

**EPIZOOTOLOGY, DEVELOPMENT, AND PATHOLOGY OF
GEOPETITIA ASPICULATA WEBSTER, 1971
(NEMATODA: HABRONEMATOIDA) IN TROPICAL
BIRDS AT THE ASSINIBOINE PARK ZOO, WINNIPEG,
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EPIZOOTIOLOGY, DEVELOPMENT, AND PATHOLOGY OF *GEOPETITIA ASPICULATA* WEBSTER, 1971 (NEMATODA: HABRONEMATOIDEA) IN TROPICAL BIRDS AT THE ASSINIBOINE PARK ZOO, WINNIPEG, CANADA

Cheryl M. Bartlett,¹ Graham J. Crawshaw,² and Ralph G. Appy¹

ABSTRACT: *Geopetitia aspiculata* was found in 12 species of Passeriformes, one species of Coraciiformes, and one species of Charadriiformes which died in a free-flight area in the Tropical House at the Assiniboine Park Zoo, Winnipeg, Canada. The nematodes occurred in chronic inflammatory cysts attached to the serosa of the posterior end of the esophagus, proventriculus, and the anterior part of the gizzard. Posterior ends of worms were observed to extend from the cyst into the lumen of the proventriculus. Birds hatched and raised in the Tropical House acquired infections, probably through the agency of feral crickets. Experimental studies showed that *G. aspiculata* developed to the infective third stage in the fat body of *Acheta domesticus* (L.). This is the first transmission cycle of *Geopetitia* spp. to be elucidated and the egg, first, third, and early fifth stages of the parasite are described. The absence of spicules in males of *G. aspiculata* is confirmed. *Geopetitia aspiculata* was probably introduced to the Zoo by infected tropical birds obtained from dealers or other zoos. The wild source of the parasite is not known since *G. aspiculata* has never been reported in wild birds; the report of *G. aspiculata* in wild Piciformes in Cuba by Barus (1971) is in error.

INTRODUCTION

Numerous periproventricular nematodes, identified herein as *Geopetitia aspiculata*, were found in various birds which died in the Tropical House at the Assiniboine Park Zoo in Winnipeg, Canada. Some infected birds had been hatched and raised in the Tropical House, indicating that infections were being acquired within the exhibit. The transmission of *Geopetitia* spp. has not previously been investigated and experimental studies were undertaken to determine potential intermediate hosts of *G. aspiculata* in the Zoo. The present paper discusses factors which probably led to the epizootic, lists the bird species involved, and describes lesions observed in infected birds. It also gives the results of studies on experimental trans-

mission and describes the post-embryonic development of the parasite.

MATERIALS AND METHODS

Encysted periproventricular nematodes found in birds which died in the Tropical House (see description below) at the Assiniboine Park Zoo, Winnipeg, Manitoba, Canada, were fixed in 10% buffered formalin and sent to the University of Guelph for identification. Worms were then transferred to 70% glycerin-alcohol and examined in glycerin. Tissues from some birds harboring these nematodes were fixed in 10% buffered formalin, sectioned at 7 μ m and stained with hematoxylin and eosin. Other worms and tissues containing worms were transferred from formalin to 2.5% cacodylate buffered glutaraldehyde for 1 hr and 1% cacodylate buffered osmium tetroxide for 12 hr. They were dehydrated in ethanol, critical point dried by CO₂ substitution, mounted on metal stubs, coated with gold-palladium and examined using a JEOL JSM 35-C scanning electron microscope.

Nematodes from some kiskadees, a greyish saltator, and a woodhoopoe (see below) were placed in plastic bags in insulated packages containing ice and shipped air express to the University of Guelph. Upon receipt, pieces of gravid female worms were placed on apple peel and contaminated peels were fed to crickets (*Acheta domesticus* (L.)) and cockroaches (*Leucophaea maderae* (Fab.) and *Periplaneta*

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americana (L.)). Arthropods were maintained at room temperature (approximately 22 C) and given dried dog food and water ad libitum. They were dissected at various intervals postinfection and examined for larval nematodes. Some larvae were fixed in hot 70% glycerin-alcohol and studied in glycerin.

