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The Discovery of a New Genus of the *Prosthenorchis Travassos*, 1915 (Acanthocephala: Oligacanthorhynchidae) Complex in North America, with the Description of *Neoprosthenorchis bifestoonatus* n. gen., n. sp. from Bobcats in Louisiana

Omar M Amin*

Institute of Parasitic Diseases, USA

***Corresponding author:** Omar M Amin, Institute of Parasitic Diseases, 11445 E. Via Linda # 2-419, Scottsdale, Arizona 85259, USA, Email: omaramin@aol.com

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Abstract

Neoprosthenorchis bifestoonatus n. gen., n. sp. (Oligacanthorhynchidae) is described from a bobcat, *Lynx rufus* (Schreber) (Felidae) in Louisiana. *Neoprosthenorchis* is the third of the genera comprising the *Prosthenorchis* Travassos, 1915 complex and the first in North America. The other two genera are *Prosthenorchis* from primates in South America and Felidae in Africa and *Paraprosthenorchis* Amin, Ha, Heckmann 2008 from Manidae in Vietnam. *Neoprosthenorchis* n. gen. is distinguished from the other genera of the complex by having two conspicuous sets of festoons at the anterior end of the trunk. The first set is made up of long and narrow vertical ribs encircling the anterior trunk and the overlaying second is a prominent horizontal undulating band. The festoons of *Neoprothenorchis bifestoonatus* n. gen., n. sp., especially the second set, are unique and different from all others previously described. Species in the two other genera normally have one crown of vertically ribbed festoons each. In addition, the North American species *N. bifestoonatus* also has a non-ornate longer than wide proboscis with 12 rows of 3 hooks with lateral slits, each without barbs but with simple heart-shaped roots and slight anterior manubria. A detailed description with line drawings and light microscope images along with the history of the concept of *Prosthenorchis* are provided.

Keywords: Oligacanthorhynchidae; *Neoprosthenorchis bifestoonatus* n. gen., n. sp., Felidae, North America.; Felidae

Introduction

The keys of Petrochenko [1], Golvan [2], and Yamaguti [3] have been insufficient to resolve the contraversies surrounding the historically confused taxonomic literature of the Oligacanthorhynchidae. Schmidt [4] studied species of all genera known then and revised the family on sound morphological lines without resorting to separating the genera on the basis of definitive hosts as have been historically done, e.g., *Oligacanthorhynchus* Travassos, 1915 from birds and *Hamanniella* Travassos, 1915 from opossums and edentates, etc. [4]. The identification of juveniles lacking

adult structures and the interpretation of proboscis hooks in spiral rows by some remains problematic.

The three original species of *Prosthenorchis* constituting the classical genus complex are *Prosthenorchis elegans* (Diesing, 1851) Travassos, 1915, *Prosthenorchis fraterna* (Baer, 1959) Schmidt, 1972, and *Prosthenorchis lemuri* Machado, 1950 (Table 1). Much has transpired over the last 50 years since Schmidt [4] published his revision. One other species of *Prosthenorchis* have been described since: *Prosthenorchis cerdocyonis* Gomes, Olifiers, Souza, Barbosa, D'Andrea & Maldonado Jr., 2015 from the crab-eating fox in Brazil (Gomes, et al. [5]). This species has all the characteristics of the genus *Prosthenorchis* outlined in Table 1 including the vertically ribbed festoons, barbed hooks and complex manubriated hook roots. *Prosthenorchis sinicus* Hu-Jiand, 1990 from a dog *Canis familiaris* Linn. in the Tacheng area of Xinjiang, China [6] is a misidentification. Most of the continuing research on acanthocephalans from this complex has dealt with *P. elegans* from New World primates covering the following selected topics. Mitochondrial DNA diversity in Colombia [7], pathology in marmosets in Brazil [8], cryptic diversity [9], case reports in tamarins *Sanguinus leucopus* (Günther) in Colombia [10], from primates in the Moscow Zoo [11], and from free-living primates in the Atlantic Forest of Bahia [12].

Our present work deals with the basic taxonomy of a new genus and species in the *Prosthenorchis* complex from a Felidaean, the bobcat, in the USA. This is the first record of any member of the *Prosthenorchis* complex in North America.

