# THE PREDOMINANT DEFINITIVE AVIAN HOSTS OF THE INVASIVE ASIAN TREMATODE, *CENTROCESTUS FORMOSANUS*, IN THE HEADWATERS OF THE COMAL, SAN ANTONIO, AND SAN MARCOS RIVERS OF CENTRAL TEXAS

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by

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San Marcos, Texas
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**ACKNOWLEDGEMENTS**

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## TABLE OF CONTENTS

## LIST OF TABLES

**Page**

## LIST OF FIGURES

## ABSTRACT

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SUPERVISING PROFESSOR: DAVID G. HUFFMAN


*Centrocestus formosanus* is a heterophyid trematode of Asian origin that encysts as a metacercaria in the gills of many fishes, and presumably uses local piscivorous birds as definitive hosts. It has been reported to be a serious pathogen of the endangered fountain darter, *Etheostoma fonticola*, in the Comal River (Comal County, Texas). The gills of the darters can be so extensively damaged by this trematode that the worm threatens the survival of the fish in its habitat during low-water conditions. The research objectives were to determine the predominant definitive host of *C. formosanus*, particularly in

the Comal River, and to recover and identify other helminths encountered in the digestive system of the birds during necropsy.

Twelve species of migratory piscivorous birds were collected and examined for adult worms. The birds were collected from three aquatic habitats between San Marcos and San Antonio, inclusively, all of which are dominated by water from the Edwards Aquifer. All birds were then transported on ice to the lab and immediately dissected. The entire digestive tract of the birds was excised, slit longitudinally, and examined briefly for helminths. Specimens found live were fixed using taxon-specific protocols. The intestine and its contents were examined under a dissecting microscope for helminths, with adults of *C. formosanus* being the focus of the search.

Based on presence/absence of adult *Centrocestus,* the avian species that was designated as the predominant definitive host for the parasite in the study area is the Green Heron (*Butorides virescens*). Of the ten Green Herons collected, two were heavily infected with heterophyid trematodes, some of which were identified as *Centrocestus* sp. No evidence was found that any of the other eleven avian species might be important definitive hosts for *C. formosanus* in the study area. Other parasites recovered during the necropsies included several species each of Trematoda, Nematoda, Cestoda, one species of Acanthocephala, and one species of *Giardia*.

# I. INTRODUCTION

*Centrocestus formosanus*, a digenetic trematode of the family Heterophyidae, was first described in Taiwan (Nishıgori 1924). The lifecycle of this parasite includes piscivorous birds as definitive hosts, fishes as second intermediate hosts, and snails as first intermediate hosts (Martin 1958).

The first records of the trematode in North America were in the early 1980's from ponds used by the tropical fish producers in Florida (Blazer and Gratzek 1985; Vogelbein and Overstreet 1988). This record was quickly followed by a report from aquaculture ponds in central Mexico in 1985 (Scholz and Salgado-Maldonado 2000). Scholz and Salgado-Maldonado (2000) also speculated that this Mexico population could have been brought in with imported snails as early as 1979. The worm has since been reported in central Texas fishes from the canal of the San Antonio Zoo (Knot and Murray 1991, cited by Mitchell et al. 2005), the San Marcos River (Mitchell et al. 2000), and in Landa Lake ın the Comal River (Mitchell et al. 2000).

The literature regarding *C. formosanus* in central Texas from 1956 through early 2004 was reviewed by Mitchell et al. (2005). The discovery of *C. formosanus* on several federally listed fishes from central and west Texas raised concern that the parasite may represent an additional threat to the survival of these fishes in the wild (M$^c$Dermott 2000). One of these species, the fountain darter, is restricted to two spring-fed rivers in central Texas, the Comal and the upper San Marcos rivers (U.S Fish and Wildlife Service 1995, cited by Mitchell et al. 2000). Since the metacercariae of the trematode encyst in the gill filaments of the host, the concern was that the infections might result in respiratory distress for the fish. Indeed, a thick layer of host reaction tissue was observed to have formed in the gills of infected fountain darters in response to the parasite (Mitchell et al. 2000). This observation led to questions concerning how the parasite population was being perpetuated in these sensitive habitats.

To date, the only definitive hosts confirmed for *C. formosanus* in the continental United States are the Green Heron (*Butorides virescens*) and the Great Egret (*Ardea alba*) (Mitchell et al. 2005). However, in Mexico, only the Striated Heron (*B. stratus*, thought by some to be synonymous with *B. virescens*) has been reported as definitive host for the parasite (Scholz and Salgado-Maldonado 2000). The information on definitive hosts is expected to be useful in any future plans to mitigate the effects of the trematode on these sensitive ecosystems.

The objectives of this research were:

1. to determine the predominant definitive host of *C. formosanus*, particularly in the Comal River, and
2. to recover and identify to a reasonable level other helminths encountered in the digestive system of the birds during necropsy.

## II. MATERIALS AND METHODS

*Bird Collection areas.*-- The San Antonio Zoo, Bexar County, Texas, pumps Edwards Aquifer water into a canal that flows through the facility and into the headwaters of the San Antonio River. Several native species of herons and egrets have rookeries in trees along the canal and forage for food in the canal. The near constant 22˚C of the well water pumped into the canal is conducive to year-round survival of the Asian snail, *Melanoides tuberculata*, which *C. formosanus* uses as first intermediate host in central Texas. Birds also were collected from Landa Lake, the headwaters of the Comal River (Comal County, Texas), and from Spring Lake and the I-35 crossing of the upper San Marcos River (Hays County, Texas). The headwaters of both rivers issue from the Edwards Aquifer and water temperatures in the rivers are generally between 21 and 24˚C year-round.

*Bird collection.*-- All birds were collected by Fish and Wildlife Service law enforcement agents or Texas Parks and Wildlife game wardens using shotguns. Birds were collected during two seasonal periods: (1) shortly after migrating into the area and prior to egg laying, and (2) after young were fledged. The collection period ranged from the end of March 2006 through the beginning of October 2006. Species targeted for collection are listed in Table 1. Immediately following collection, the birds were enclosed in a water-tight plastic bag, placed on ice, and transported back to the National Fish Hatchery and Technology Center (NFHTC) in San Marcos, Texas. Necropsy was started within 1.5 h of collection.

Table 1. List of birds and numbers collected from Landa Lake, San Antonio Zoo, and Spring Lake.

| Scientific Name | Common Name | Number Collected | | |
|---|---|---|---|---|
| | | SA Zoo | Landa Lake | Spring Lake |
| *Butorides virescens* | Green Heron | 2 | 5 | 3 |
| *Bubulcus ibis* | Cattle Egret | 2 | | |
| *Egretta thula* | Snowy Egret | 2 | | |
| *Egretta caerulea* | Little Blue Heron | 2 | | |
| *Ardea alba* | Great Egret | 2 | | |
| *Podilymbus podiceps* | Pied-billed Grebe | | 2 | |
| *Anas* spp. | Various hybrid ducks | | 2 | |
| *Nyctanassa biolacea* | Yellow-crowned Night Heron | | 2 | |
| *Phalocrocorax auritus* | Double-crested Cormorant | | 1 | |
| *Phalocrocorax brasilianus* | Neotropic Cormorant | | 1 | |
| *Ardea herodias* | Great Blue Heron | | 1 | |
| *Ceryle alcyon* | Belted Kingfisher | | 1 | |

*Examination for Parasites.*-- The eyes and mouths of most birds were examined for helminths. The stomach, intestines, and colon were removed, and placed into separate trays on ice pending examination for parasites. The stomach was opened from end to end with a longitudinal incision, and the contents and lining briefly examined. The entire intestine of small birds was cut into approximately 5-cm segments. These segments were then opened with a longitudinal incision, placed in a Petri dish, rinsed with saline, scraped gently with a blade, rinsed again, and the mucosa and intestinal contents thoroughly examined under a dissecting microscope. The intestine of larger birds was sectioned into anterior, middle, and posterior segments. Then, three or more subsegments 5 cm long from each of the three major segments were examined as above. The entire colon of all birds was thoroughly examined after opening with a longitudinal incision.

*Processing of Parasites.--* Removed parasites were separated into Petri dishes containing physiological saline until the examination of the source organ was completed, then killed by flooding with hot water. In most cases, living representatives of a taxon were studied and photographed with a compound microscope. After killing, the worms were fixed in vials of either 95% ethanol (for subsequent DNA analysis) or 10% formalin (for subsequent presumptive identification and possible permanent mounting in balsam). The trematode specimens that were thought to be *C. formosanus* were transferred to 70% glycerinated ethanol in vials and sent to R.M. Overstreet for confirmation of our presumptive identification. Other parasites found in the intestine were fixed and preserved by appropriate procedures for later identification.

*Determination of Avian Host.--* The species with the highest number of gravid *Centrocestus* adults will be considered as the dominant definitive avian host for the worm in this area. The identification of *Centrocestus* was accomplished by studying various morphological features known to be diagnostic for the genus (Chen 1942, Hernández, Díaz, and Bashirullah 2003, Nishigori 1924). All measurements were accomplished using a calibrated compound microscope.

*H5N1 Containment Protocols.--* Safety precautions were taken to reduce likelihood of contact with the avian influenza virus (H5N1). Safety material employed included nitrile gloves, N95 respirators, and disposable outer garments as specified in

Delaney (2004). These disposable items were discarded at the end of each session, and all lab equipment potentially contaminated during use was disposed of, or washed with dish soap. The bird carcasses were buried at the hatchery.

*Required Permits.*-- Permits authorizing these collections include Federal Fish and Wildlife Permit (No. MB042553-1) and a Texas Parks and Wildlife Permit (No. SPR-0390-045). These permits were issued to Dr. Thomas Brandt (NFHTC).

## III. RESULTS

Intestinal helminthes were observed in all but two of the twenty-eight birds collected; only a Little Blue Heron and a Cattle Egret appeared to have none.

The only observed *Centrocestus* infections were in Green Herons. The first four of the ten Green Herons collected [24 MAR 06(1), 07 APR 06(2), 14 APR 06(1)] were infected with *Centrocestus*; however, the specimens recovered from these four birds were fixed incorrectly due to improperly prepared fixation reagents, and had deteriorated before the error was noticed. Fortunately, high-power photomicrographs had been taken of some of these worms while still alive, and review of these photomicrographs (Figure 1) revealed that many of them were, indeed, *Centrocestus*, and the remainder appeared to be *Ascocotyle* (*Phagicola*) sp. Subsequent review of research logs and low-power photomicrographs of the colons of these birds (Figure 2) indicated that at least two of these Green Herons were heavily infected with heterophyids, with one bird having 200+ worms, and another estimated to have over 300.