Some larvae recovered from crickets which had been fed fragments of worms from a kiskadee were placed in 0.75% physiologic saline and administered orally to two cutthroat finches (*Amadina fasciata* (Gmelin)) purchased at a local pet store. Finches were housed together and fed canary food. One finch received eight molting second-stage larvae and was killed 60 days postinfection. The other finch received six third-stage larvae and was killed 35 days postinfection. Two additional cutthroat finches purchased at the same pet store were used as controls and were killed 60 days after they were purchased. All finches were examined and nematodes recovered were fixed in hot 70% glycerin-alcohol and studied in glycerin.

Measurements of worms are either given as mean, followed by range in parentheses ($n > 3$) or listed ($n \leq 3$).

The paratype specimens of *Geopetitia aspiculata* in the U.S. National Parasite Collection (No. 38988) were examined.

Description of the Tropical House

The Tropical House at the Assiniboine Park Zoo consisted of a large (approximately 1,100 m²) free-flight flight area containing tropical vegetation, reptiles, mammals, and birds. Among the latter were species of Ciconiiformes, Anseriformes, Galliformes, Charadriiformes, Columbiformes, Cuculiformes, Coraciiformes, Piciformes, and Passeriformes. During the summer these birds had access also to two 46-m² outdoor flight cages. Separate enclosed exhibits within the building housed additional mammalian and avian species. Food given to the various animals included chopped fruit, seeds, dog chow, ground meat and "farm-raised" crickets. Feral invertebrates in the building included cockroaches, ants, woodlice and beetles. It is thought that escapees among the farm-raised crickets may have established reproducing populations in the exhibit.

RESULTS

Diagnosis

Periproventricular nematodes found in birds at the Assiniboine Park Zoo were identified as *Geopetitia aspiculata* Webster, 1971 (Spirurida, Habronematoidea,

Tetrameridae, Geopetitiinae). Diagnoses were based on Chabaud (1975) and Webster (1971). Mature adult specimens were similar morphologically to the paratypes of *G. aspiculata*; males lacked spicules.

Infected birds

The following species of birds which died at the Assiniboine Park Zoo during 1976–1983 were infected with *G. aspiculata* (common name and number infected are given in parentheses).

PASSERIFORMES—Tyrannidae: 1) *Pitangus sulphuratus* (L.) (kiskadee, 8); Emberizidae: 2) *Tachyphonus rufus* (Boddaert) (white-lined tanager, 4), 3) *Saltator coerulescens* Vieillot (greyish saltator, 1), 4) *Thraupis palmarum* (Wied) (palm tanager, 1), 5) *Thraupis episcopus* (L.) (blue-gray tanager, 1); Muscicapidae: 6) *Myiophoneus caeruleus* (Scopoli) (blue whistling thrush, 2), 7) *Minla cyanouroptera* (Hodgson) (blue-winged siva, 1); Irenidae: 8) *Irena puella* (Latham) (fairy bluebird, 1), 9) *Chloropsis hardwickei* Jardine and Selby (orange-bellied leafbird, 1); Pycnonotidae: 10) *Pycnonotus cafer* (L.) (red-vented bulbul, 1), 11) *Pycnonotus jocosus* (Linnaeus) (red-whiskered bulbul, 1); Ptilonorhynchidae: 12) *Sericulus chrysocephalus* (Lewin) (regent bowerbird, 1). CORACIIFORMES—Upupidae: 13) *Phoeniculus purpureus* (Miller) (green woodhoopoe, 1). CHARADRIIFORMES—Charadriidae: 14) *Hoplopterus armatus* (Burchell) (black-smith plover, 1).

All infected birds had been housed in the free-flight area of the Tropical House. When found, birds were either dead or moribund. Zoo records showed that most infected birds did not exhibit previous signs of illness. One kiskadee, however, had an enlarged abdomen which showed radiographic evidence of an intraabdominal mass. The kiskadee had a normal appetite but died several wk later. Records also showed that two of the birds had been wild-caught, 10 had been hatched and