Materials and Methods

Collection

We have only one adult female in the ovarian ball stage that was collected from a bobcat, *Lynx rufus* (Schreber) (Felidae) in Louisiana by Dr. Richard L. Buckner. The specimen was already whole mounted in Canada balsam on a microscope slide labeled "*Oncicola*, *Lynx rufus* intestine, Oligacanthorhynchida, JW 20, RLB 1474" and the glass slide was etched with the same "RLB 1474." Measurements are in micrometers, unless otherwise noted; the range is followed by the mean values between parentheses. Width measurements represent maximum width. Trunk length does not include proboscis, neck, or bursa.

Line Drawings

Line drawings were created by using a Ken-A-Vision micro projector (Ward's Biological Supply Co., Rochester, N.Y.) which uses cool quartz iodine 150W illumination. Images of the stained whole mounted specimen were projected vertically on 300 series Bristol draft paper (Starthmore, Westfield, Massachusetts), then traced and inked with India ink. The completed line drawings were subsequently scanned at 600 pixels on a USB then downloaded on a computer.

Optical Microscope Images

Optical microscope images were obtained using a BH2 light Olympus microscope (Olympus Optical Co., Osachi-shibamiya, Okaya, Nagano, Japan) attached to an AmScope 1000 video camera (United Scope LLC, dba AmScope, Irvine, California), linked to an ASUS laptop equipped with HDMI high-definition multimedia interface system (Taiwan-USA, Fremont, California). Images from the microscope were transferred from the laptop to a USB and stored for subsequent processing on a computer. This specimen was not perfect but informative.

Results

Most species previously ascribed to *Prosthenorchis* are indistinguishable from *Oncicola* Travassos, 1916 except for 3 species noted by Schmidt [4] to have conspicuous festooned collar, e.g., *P. elegans*, *P. fraterna*, and *P. lemuri* (Table 1). Only one additional species, *Prosthenorchis cerdocyonis* was recently described since from a fox in Brazil by Gomes, et al. [5]. Another species, "*Prosthenorchis sinicus*" described from a dog in Xinjiang, China by Hu-Jiand [6] is a misidentification. It does not have a festooned collar and appeared to belong in *Oncicola*; see Figs. 1A & 1B in Hu-Jiand [6]. Van Cleave's [13] extensive treatment of the Acanthocephala of North American mammals referred only to the one species of *Oncicola* known in North America, *O. canis* (Kaupp, 1909) from domestic dog, cats, coyote, and a "lynx." Van Cleave [13] made no reference to the recovery of any specimens of *Prosthenorchis.* In this presentation, we describe *Neoprosthenorchis bifestoonatus* n. gen., n. sp. from a bobcat, *Lynx rufus* (Schreber) (Felidae) in Louisiana. This is the first record of any member of the *Prosthenorchis* complex in North America.

Description

Neoprosthenorchis n. gen. (Figs. 1-15)

Diagnosis: Female with short-medium trunk, broader anteriorly, gradually tapering posteriorly. Anterior trunk with two sets of festoons. First set of many vertical long and narrow ribbed festoon with large rimmed sensory stomata overlayed by a second set of undulating horizontal festoon, both encircling anterior trunk near its anterior end. Proboscis sub-globular, non-ornate. Rooted hooks in 12 alternating rows with 3 hooks each in dome-shaped teguments, sharply curved, smaller posteriorly, with lateral slits and without barbs. Hook roots simple, heart-shaped and blunt posteriorly