Other intestinal helminths recovered from collected birds included several species each of other trematodes, cestodes, nematodes, and acanthocephalans. These other parasites were identified using a variety of taxonomic keys. Some parasites were easily identified down to genus, while others could only be identified down to class, order, or family without additional resources. The numbers of birds of each species positive for each of the four major helminth groups are given in Table 2.

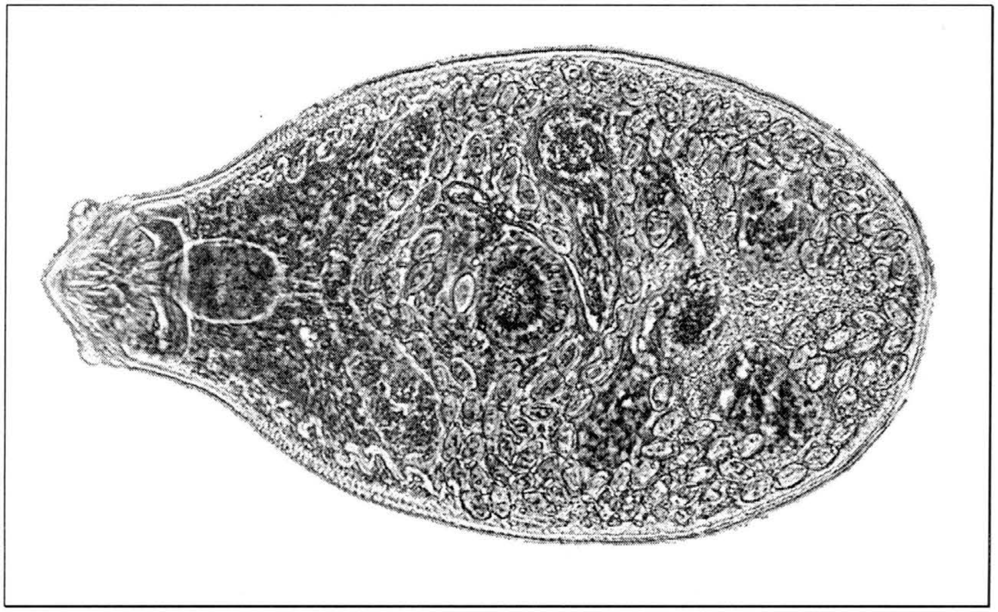

Figure 1. *Centrocestus* sp. found in a Green Heron from Landa Lake.

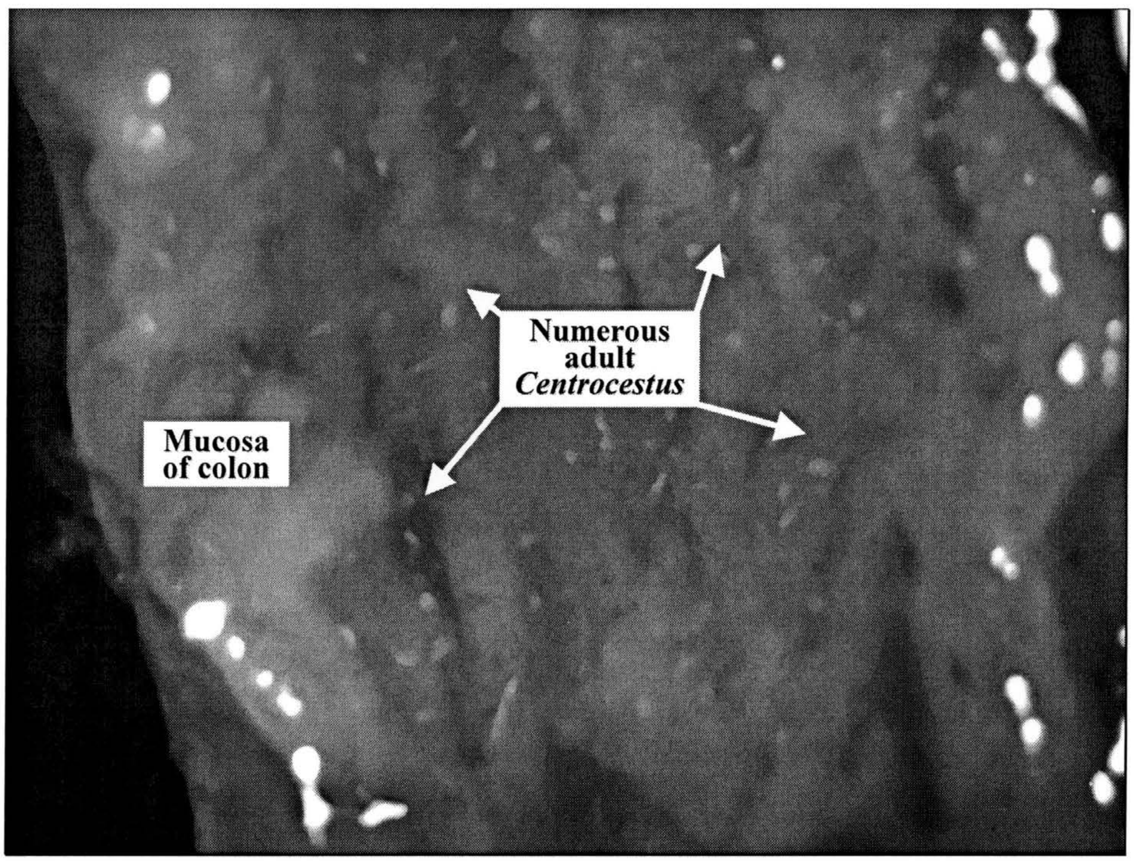


Figure 2. Colon of a Green Heron from San Antonio Zoo showing heavy infection with *Centrocestus* sp.

Table 2. Number of birds of each species positive for each of the four major helminth groups.

| | Numbers of Birds Positive by Bird Species | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Green Heron (10)[1] | Cattle Egret (2) | Snowy Egret (2) | Little Blue Heron (2) | Great Egret (2) | Pied-billed Grebe (2) | Various hybrid ducks (2) | Yellow-crowned Night Heron (2) | Double-crested Cormorant (1) | Neotropic Cormorant (1) | Great Blue Heron (1) | Belted Kingfisher (1) | **Total Birds Positive (28)** |
| Trematodes (other) | 6 | 1 | | 1 | 2 | | 1 | 1 | 1 | 1 | 1 | 1 | **16** |
| Cestodes | 6 | | | | | 2 | 1 | 2 | 1 | 1 | 1 | | **14** |
| Nematodes | 4 | | 1 | 1 | 2 | | 1 | | | | 1 | 1 | **11** |
| Acanthocephalans | 1 | | | | | | 1 | | | | | | **2** |
| **Total Groups** | **4** | **1** | **1** | **2** | **2** | **1** | **4** | **2** | **2** | **2** | **3** | **2** | |

[1] $(n)$ = number of birds examined this species

Some worms, initially identified as *Centrocestus*, were subsequently determined to be *Ascocotyle*[2]. These genera are very similar in appearance, as can be surmised from Figure 1 and Figure 3, and differentiation of specimens into proper genus presented considerable difficulties. At least two species of *Ascocotyle* were recovered from six Green Herons. At least one of these species is in the subgenus *A.* (*Phagicola*).

Some other recovered trematodes have also been identified to family or below. One *Notocotylus* species, shown in Figure 4, was found in a hybrid duck from Landa Lake.

Several specimens in the echinostome genus *Echinochasmus* (Figure 5) were recovered from three birds; a Green Heron and a Great Egret from the San Antonio Zoo, and a Double-crested Cormorant from Landa Lake. At least two, and perhaps three species of *Echinochasmus* (*E. donaldsoni, E. leopoldinae,* and/or *E. macrocaudatus*) are represented among these specimens. The species *E. donaldsoni* and *E. leopoldinae* are very similar in appearance, and have the same number of collar spines (20). One of the main distinctions between the two is the size of the collar spines (Scholz, Ditrich, and Vargas-Vázquez 1996). Attempts to differentiate between the two species with the specimens available have been unsuccessful, but later study of other specimens may yield sufficient information necessary for specific diagnosis. Some echinostome specimens were assigned to *Echinochasmus macrocaudatus*, because they had 22 readily countable collar spines (Ditrich, Scholz, and Vargas-Vázquez 1996). Another echinostome genus (*Echinostoma*, Figure 6) was recovered from a hybrid duck from Landa Lake.

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[2] Robin Overstreet, personal communication

Numerous species of strigeoid trematodes were recovered. *Uvulifer ambloplites,* shown in Figure 7, was recovered from a Belted Kingfisher from Landa Lake. *Clinostomum complanatum* (Figure 8) was recovered from the gullet of a Great Blue Heron from Landa Lake. *Posthodiplostomum minimum* was recovered from a Great Egret from the San Antonio Zoo (Figure 9). Several other unidentified strigeoid species were recovered from various birds, and specimens of three of these species are shown in Figure 10.

Another trematode species recovered from a Green Heron from Landa Lake has not been identified further than Class Trematoda. The peculiar morphology of the excretory bladder of this species is shown in Figure 11.

Fourteen of the birds were infected with cestodes, some of which were identifiable to family or below. A Yellow-crowned Night Heron from Landa Lake was infected with a dilepidid species as shown in Figure 12. Many of the worms in this bird were observed to have their elongate rostellar proboscides deeply imbedded in the crypts of the intestine (Figure 13). The craspedote proglottids and excretory canals of this cestode are readily discernible in Figure 14. Figure 15 shows the strobila and bulb of the scolex of this same species. The calcareous granules of this worm were very abundant, and distributed in a pattern that varied consistently along the strobila. The morphology and distribution pattern of the granules in one section of a strobila are evident in Figure 16. Other morphological features of this peculiar worm, including the bulb of the scolex, the stem of the rostellum, the armed rostellum, and the cone-shaped rostellar apex can be seen in Figure 17 and Figure 18.