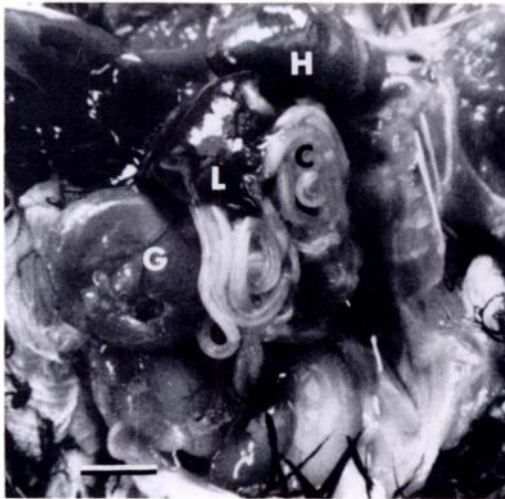


FIGURE 1. *Geopetitia aspiculata* partially removed from membranous cyst (C) in red-vented bulbul. H = heart, L = liver, G = gizzard. Bar = 0.5 cm.

raised in the Tropical House and the remainder obtained from dealers or other zoos. Ages of the birds ranged from 4 wk to 9 yr and infections occurred in both male and female birds.

Gross observations

The extent of lesions associated with *G. aspiculata* varied among the infected birds. The smallest lesions were two 2-mm² white plaques on the serosal surface of the proventriculus of a 4-wk-old white-lined tanager. Larger lesions, however, occurred in most birds and consisted of cysts attached to the serosa of the posterior end of the esophagus, the proventriculus, and the anterior part of the gizzard. In some cases, adhesions had formed between the cyst and the serosa of the liver and spleen. All cysts contained nematodes. In some birds, the cyst was delicate and membranous and the worms inside could readily be seen (Fig. 1). In other birds, the cyst was a hard, friable mass and the presence of worms was obscured (Fig. 2); such infections were presumably older than those with membranous cysts. Worms within cysts were extensively coiled and en-

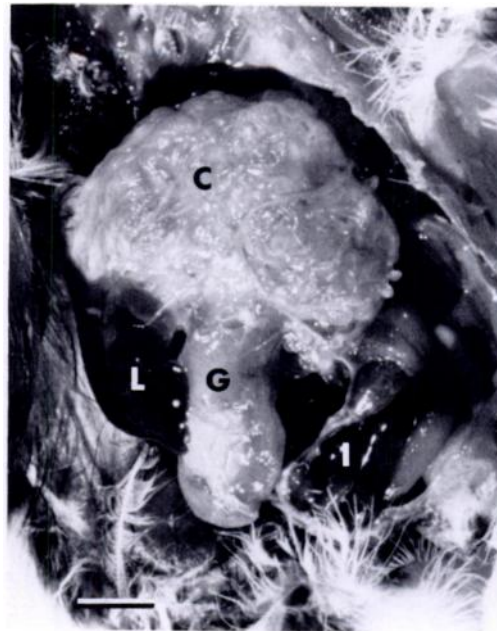


FIGURE 2. *Geopetitia aspiculata* in friable cyst (C) in blacksmith plover. L = liver, G = gizzard, I = intestine. Bar = 0.5 cm.

twined and the posterior extremities of many males and females extended, in clusters, through the wall into the lumen of the proventriculus (Figs. 3, 4). Some worms within membranous cysts could be removed intact. Only fragments of worms could generally be obtained from fibrous cysts. Thus, intensity of infection was not precisely determined. However, on the basis of the number of anterior ends of worms found, intensity was estimated to range from six to 50. The larger cysts occupied frequently much of the abdominal cavity.

Histologic observations

The histologic descriptions below pertain to tissues from birds in which cysts were hard and friable. Tissues from birds with membranous cysts were not available for histologic examination.

There was loss of structural integrity in all layers of the wall of the proventriculus in areas penetrated by posterior ends of



FIGURE 3. Posterior ends of *Geopetitia aspiculata* in lumen of proventriculus of white-lined tanager. Bar = 500 μ m.

worms (Fig. 5). Fibrinous exudate and numerous fibroblasts, heterophils, macrophages, giant cells, and lymphocytes were present adjacent to worms. Areas of proventricular wall not immediately adjacent to worms appeared normal. Fibrinous exudate, scattered heterophils, and sloughed proventricular gland cells were present in the proventricular lumen.