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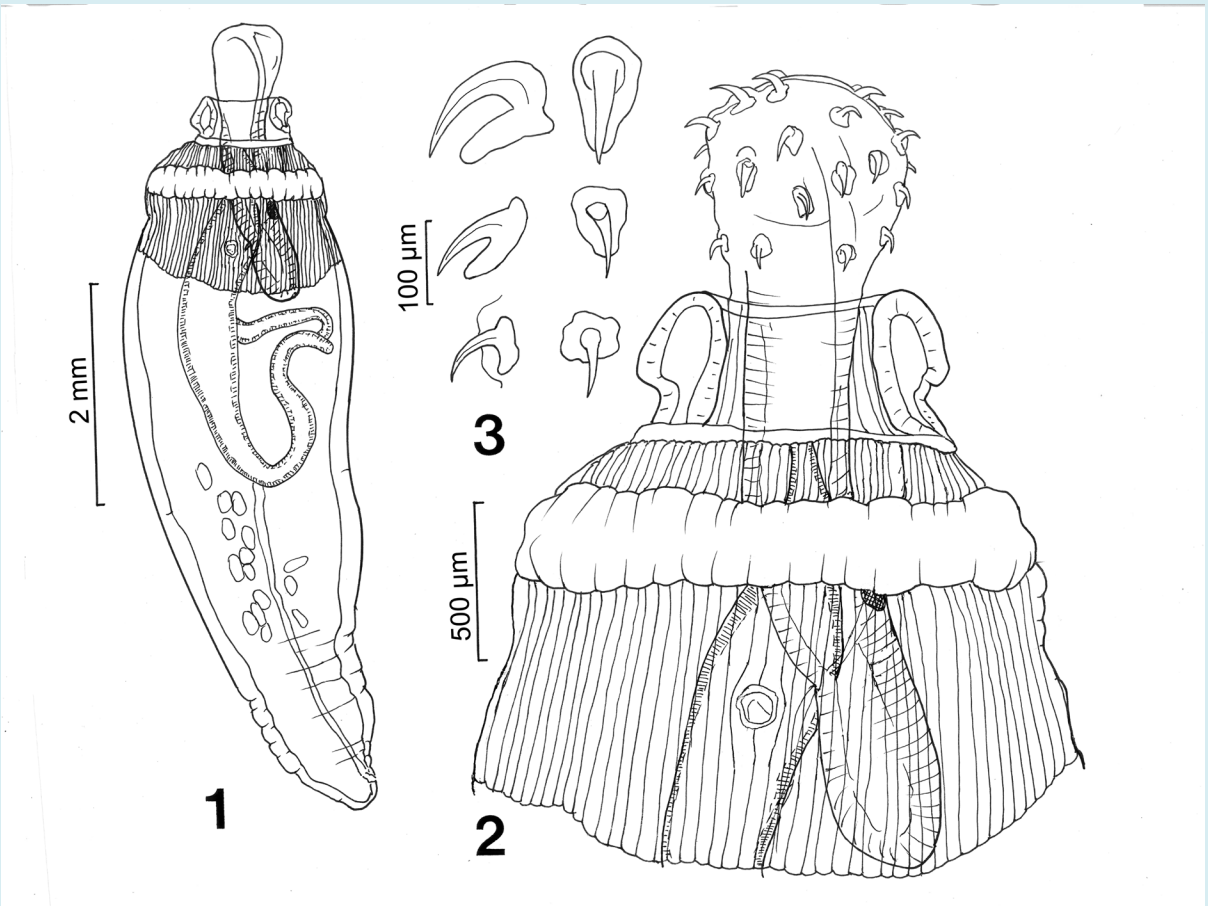
to rosette-shaped. Receptacle thick walled with no outer wall and cephalic ganglion near middle of ventral inner surface. Lemnisci relatively long, thick anteriorly, gradually tapering posteriorly. Protonephridia obscured. Gonopore subterminal. Specimen with only ovarian balls; no eggs.

Type species: *Neoprosthenorchis bifestoonatus* n. sp.

Remarks: This acanthocephalan represents the third genus in the *Prosthenorchis* complex and the first to be found in North America. The other two genera are known from primates and Felidae in South America and Africa and in Manidae in Vietnam (Table 1). *Neoprosthenorchis* has many common features with the two other genera but is distinctly different in having two sets of unusually shaped festoons as opposed to one. Species of "*Prosthenorchis*" that lacked festoons have all been relegated to other oligacanthorhynchid genera such as *Oncicola*, *Pachysentis* Meyer, 1931, and *Neoncicola* Schmidt, 1972. The genus *Neoprosthenorchis* n. gen. is further distinguished from *Prosthenorchis* and *Paraprosthenorchis* by non-ornate longer than wide proboscis, non-barbed hooks, simple hook roots, size of lemnisci, subterminal gonopore, smaller worm size, and host and geographical distribution.