Another cestode species, *Lineolepis* sp., from the family Hymenolepididae, was recovered from a Green Heron from Landa Lake (Figure 19). Figure 20 shows the scolex and the circlet of rostellar spines, while Figure 21 shows the armed and distinctly long cirrus of this worm.

A dioecious cestode with unilateral genital openings and four suckers on an unarmed scolex was recovered from a Green Heron from the San Antonio Zoo (Figure 22). The worm is provisionally placed in the family Dioecocestidae, although not all of the features match any known dioecocestid genus. The strobila of this cestode is shown in Figure 23, while Figure 24 shows the proglottids with unilateral cirri. A microphotograph of the very small eggs of this same cestode is provided in Figure 25.

Several species of nematodes and acanthocephalans were also recovered from various birds (Table 2), but identifications have not yet been attempted on these specimens.

One Cattle Egret from the San Antonio Zoo was heavily infected with the flagellate *Giardia* (Figure 26), and was presumptively identified as *G. ardeae*.

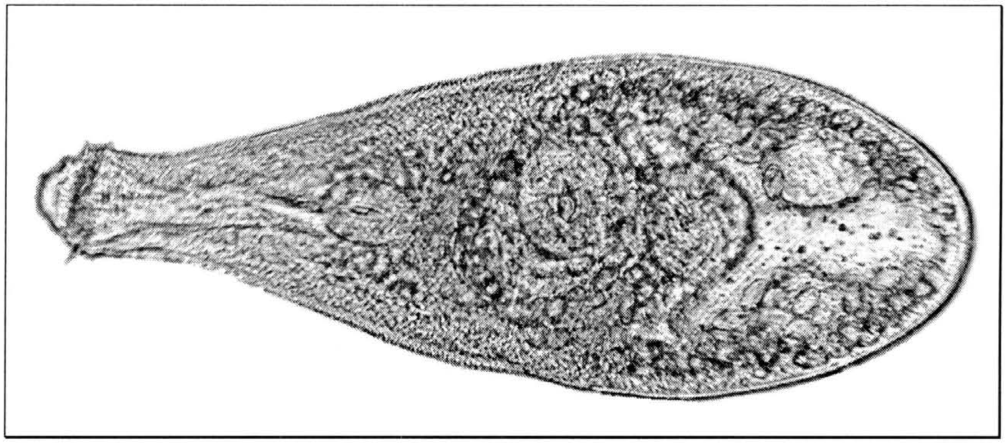

Figure 3. *Ascocotyle* sp. recovered from a Green Heron from Landa Lake.

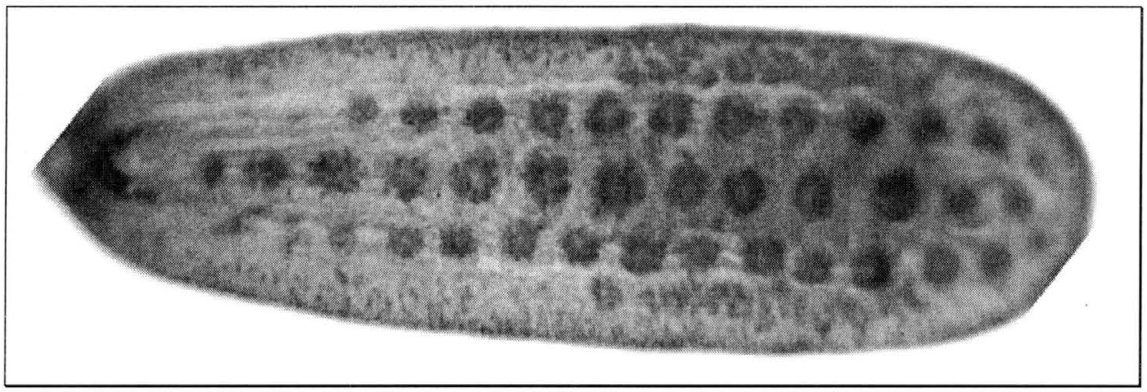

Figure 4. N*otocotylus* sp. recovered from a hybrid duck from Landa Lake.

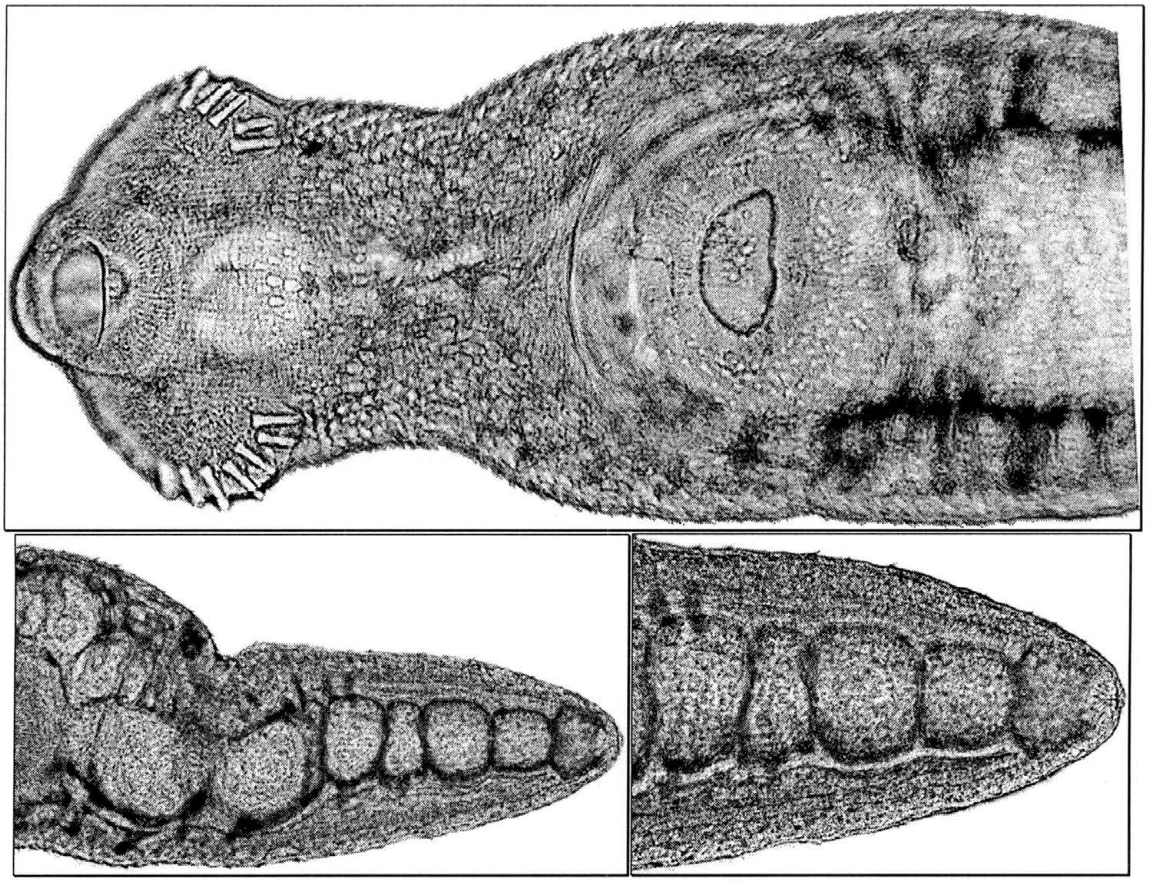

Figure 5. *Echinochasmus* species found in a Green Heron from San Antonio Zoo.

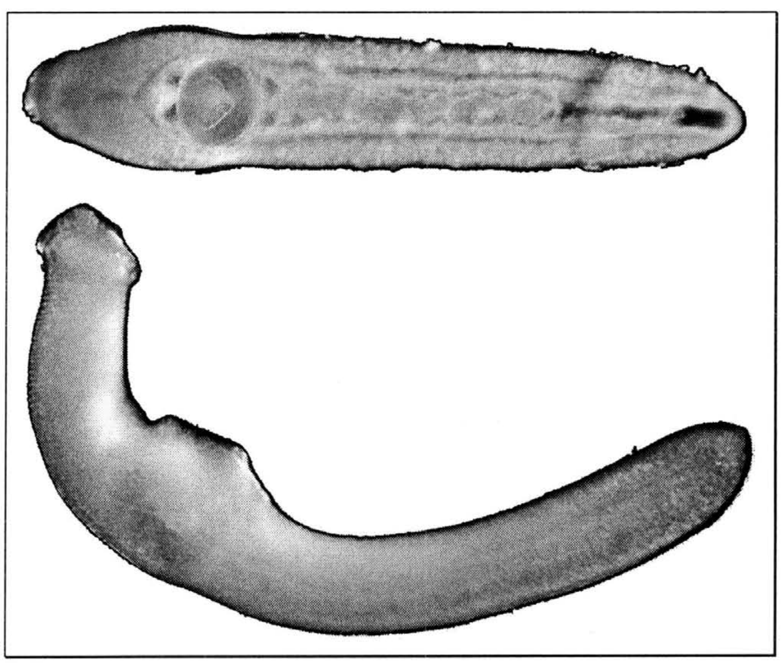

Figure 6. *Echinostoma* species found in a hybrid duck
from Landa Lake.