Within the cyst, worms were surrounded by fibrinous exudate (Fig. 6) or granulation tissue. Areas of granulation tissue contained numerous scattered fibroblasts, macrophages, giant cells, heterophils, and lymphocytes. Some worms were surrounded by extensive collections of erythrocytes, degranulated heterophils, and giant cells; some of these worms were live (Figs. 7–9), others dead (Fig. 10). In some birds, worms were also present in the parenchyma of the liver. A few nematode eggs were observed in some cysts. Masses

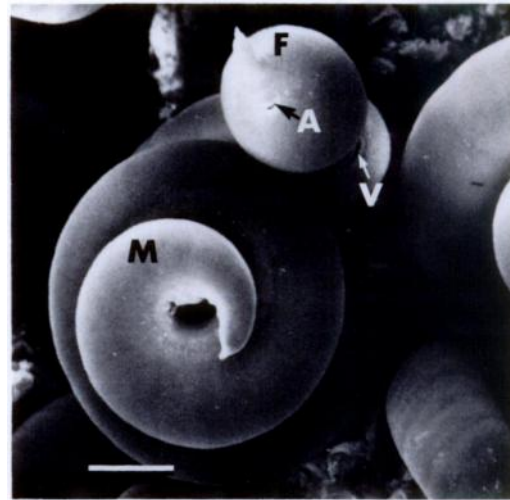


FIGURE 4. Posterior end of male (M) and female (F) *Geopetitia aspiculata* from white-lined tanager. Note anus (A) and vulva (V) of female. Bar = 100 μ m.

of eggs, within degenerate female worms, were also observed.

Development in the intermediate host

General: Larvae of *G. aspiculata* were found in amber colored capsules in the fat body of eight of 52 crickets. A cricket examined at 13 days contained 22 first-stage larvae. Two crickets examined at 27 days contained two and 24 molting second-stage larvae. A cricket examined at 39 days contained 10 molting second-stage larvae and five third-stage larvae. Two crickets examined at 42 days contained two and six molting second-stage larvae. Finally, two crickets at 48 days contained one and five third-stage larvae. Larvae were not found in 16 *L. maderae* and 22 *P. americana*.

Egg (Fig. 11): Eggs larvated, oval-shaped, with smooth shells. Measurements (in μ m) as follows: 1) from uterus of female from *Irena puella* ($n = 10$), 48 (46–50) by 26 (24–28); 2) from uterus of female from *Tachyphonus rufus* ($n = 10$), 48 (46–50) by 26 (23–28); 3) from uterus of female paratype from *Coerulea coerulea* ($n = 10$), 45 (43–49) by 27 (23–28).

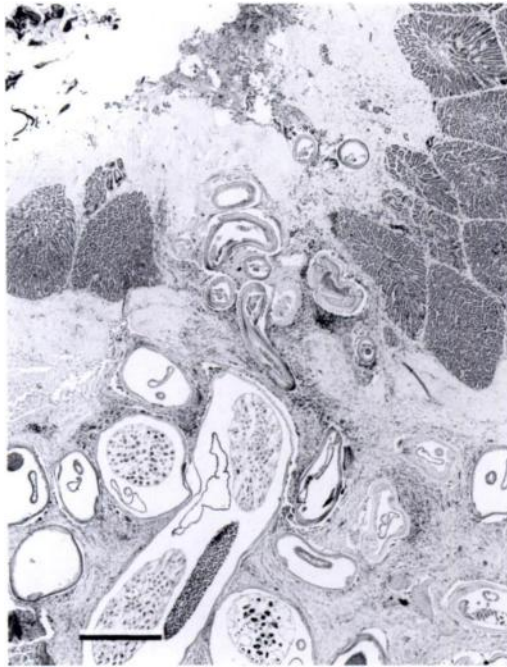


FIGURE 5. *Geopettia aspiculata* protruding from cyst through wall of proventriculus of white-lined tanager. Bar = 300 μ m.



FIGURE 6. *Geopettia aspiculata* surrounded by fibrinous exudate (E) and heterophils (H) in cyst in white-lined tanager. Bar = 300 μ m.

First-stage larva (Fig. 12) ($n = 1$, 13 days): Total length 340 μ m. Maximum width 30 μ m. Cuticle thin, with fine transverse striations. Anterior end tapered. Anterior extremity with refractile cephalic tooth and associated base. Sixteen rows of delicate spines present immediately posterior to anterior extremity. Nerve ring 80 μ m from anterior extremity. Excretory cell opening to exterior through horseshoe-shaped sinus with refractile walls; excretory pore 103 μ m from anterior extremity. Esophagus 145 μ m long, with marked dilation at posterior extremity. Esophageal-intestinal junction distinct. Intestine with large nuclei, few in number. Genital primordium near posterior third of intestine, ventral in position. Rectum large, with three large nuclei. Hyaline plug protruding from anus. Tail 48 μ m long.