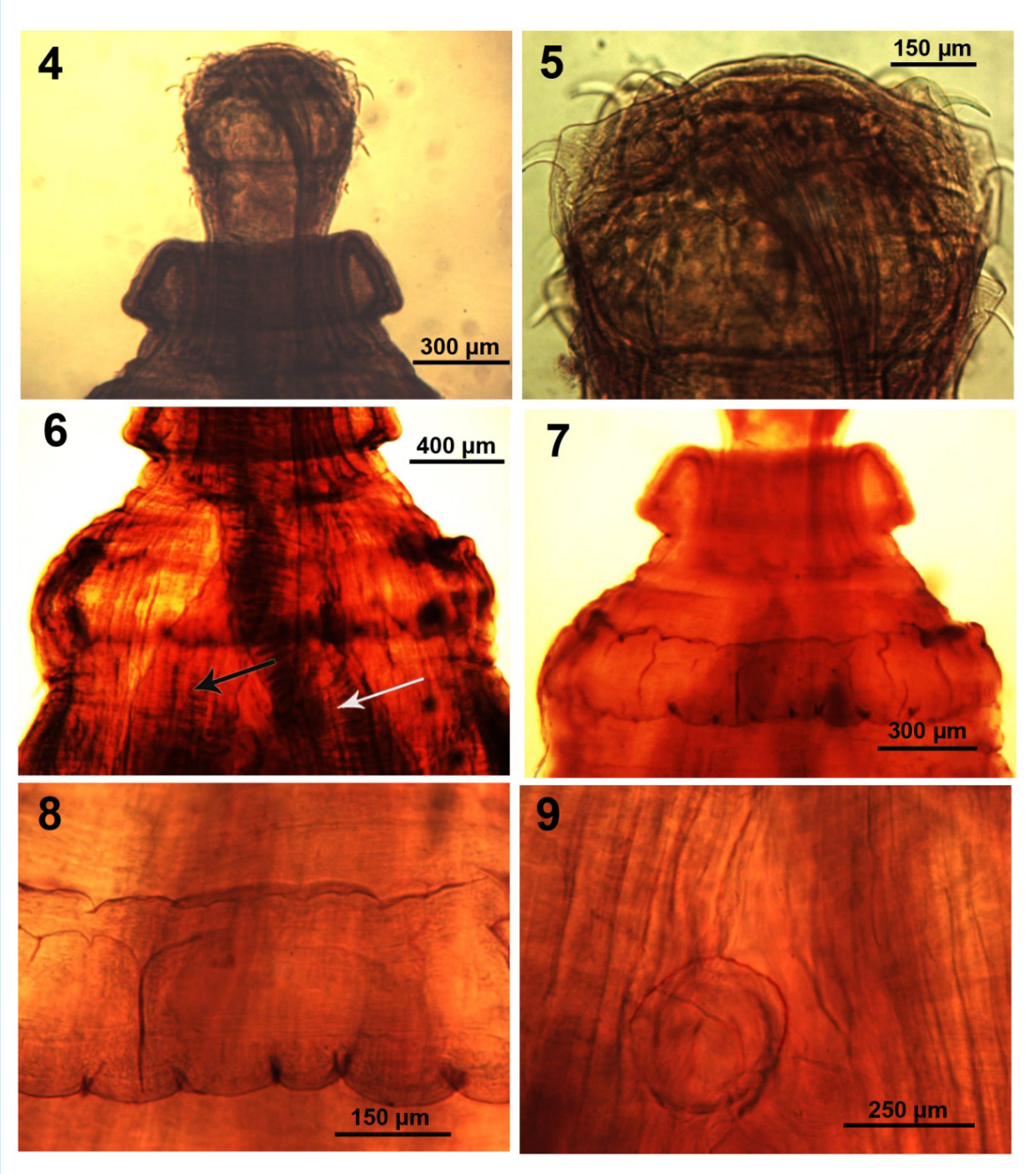
Description

***Neoprosthenorchis bifestoonatus* n. sp.**



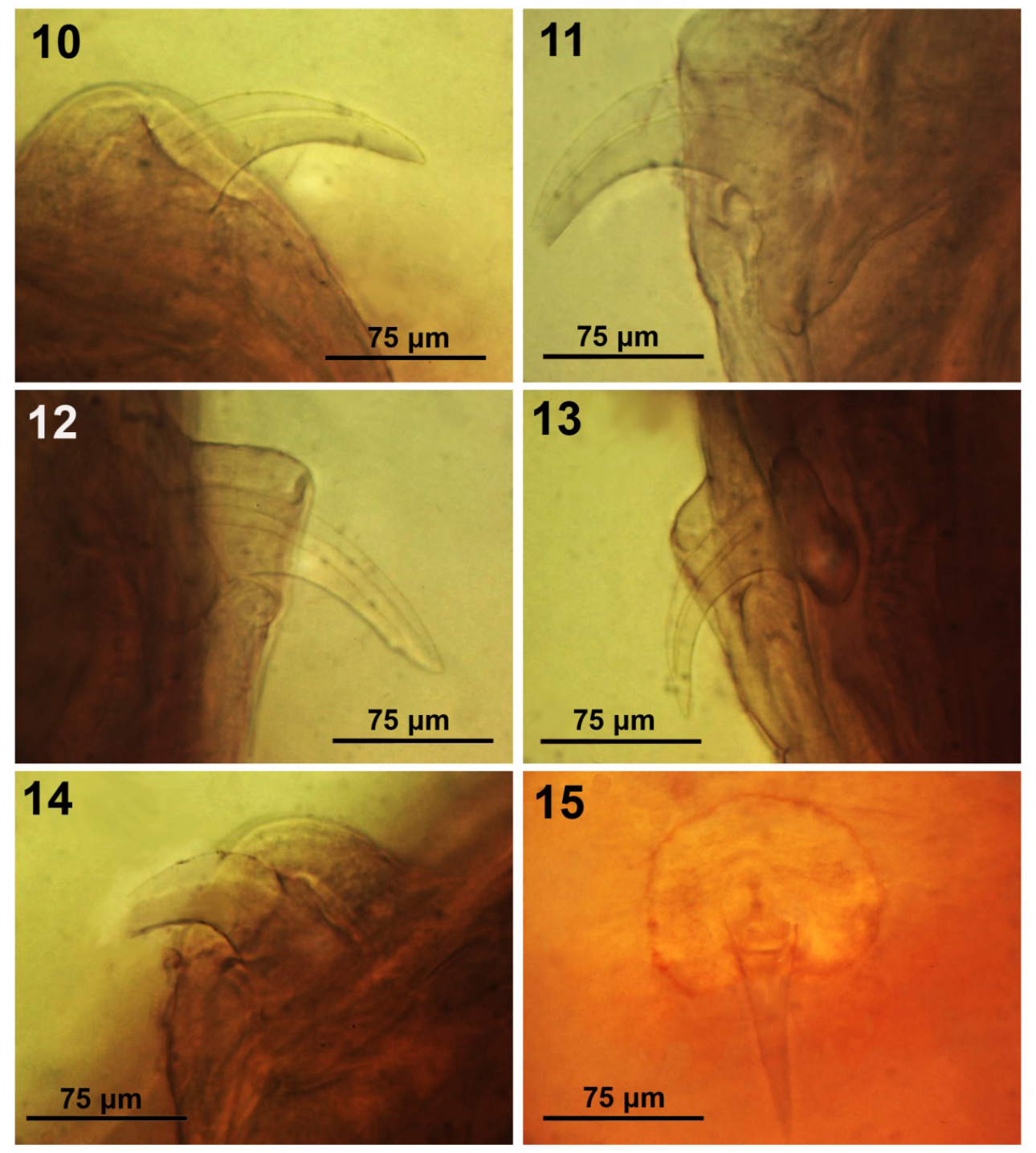
Figures 1-3: Line drawings of the holotype female of *Neoprosthenorchis bifestoonatus* n. gen., n. sp. from the bobcat, *Lynx rufus* in Louisiana. **1.** The holotype female. Note the unique shape and position of the festoons, the anteriorly thick lemniscus, the subterminal gonopore and the long slender uterus. **2.** A higher magnification of the praesoma showing details of the two festoons and the large orifice of the sensory pore. Note the cephalic ganglion near the middle of ventral inner surface of the receptacle just below the horizonal festoon. **3.** Anterior, middle, and posterior hooks in profile and in face view.

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Figures 4-9: Light microscope images of the holotype female of *Neoprosthenorchis bifestoonatus* n. gen., n. sp. from the bobcat, *Lynx rufus* in Louisiana. **4**. The praesoma. Anterior trunk is slightly retracted. **5.** Anterior proboscis. Note the strong retractor muscles. **6.** Anterior trunk. Image focuses on internal anatomy. Note the comparable size of the lemniscus (black arrow) and the proboscis receptacle (white arrow). 7. Anterior trunk. Image focuses on external surface. Note the horizontal undulating festoon encircling the anterior trunk. **8.** High magnification of the horizontal festoon. **9.** Part of the underlying vertical lamellated festoon and the large sensory orifice.