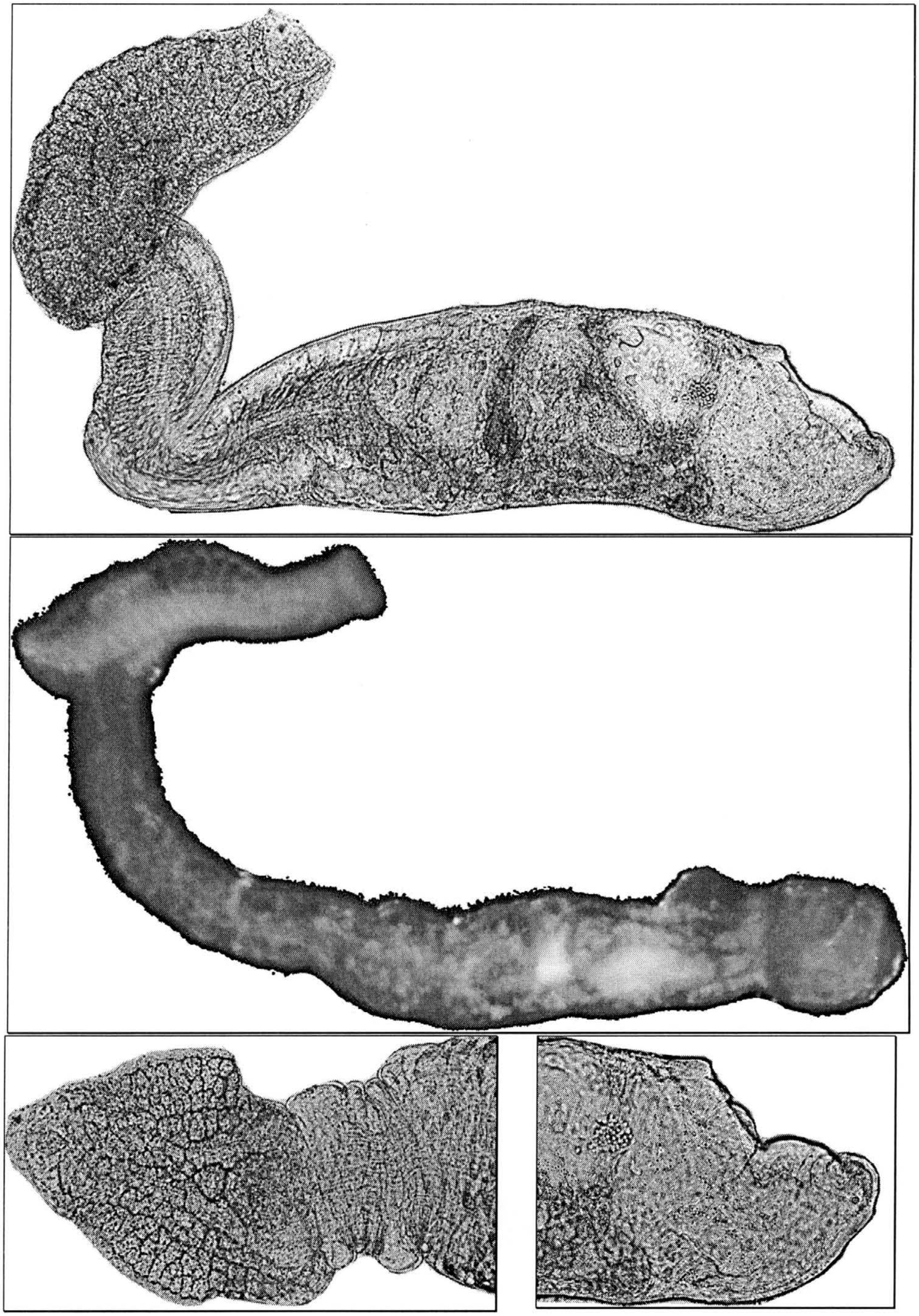

Figure 7. *Uvulifer ambloplites* from a Belted Kingfisher from Landa Lake.

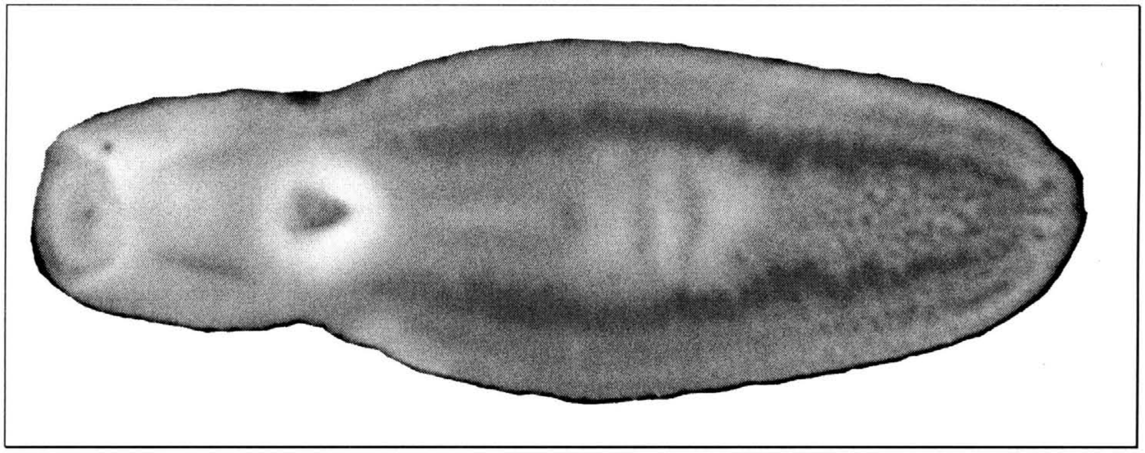

Figure 8. *Clinostomum* species found in a Great Blue Heron from Landa Lake.

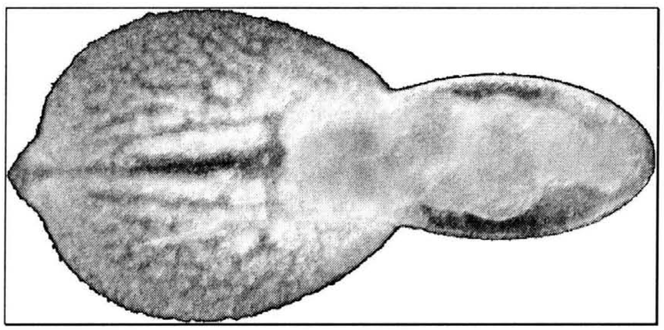

Figure 9. *Posthodiplostomum minimum* from a Great Egret from the San Antonio Zoo.

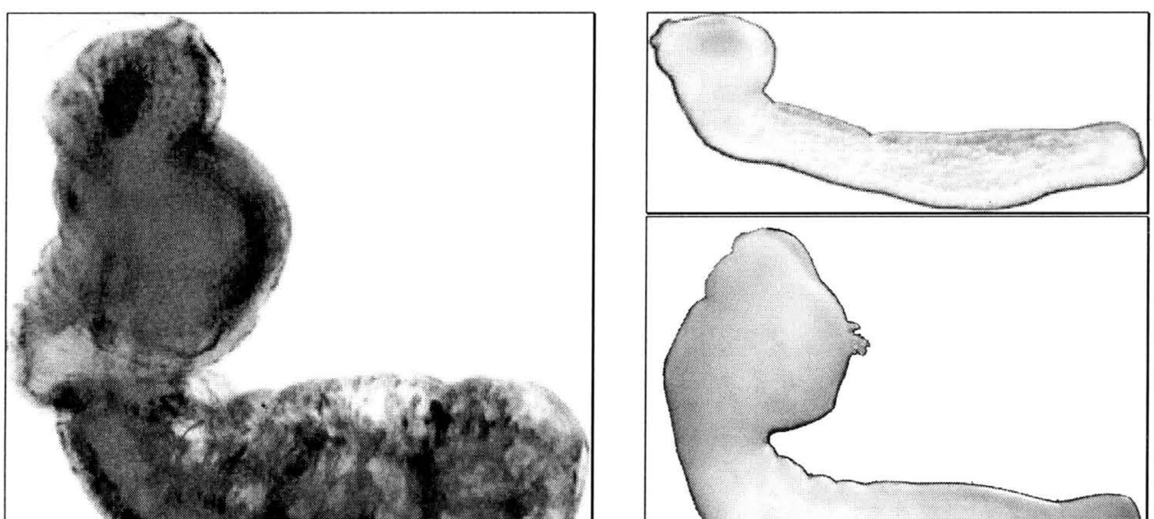

Figure 10. Images of three of the unidentified strigeoid trematodes from various birds.

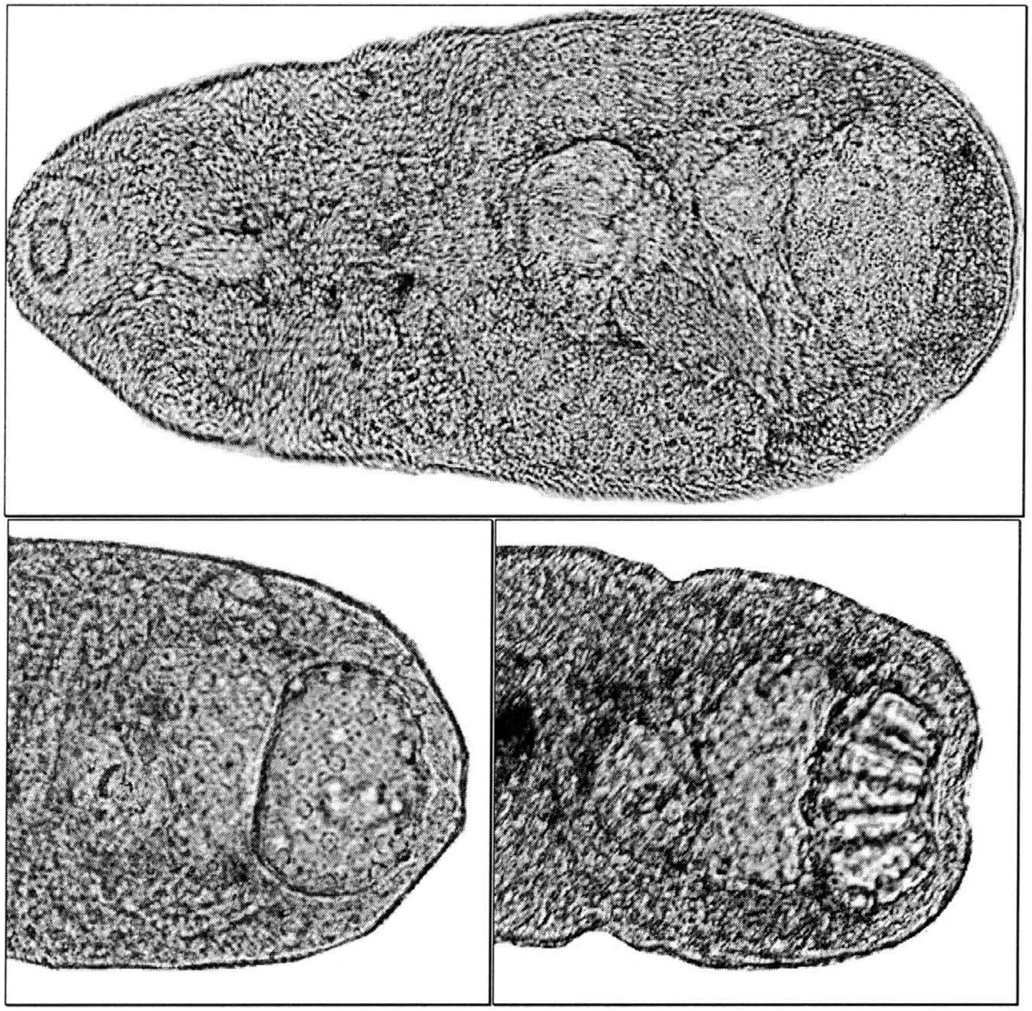

Figure 11. Trematode "A" from a Green Heron from Landa Lake, showing peculiar excretory bladder morphology.