Molting second-stage larva ($n = 10$, 27 days): Cuticle loose at anterior and pos-

terior extremities. Total length 1.2 (0.9–1.3) mm. Maximum width 36 (31–40) μ m.

Third-stage larva (Figs. 13–18) ($n = 233$, 39♀, 39 days): Total length 1.4 (1.4–1.5) mm. Maximum width 41 (39–42) μ m. Cuticle thick, with transverse striations. Cuticle in each lateral field forming two raised ridges running longitudinally along body from nerve ring to slightly anterior to anus. Anterior end of body tapered. Anterior extremity with two small, flat-topped pseudolabia, two amphids, and four pair papillae. Buccal cavity present; walls of anterior 10 (8–12) μ m of cavity composed of cuticle similar in appearance to that of body wall, walls of posterior 5 (4–5) μ m of cavity composed of refractile cuticle. Nerve ring 100 (90–108) μ m from anterior extremity. Excretory pore present immediately behind nerve ring. Deirids present near excretory pore. Esophagus divided; muscular portion narrow and

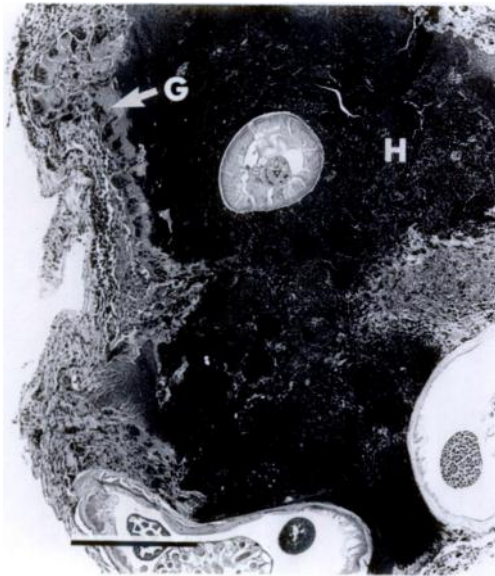


FIGURE 7. Degranulated heterophils (H) and giant cells (G) surrounding *Geopettitia aspiculata* in cyst in white-lined tanager. Bar = 200 μ m.

130 (126–153) μ m long, glandular portion broad and 265 (249–276) μ m long. Esophageal–intestinal junction distinct. Genital primordium of male 0.6, 0.6 mm from posterior extremity. Genital primordium of female 130, 141, 141 μ m from posterior extremity. Tail of male 75, 69 μ m long. Tail of female 69, 50, 50 μ m long. Posterior end tapered; extremity nipple-like.

Development in the final host

Third-stage larva (Figs. 19, 20): Third-stage *G. aspiculata* were found in addition to adult nematodes in a white-lined tanager from the Assiniboine Park Zoo. These specimens ($n = 3\delta\delta, 3\varphi\varphi$) were morphologically similar to third-stage larvae from crickets. Their total length was 1.7 (1.5–1.9) mm and maximum width was 52 (48–56) μ m.

Young fifth stage (Figs. 21, 22): One young adult male and one young adult female *G. aspiculata*, described below, were found in a membranous cyst on the proventricular serosa of the cutthroat finch given third-stage larvae. The caudal ex-

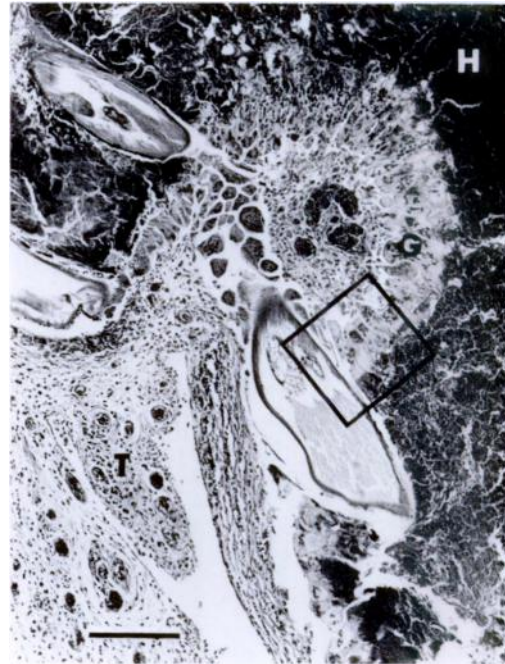


FIGURE 8. Heterophils (H), giant cells (G), and granulation tissue (T) around *Geopettitia aspiculata* in cyst in blue-winged siva. Enlargement of box, see Figure 9. Bar = 150 μ m.