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Figures 10-15: Light microscope images of the holotype female of *Neoprosthenorchis bifestoonatus* n. gen., n. sp. from the bobcat, *Lynx rufus* in Louisiana. **10-14.** Lateral view of anterior, middle and posterior hooks and roots, in the same order. All hooks show the lateral slits or furrows. **15.** Face view of a basal hook showing the frontal shape of the root.

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Female (based on 1 female in the ovarian ball stage). Trunk small-moderate widest in anterior third with thick integument (Fig. 1), 7.50 long by 2.15 wide. Proboscis sub-globular 625 long by 650 wide, with 12 extremely alternating rows of 3 hooks each showing marked size variations (Figs. 2-5). Hooks not barbed, extremely curved, with lateral slits, largest anteriorly, in dome-shaped bulbs (Figs. 2,3), vary in size in alternate rows. Roots simple posteriorly directed and blunt heart-shaped with small anterior manubria. Anterior hooks largest (Figs. 10,11), 145-156 long. Middle hooks (Fig. 12) 124-146 long. Basal hooks (Figs.13-15) 94-109 long on both dorsal and ventral sides (Fig. 2). Neck 550 long by 750 wide at base. First set of festoons at anterior end of trunk of about 80 longitudinal ribs 1.50-1.60 mm long by 0.041-0.062 mm wide with paired rimmed sensory pore 260 in orifice diameter (Figs. 2,9). Second set of festoons of 33 undulating segments of horizontal band 1.75 mm wide by 0.27-0.37 mm long vertically, at anterior end of first set of festoons (Figs. 2,7,8). Thick-walled receptacle 2.00 mm long by 0.40 mm wide (Fig. 6, white arrow) with cephalic ganglion near middle of its ventral inner surface. Lemnisci 6.00 long by 0.50 wide anteriorly (Fig. 6, black arrow). Reproductive system with subterminal gonopore and long uterus reaching posterior end of receptacle (Fig. 1).

Taxonomic Summary

Type host: Bobcat, *Lynx rufus* (Schreber) (Felidae).
Type locality: Louisiana, USA.
Specimen: Deposited at the Harold W. Manter Laboratory (HWML) of Parasitology Coll. no. HWML 216486 (accessioned P-2023-046)., Nebraska State Museum, Lincoln, Nebraska.
Etymology: The new genus is named for its similarities to species in the genus *Prosthenorchis* and the species named for its unique double festoon sets.
ZooBank LSID#AB254E1-5021-42ED-B80C-424E556731FB

Remarks

Neoprothenorchis bifestoonatus n. gen., n. sp. is distinguished from all other species of the *Prosthenorchis* group; in having two unique sets of festoons and hooks with lateral slits. The first set of festoons is made up of long and narrow vertical ribs encircling the anterior trunk just posterior to the neck and the overlaying second set is a prominent horizontal undulating band encircling the first set of vertical festoons near its anterior margin (Fig. 2).

Genera	***Neoprosthenorchis* n. gen.**	***Paraprosthenorchis***	***Prosthenorchis****
Species	*N. bifestoonatus* n. gen., n. sp.	*P. ornatus*	*P. elegans* (Deising, 1851) *P. fraterna* (Baer, 1959) *P. lumuri* Machado, 1950
Sources	This paper	Amin, et al. (2008)	Schmidt (1972); Baer (1959); Machado (1950)
Trunk length (mm)	Female about 8.00 mm	Females over 200 mm	Up to 50 mm long
Festoons	2 sets of longitudinal & horizontal festoons near anterior end of trunk	1 set of longitudinal festoons at anterior end of trunk	1 set of longitudinal festoons at anterior end of trunk
Proboscis	Sub-globular, not ornate, slightly longer than wide	Sub-globular, ornate with beady rings around hooks	Not ornate, sub-globular
Proboscis hooks	3 in each of 12 alternating rows	3 in each of 16 rows	3 in each of 12 rows
Barbs on hooks	Absent	Absent	Present
Hook root	Simple, heart-shaped, bluntly pointed. minimal manubria	Simple, heart-shaped, bluntly pointed. No manubria	Manubria large & complex specially anteriorly
Posterior hook root	Heart-shaped-rosette	Large, oblong horizontally	Small, discoid
Lemnisci	Unequal. Long & thick, tapering posteriorly	Subequal. Very long & slender with giant nuclei in swellings	Equal, moderate to very long & slender, multi nucleated
Protonephridia	Obscured	Gill-like, capsular on uterine bell in females	Small, gill-like, curved on uterine bell in females
Female gonopore	Subterminal	Terminal	Subterminal
Host & distribution	Bobcat, USA	Manidae in Vietnam	Primates in South America & Felidae in Africa
Intermediate hosts	Unknown	Ants and termites	Cockroaches & beetles

**Prosthenorchis cerdocyonis* Gomes, Olifiers, Souza, Barbosa, D'Andrea & Maldonado Jr., 2015 from the crab-eating fox in Brazil is not included.