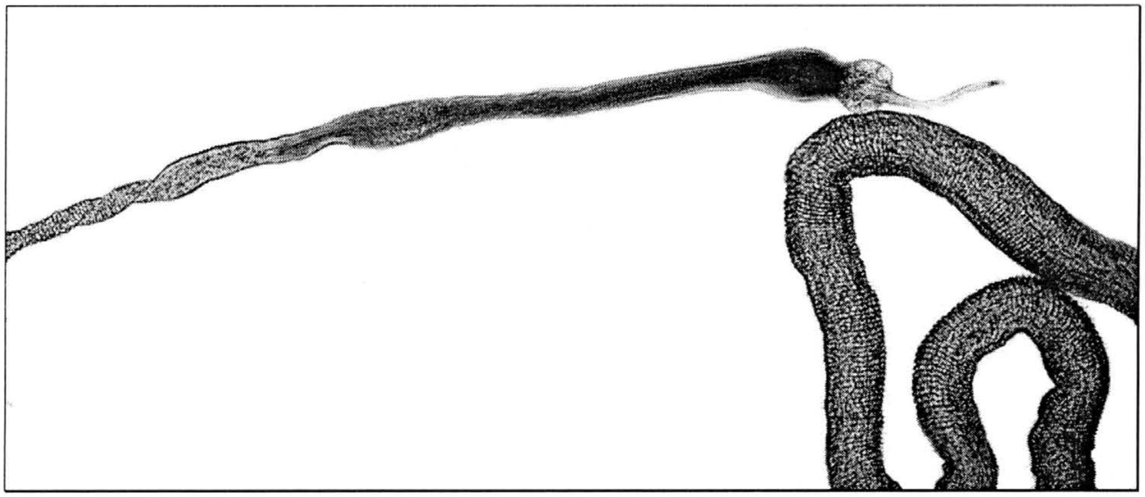

Figure 12. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake.

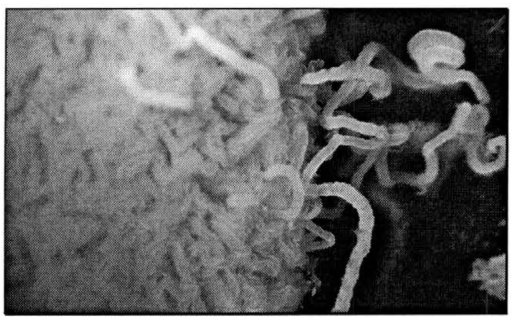

Figure 13. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; worms in intestine.

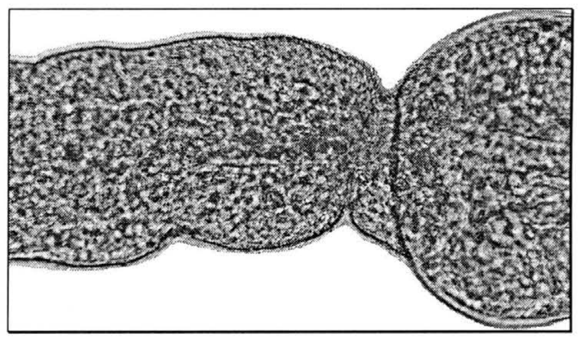

Figure 15. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; strobila, and bulb of scolex.

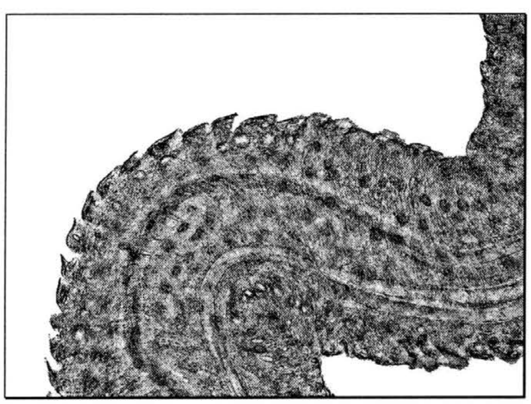

Figure 14. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; proglottids and excretory canals.

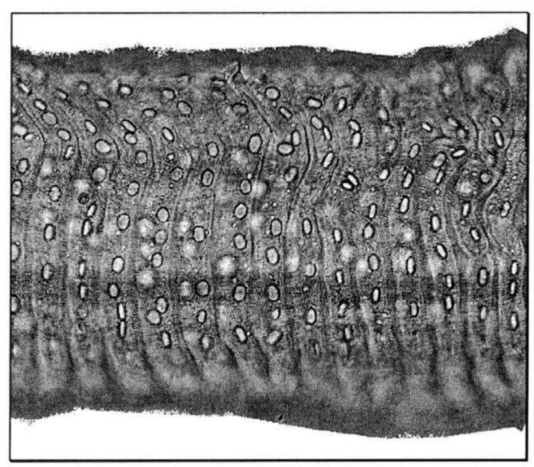

Figure 16. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; calcareous granules.

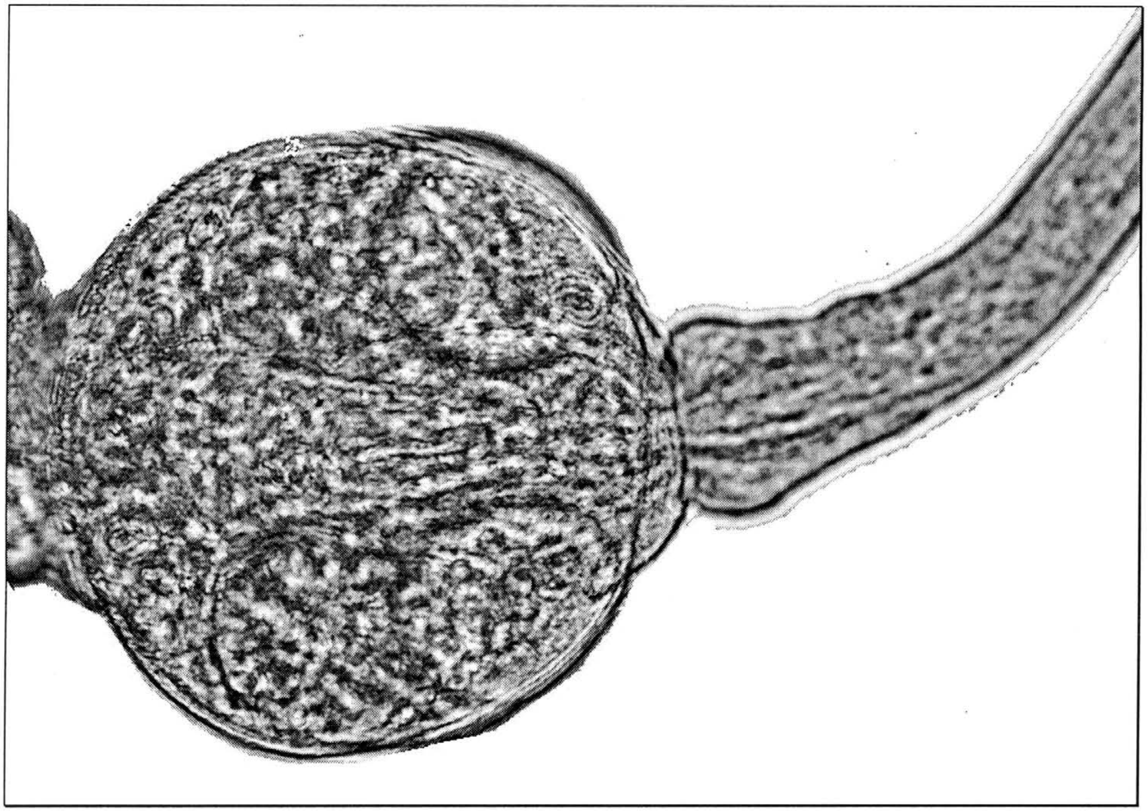

Figure 17. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; bulb of scolex and stem of rostellum.

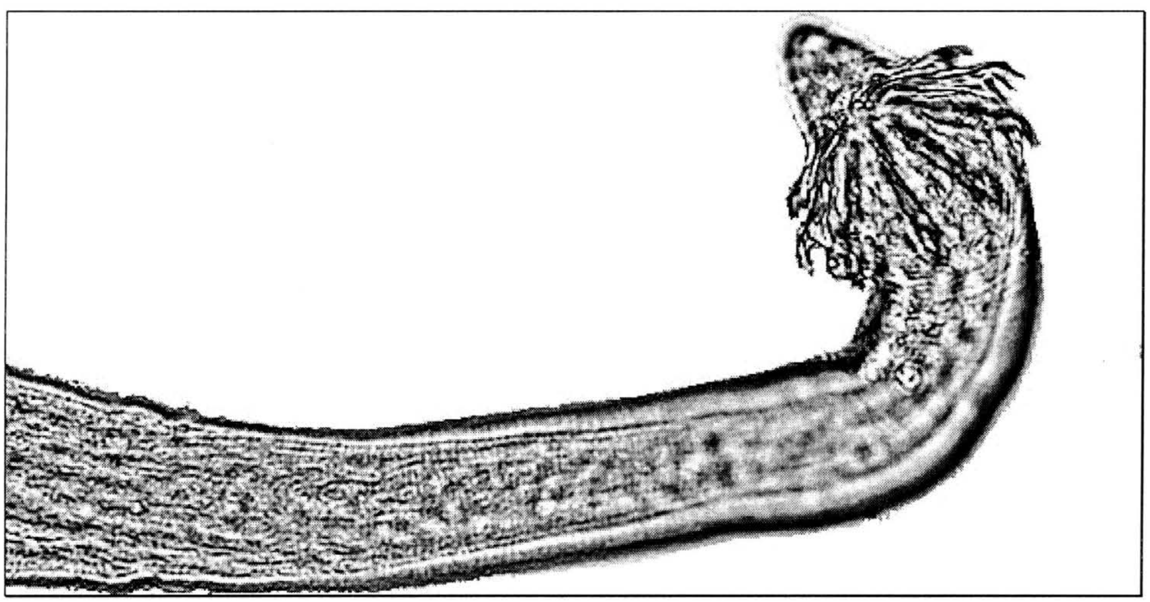

Figure 18. Dilepidid cestode from a Yellow-crowned Night Heron from Landa Lake; armed rostellum with cone-shaped apex.

