes flavigula, also have different spicule tip morphology.

*Skrjabingylus petrowi* appears to have several distal projections of the alae and spicule column, and *S. ryjikovi* has rounded tips (Kontrimavichus, 1961; Kontrimavichus et al., 1985).

A prominent, spikelike projection has been observed at the tail tip of males in the other 5 Skrjabingylus spp., but this structure is absent in S. santaceciliae. Morphometrics also distinguish S. santaceciliae n. sp. from the other species (Table I). For example, the spicules of S. chitwoodorum are almost twice the length of those in S. santaceciliae, whereas the gubernaculum length is similar in both species. Male and female length, esophagus length, and the distances to the anterior end of both nerve ring and excretory pore of S. chitwoodorum are also greater than in S. santaceciliae. These measurements in S. santaceciliae overlap slightly with those of S. nasicola. However, in comparison with S. santaceciliae, the spicules and gubernacula of S. nasicola (reported only from Mustela spp.) and S. lutrae are much shorter. In contrast, the spicules of S. petrowi are slightly longer, and those of S. ryjikovi much longer than those of S. santaceciliae. Other measurements that differentiate these 2 Palearctic species from Martes hosts from S. santaceciliae include body and esophageal length in both sexes.

### DISCUSSION

The range of *M. macroura* extends from the southwestern United States to Costa Rica (Reid, 1997). Thus, if *S. santaceciliae* is host specific, it was discovered at the southern part of its host range. Lesions attributed to *Skrjabingylus* spp. infections have been reported in *M. macroura* from Mexico (Kirkland and Maldonado, 1988) and may have been caused by *S. santaceciliae*. However, confirmation of pathology due to *S. santaceciliae* and documentation of the geographic range of this nematode require additional collection, careful diagnosis, and documentation of tissue damage.

Based on DNA sequences from *S. chitwoodorum*, there is evidence that the genus *Skrjabingylus*, although belonging to

the monophyletic Metastrongyloidea, is not represented in the clade that includes most of the other metastrongyloid families (Carreno and Nadler, 2003). Species from this genus have only been reported from the frontal sinuses of their mustelid hosts, and many host species remain unsampled. Survey of additional mustelids is necessary to obtain more information on biodiversity in species of *Skrjabingylus* and for understanding the phylogeny of these parasites.

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