Table 1: Comparison between the genera and the classical species of the *Prosthenorchis* complex (Oligacanthorhynchidae).

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The first set of festoons may be hard to distinguish as it is more deeply embedded in the integument. Species in the two other genera normally have one crown of vertically ribbed festoons each. Some structures such as hook sizes of *N. bifestoonatus* overlap with those in *Paraprosthenorchis ornatus* Amin, Ha, Heckmann 2008 and *P. elegans*, *P. fraterna*, *P. lemuri* (Table 1) and *P. cerdocyonis* [4,14,15] that have the same type of festooned collar totally different from those of *Neoprosthenorchis.* Compared to *N. festoonatus*, *P. ornatus* the trunk is much longer, the hooks are without barbs, in beady grids and in 16 rows, posterior hooks with horizontally oblong root, and the lemnisci are very long and slender with giant nuclei in swellings. In the 4 known species of *Prosthenorchis*, the trunk is up to 50 mm long, hook tips with barbs, hooks roots complex with large manubria, lemnisci are slenderer and may reach the posterior end of trunk, and are found in primates or carnivores in South America and Felidae in Africa. In *N. festoonatus*, the lemnisci are as robust anteriorly as the proboscis receptacle and truncated.

Discussion

Considering the unique pattern of festoons in *Neoprosthenorchis* that are unlike any of those previously described in other acanthocephalans, we redefine the term festoon to indicate "an outcrop of a sculptured decorative curved tegument-based chains or strings in variably shaped vertical or circular arrangements at the anterior end of the trunk of acanthocephalans." This definition applies to the analogous festoons in oligacanthorhynchid acanthocephalans but comparable definitions are found for garlands, architecture, insects and many other contexts in various dictionaries.

Paraprosthenorchis ornatus was reported from the pangolin *Manis pentadactyla* Linn. in Vietnam. The only other oligacanthorhynchids reported from pangolins are *Nephridiorhynchus palawanensis* Tubangui and Masilungan, 1938 collected from *Manis javanica* Desmarest in Palawan Island, the Philippines, and *Oligacanthorhynchus gerberi* (Baer, 1959) Schmidt, 1972 and *Oligacanthorhynchus manisensis* (Meyer, 1931) Schmidt, 1972, from *Manis tricuspis* Rafinesque in Africa. These forms have non-ornate proboscides with 12 rows of 3 or 4 hooks each; the female protonephridia occur as a compact closed capsule and lack festooned collar [16].

Schmidt [4] summarized the status of the genus *Prosthenorchis* and its 3 species known then, e.g., *P. elegans*, *P. fraterna*, and *P. lemuri.* The fourth known species of this genus, *P. cerdocyonis* from the crab-eating fox *Cerdocyon thous* Linn. in the Brazilian Pantanal Wetlands has 12 rows of 3 proboscis hooks each is distinguished from the other 3 species of *Prosthenorchis* by its smaller trunk, longer lemnisci reaching posterior end of the body as they do in *P. fraterna*, prominent paired ducted papillae at the base of proboscis, about 24 irregular festoons with transverse divisions below ribbed neck (Fig. 9), larger eggs, and host and geographical distribution. The number of festoons in the other 3 known species of *Prosthenorchis* have up to 23 vertical ribs and about 35 ribs in *Paraprosthenorchis* [16]. Having only one whole-mounted female specimen provided by Richard Buckner does not provide the opportunity to create SEM, Energy Dispersed x-ray or molecular analysis. The light microscopy images helped elucidate some of the morphological features depicted by line drawings. The implications to the evolutionary tree of oligacanthorhynchid acanthocephalans are enormous but regrettably, one whole mounted specimen will not help elucidate its genetic profile.

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Conflicts of Interest: The authors declare no conflicts of interest.

Ethical Approval: The authors declare that they have observed all applicable ethical standards.

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