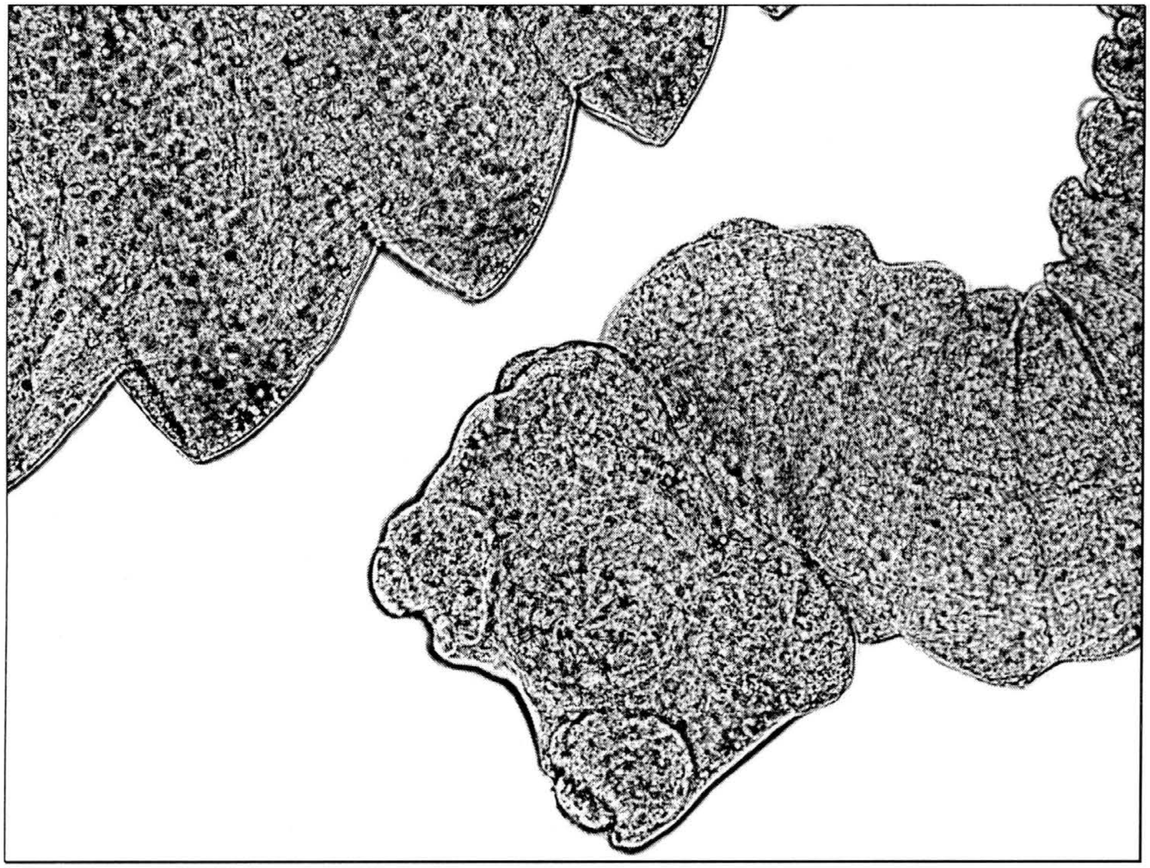

Figure 19. *Lineolepis* sp. (Hymenolepididae) from a Green Heron from Landa Lake.

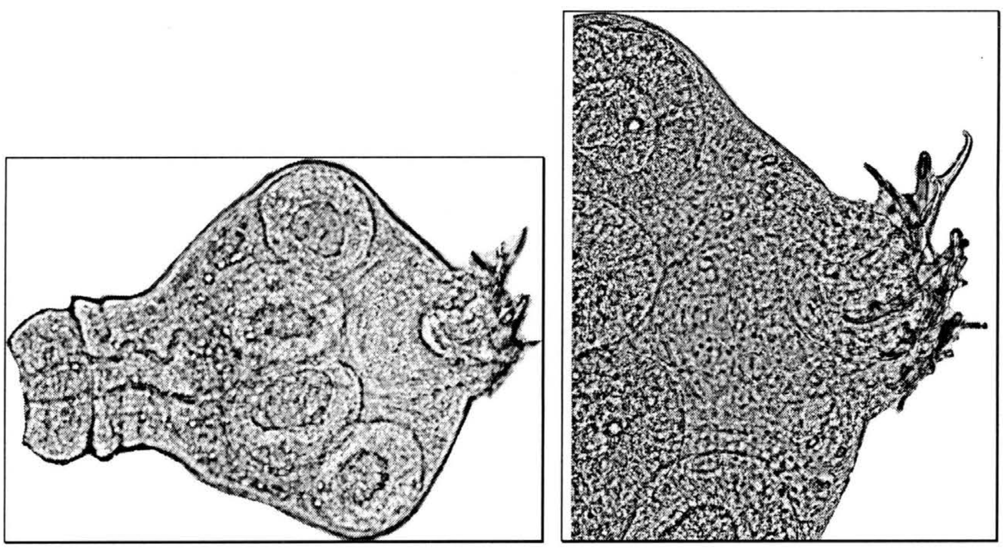

Figure 20. *Lineolepis* sp. (Hymenolepididae) from a Green Heron from Landa Lake, showing scolex and armed rostellum.

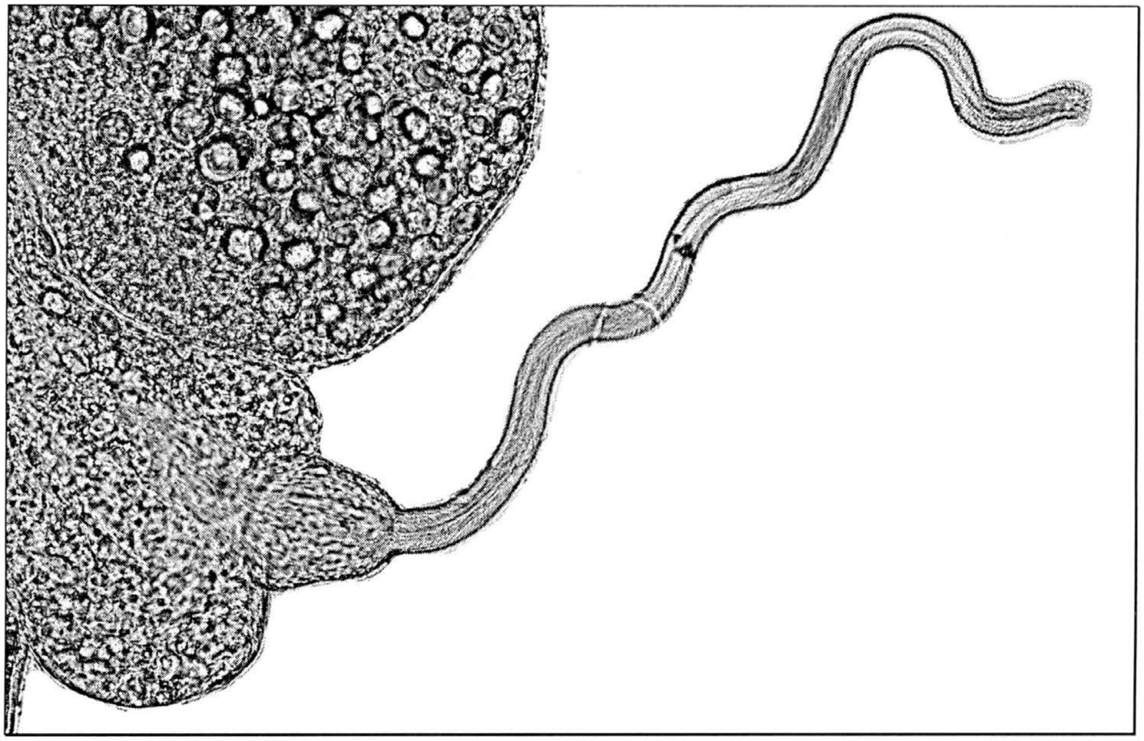

Figure 21. *Lineolepis* sp. (Hymenolepididae) from a Green Heron from Landa Lake, showing cirrus.

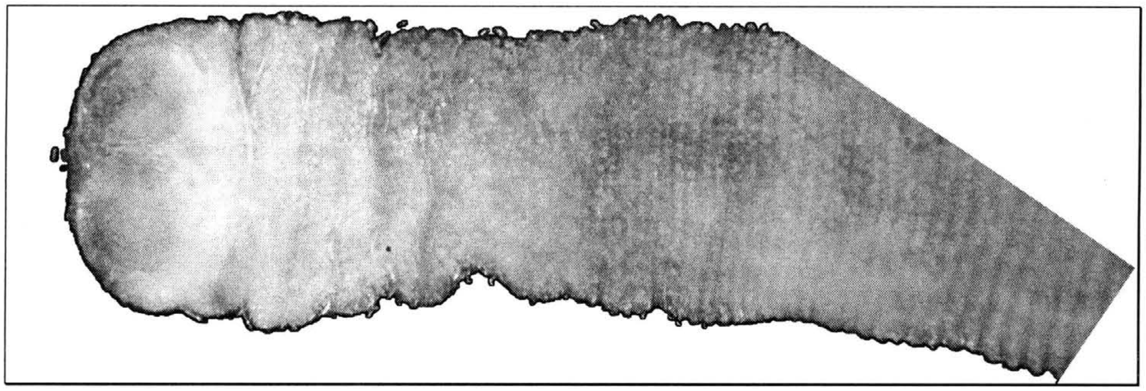

Figure 22. Dioecocestid? tapeworm recovered from a Green Heron from the San Antonio Zoo; scolex.

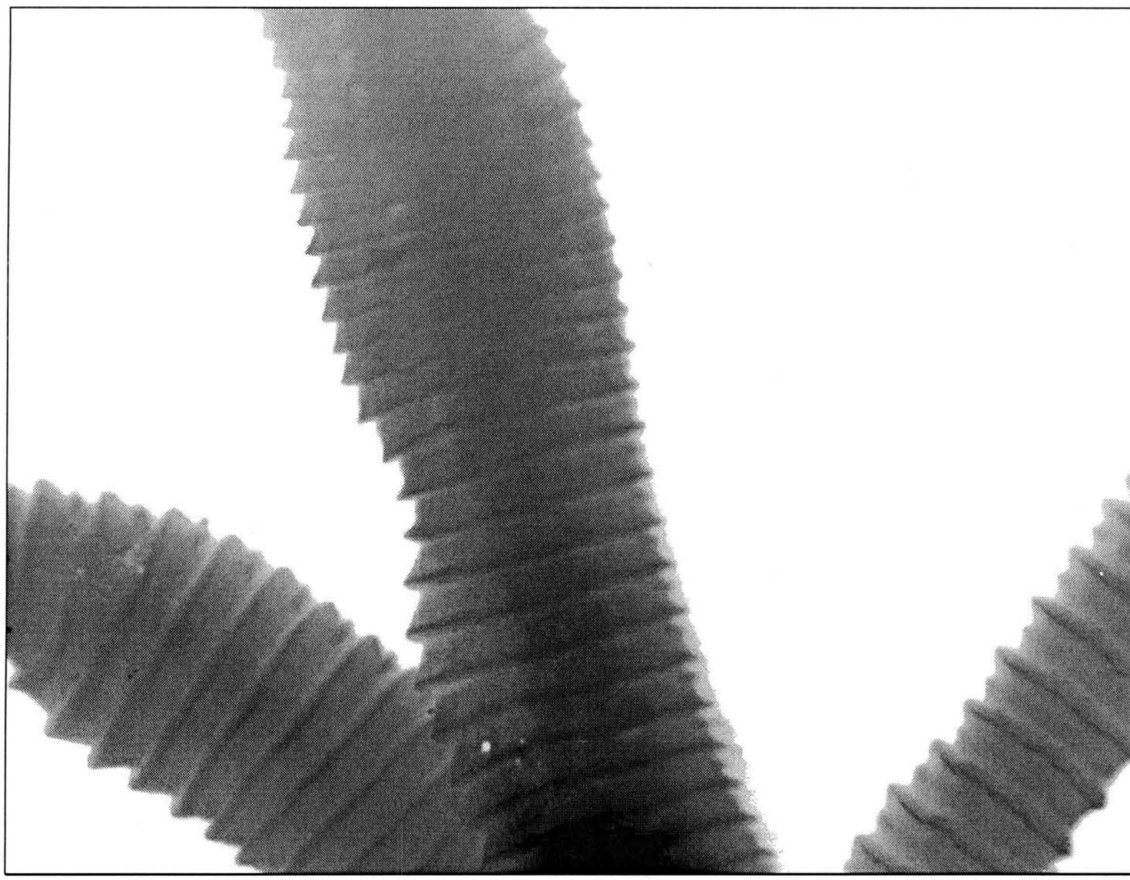

Figure 23. Dioecocestid? tapeworm recovered from a Green Heron from the San Antonio Zoo; female strobilus.

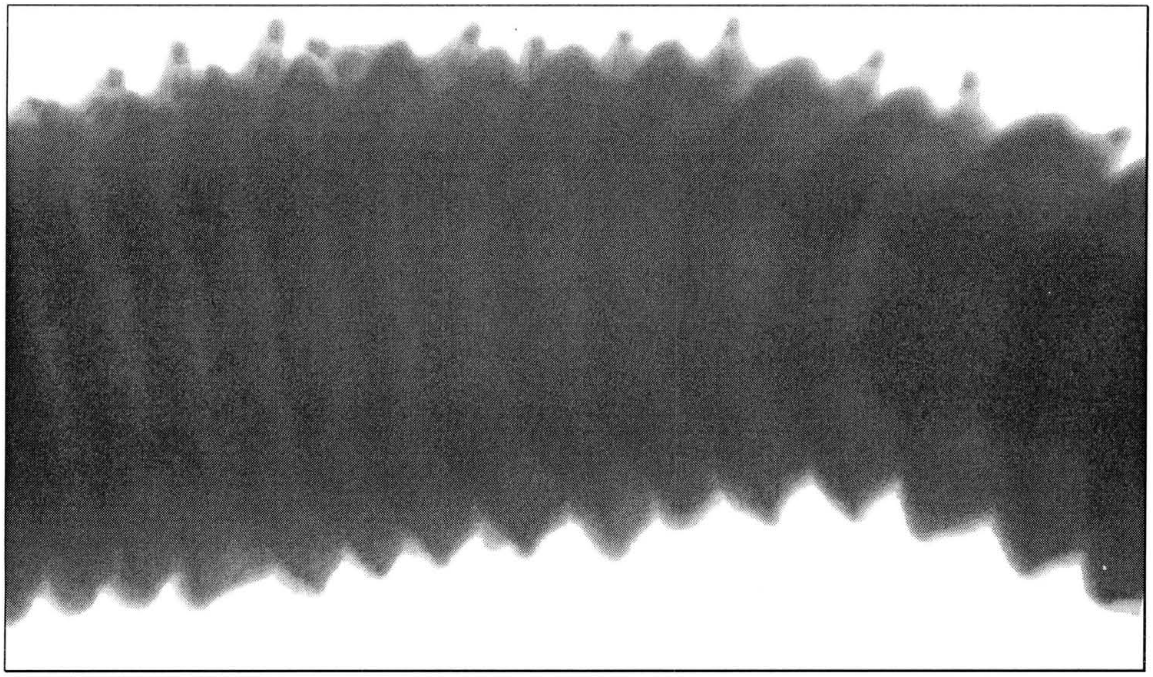

Figure 24. Dioecocestid? tapeworm recovered from a Green Heron from the San Antonio Zoo; male strobilus showing unilateral cirri.

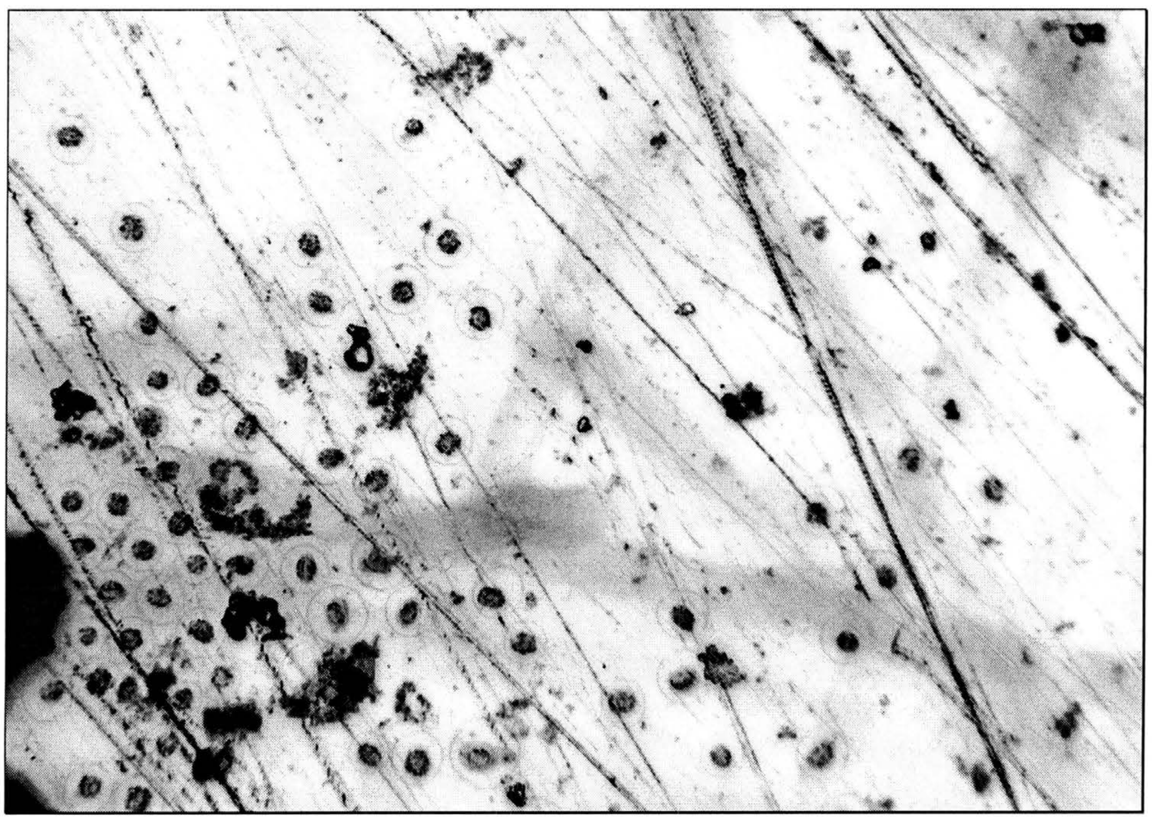

Figure 25. Dioecocestid? tapeworm recovered from a Green Heron from the San Antonio Zoo; eggs.

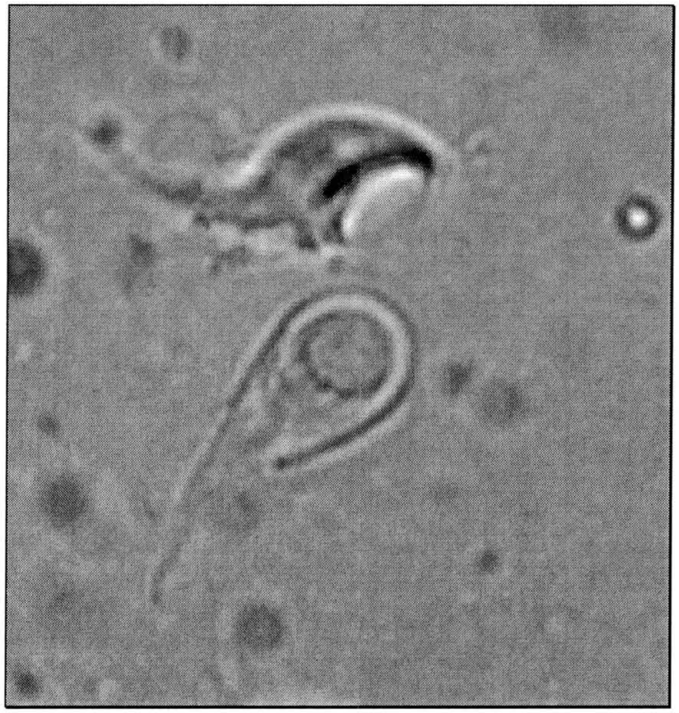

Figure 26. *Giardia* species found in a Cattle Egret from the San Antonio Zoo.

## IV. DISCUSSION

*The Definitive Host of* Centrocestus formosanus *in Central Texas*

Although heterophyid trematodes are commonly encountered, the taxonomy of the family Heterophyidae is still unsatisfactory (Scholz 1999), despite the voluminous taxonomic literature that focuses on this family. Since *Centrocestus formosanus* is the principal species in this study, criteria for differentiating between *Centrocestus* and *Ascocotyle* was determined to be of prime importance, but has proven to be problematic. Traditional morphological criteria that are often used to assign a specimen to a species in either of these genera have met with mixed results in this study. Both genera are characterized as being pyriform in shape, having two testes positioned directly opposite each other at the posterior end of the body, having at least one ring of conspicuous spines surrounding the oral sucker, and having similar juxtaposition of most other organs (Nishigori 1924, Scholz 1999). Oral spine count and distribution can be crucial for differentiation of some species of heterophyid genera, but these spines are difficult to count on many specimens, and are often lost in processing (Scholz et al. 1997a).

After studying *C. formosanus* in Mexico, Scholz and Salgado-Maldonado (2000) concluded that the oral sucker is surrounded by 32 circumoral spines arranged in two unbroken circlets of 16. Chen (1942) noted that the spines of the anterior circlet are longer and heavier than those of the posterior circlet. *Ascocotyle* species, on the other hand, usually have one row of sixteen spines; however, one species, *Ascocotyle (Phagi-*

*cola) inglei* has one row of nineteen spines (Scholz 1999), and others may also have an additional incomplete circlet of two to six spines. Another species, *Ascocotyle* (*A.*) *nunezae*, is described by Scholz et al. (1997b) as having an oral sucker surrounded by 32-37 circumoral spines arranged in 1 complete row of 25-27 spines and 6-10 accessory spines on dorsal side. In addition, the taxonomic literature regarding species of *Ascocotyle* sometimes recommends diagnostic criteria that lead to erroneous assignment of specimens to species (Scholz 1999). In addition to these confusing factors, some authors place the taxon *Phagicola* at the level of genus, while others consider it to be a subgenus of *Ascocotyle* (Scholz 1999).

The detailed morphology of the terminal genitalia is of prime importance in the taxonomy of *Ascocotyle* and other heterophyids (Scholz 1999), but these important details have been overlooked in most of the literature. Such detailed morphological studies, which generally require serial sectioning, are also beyond the scope of the present study.

In this study, all worms identified as *Centrocestus* had: (1) two unbroken circlets of about sixteen spines, each with spines of the anterior circlet larger than those of the posterior circlet (Chen 1942), (2) a completely spinose body (Chen 1942), (3) morphological measurements comparable to those provided by Hernández, Díaz and Bashirullah (2003), and (4) organ juxtaposition consistent with that described by Nishigori (1924). Once a specimen has been assigned to the genus *Centrocestus*, the issue of prime importance then becomes finding reliable criteria for distinguishing between *C. formosanus* and some other known (or perhaps unknown) species of *Centrocestus*.

When this study was initiated, the heterophyid problem in the fountain darters of the Comal River had been under study by various researchers since May 1997, and the worm was first identified by Mitchell et al. (2000) as *C. formosanus*. Most of the previous studies focused on the larval stages of the worm. Since the present study began, with a focus on the adults of the heterophyid parasite, the specific identity of the worm as *C. formosanus* has become increasingly suspect. Not only has it been difficult to identify any of the adult heterophyids recovered in this study to genus, but all of the previous literature that focuses on *C. formosanus* as the cause of the problem in the Comal River is now suspect. Perhaps the mixed conclusions in previous studies are not due as much to environmental variables as it is to a previously undiagnosed mix of trematode species in the data. Therefore, a focus on *C. formosanus* as the species of interest is perhaps premature until the taxonomic confusion between the genera *Centrocestus* and *Ascocotyle* is resolved, and the North American species in the genera are described with reliable characters.

*Implications for Possible Management of the Heterophyid problem in Central Texas*

Controlling the heterophyid problem in the study area may be a complicated task. Since representatives were only found in Green Herons in the study area, it is considered the definitive host for the area. However, both *Centrocestus* and *Ascocotyle* (*Phagicola*) were found in Green Herons during the present study. If both genera are found in the fountain darter, then the problem is much bigger than *C. formosanus*, and perhaps the likelihood of the fountain darter becoming locally extinct is even greater than previously anticipated. This would mean there are two different problems working against the same endangered species. However, if only *Centrocestus* is found within the fountain darter, then there is at least one other fish in the study area that is infected with *Ascocotyle* (*Phagicola*).

## V. CONCLUSION

*Centrocestus formosanus* has become a serious pathogen of many fishes. It can be transported to aquatic habitats that possess endangered species. This can lead to extinction of those sensitive animals. The San Marcos, Comal, and San Antonio Rivers, are dominated by water from the Edwards Aquifer. The San Marcos and Comal rivers serve as natural habitat for the endangered fountain darter. If the heterophyid community were to continue expanding, it could lead to local extinction of the darters in these sensitive habitats. However, several other heterophyid species also were found in the Green Heron, and these two species are very similar, leading to easily confusing one for the other. It is still possible that only *C. formosanus* infects the fountain darter, and the other heterophyid species are infecting other local fishes. But at this writing, the question of the identity of the causative agent in the metacercarial disease of the fountain darter gills remains unanswered.

Based on presence/absence of adult worms tentatively identified as *C. formosanus*, the avian species that is herein designated as the predominant definitive host for the parasite in the study area is the Green Heron (*Butorides virescens*). No evidence was found that any of the other eleven avian species might be important definitive hosts for the parasite, because no worms identified as heterophyids were recovered from any of them in this study.

Other parasites recovered during the necropsies include several species each of Trematoda, Nematoda, Cestoda, two species of Acanthocephala, and one species of *Giardia*. More research needs to be done on further identifying parasites collected from intestines of birds in the study area to make a parasite list for each host.

## LITERATURE CITED


Blazer, V.S., and J.B. Gratzek. 1985. Cartilage proliferation in response to metacercarial infections of fish gills. Journal of Comparative Pathology 95:273-280.

Chen, H.T. 1942. The metacercaria and adult of *Centrocestus formosanus* (Nishigori 1924), with notes on the natural infection of rats and cats with *C. armatus* (Tanabe, 1922). Journal of Parasitology 28:285-298.

Delaney, L.J. 2004. Avian Influenza Update: Worker Health and Safety. NIOSH. Available: www.cdc.gov/flu/pp/worker_health_safety_11_2004.pdf. (January 2005).

Hernández, Luz E., Marcos T. Díaz , and Abul K. Bashirullah. 2003. Description of different developmental stages of *Centrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae). Revista Cientifica 13(4):285-292.

Martin, W.E. 1958. The life history of some Hawaiian heterophyid trematodes. Journal of Parasitology 44:305-318.

McDermott, K.S. 2000. Distribution and infection relationships of an undescribed digenetic trematode, its exotic intermediate host, and endangered fishes in springs of West Texas. Thesis, Southwest Texas State University. 26pp.

Mitchell, A.J., R.M. Overstreet, A.E. Goodwin, and T.M. Brandt. 2005. Spread of an exotic fish-gill trematode: A far-reaching and complex problem. Fisheries 30(8):11-16.

Mitchell, A.J., M.J. Salmon, D.G. Huffman, A.E. Goodwin, and T.M. Brandt. 2000. Prevalence and pathogenicity of a heterophyid trematode infecting the gills of an endangered fish, the fountain darter, in two central Texas spring-fed rivers. Journal of Aquatic Animal Health 12:283-289.

Nishigori, M. 1924. On a new trematode *Stamnosoma formosanum* n. sp., and its life history. Taiwan Igakkai Zasshi 234:181-228.

Scholz, Tomáš. 1999. Taxonomic study of *Ascocotyle (Phagicola) longa* Ransom, 1920 (Digenea: Heterophyidae) and related taxa. Systematic Parasitology 43:147-158

Scholz, Tomáš, Joaquin Vargas-Vázquez, Victor Vidal-Martinez, and Leopoldina Aguirre-Macedo. 1997a. Species of *Ascocotyle* Looss, 1899 (Digenea: Heterophyidae) from the Yucatan Peninsula, Mexico. Systematic Parasitology 36:161-181.

Scholz, Tomáš, Joaquin Vargas-Vázquez, Victor Vidal-Martinez, and Leopoldina Aguirre-Macedo. 1997b. Ascocotyle (A.) nunezae n. Sp. (Digenea: Heterophyidae) from Yucatan, Mexico. Journal of Parasitology 83(1):141-147.

Scholz, Tomáš, O. Ditrich, and J. Vargas-Vázquez. 1996. *Echinochasmus leopoldinae* n. sp. (Trematoda: Echinostomatidae) and data on its life-cycle. Systematic Parasitology 33:157-165.

Scholz, T., and G. Salgado-Maldonado. 2000. The introduction and dispersal of C*entrocestus formosanus* (Nishigori, 1924) (Digenea: Heterophyidae) in Mexico: a review. American Midland Naturalist 143:185-2020.

Vogelbein, W.K., and R.M. Overstreet. 1988. Life-history and pathology of a heterophyid trematode infecting Florida-reared ornamental fishes. International Association for Aquatic Animal Medicine Proceedings 19:138.

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