tremitry of each worm protruded into the proventricular lumen. Nematodes were not found in the cutthroat finch given second-stage larvae nor in the two control finches. Young adult worms, of slightly smaller size than those in the infected cutthroat finch, were found along with mature adults in a blacksmith plover from Assiniboine Park Zoo. **Male:** Total length 8.8 mm. Maximum width 230 μ m. Monorchic, sperm present in vas deferens. Posterior end of body coiled $1\frac{1}{2}$ times. Tail 180 μ m long. Spicules absent. Large circumanal cuticular inflation present. Papilla-like swelling present immediately anterior to anus. Eight pair of caudal papillae present. **Female:** Total length 15.8 mm. Maximum width 250 μ m. Vulva sub-lateral, situated in constriction 250 μ m from posterior extremity. Vagina long, directed anteriorad. Didelphic and amphidelphic. Oocytes present in growth zone

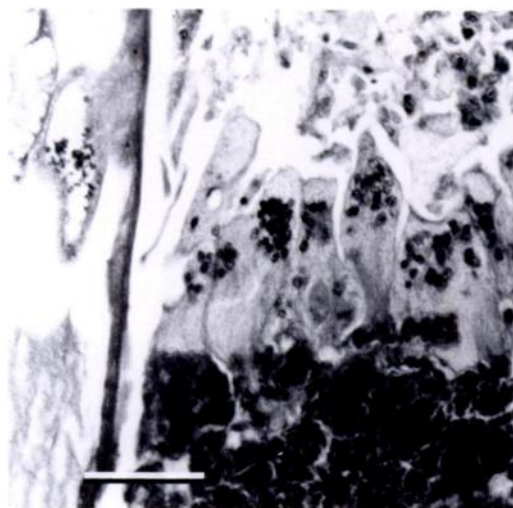


FIGURE 9. Enlargement of box in Figure 8 showing giant cells. Bar = 50 μ m.

of ovary. Eggs not present in uterus. Body swollen between vulva and anus.

Mature fifth stage (Figs. 23, 24): Anterior extremities of mature adults from a kiskadee from the Assiniboine Park Zoo were studied to clarify the cephalic morphology of *G. aspiculata*: two tri-lobed pseudolabia bordering large, oval oral opening. Pair of small papillae present on each pseudolabium. Four pairs papillae present at base of pseudolabia. Buccal cavity present, 10–15 μ m long.

Deposition of specimens

The following specimens of *G. aspiculata* were deposited in the United States National Parasite Collection (Beltsville, Maryland 20705, USA):

- 1) No. 78137. Third stage from *Acheta domesticus*.
- 2) No. 78131. Third stage from *Tachyphonus rufus*.
- 3) No. 78132. Young adults from *Tachyphonus rufus*.
- 4) No. 78133. Young adults from *Hoplopterus armatus*.
- 5) No. 78138. Young adults from *Amdina fasciata*.

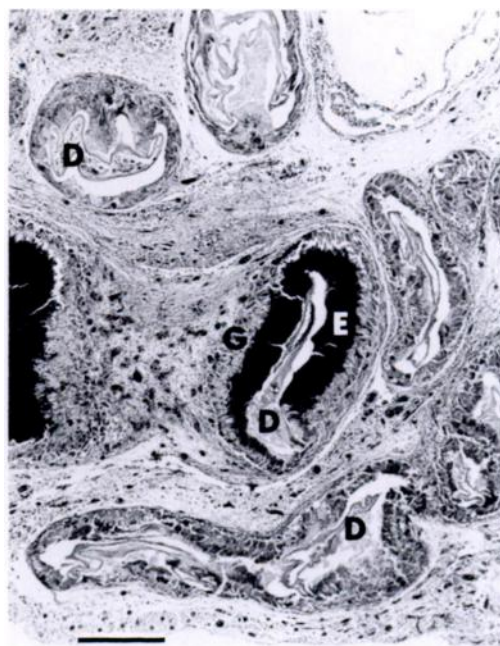
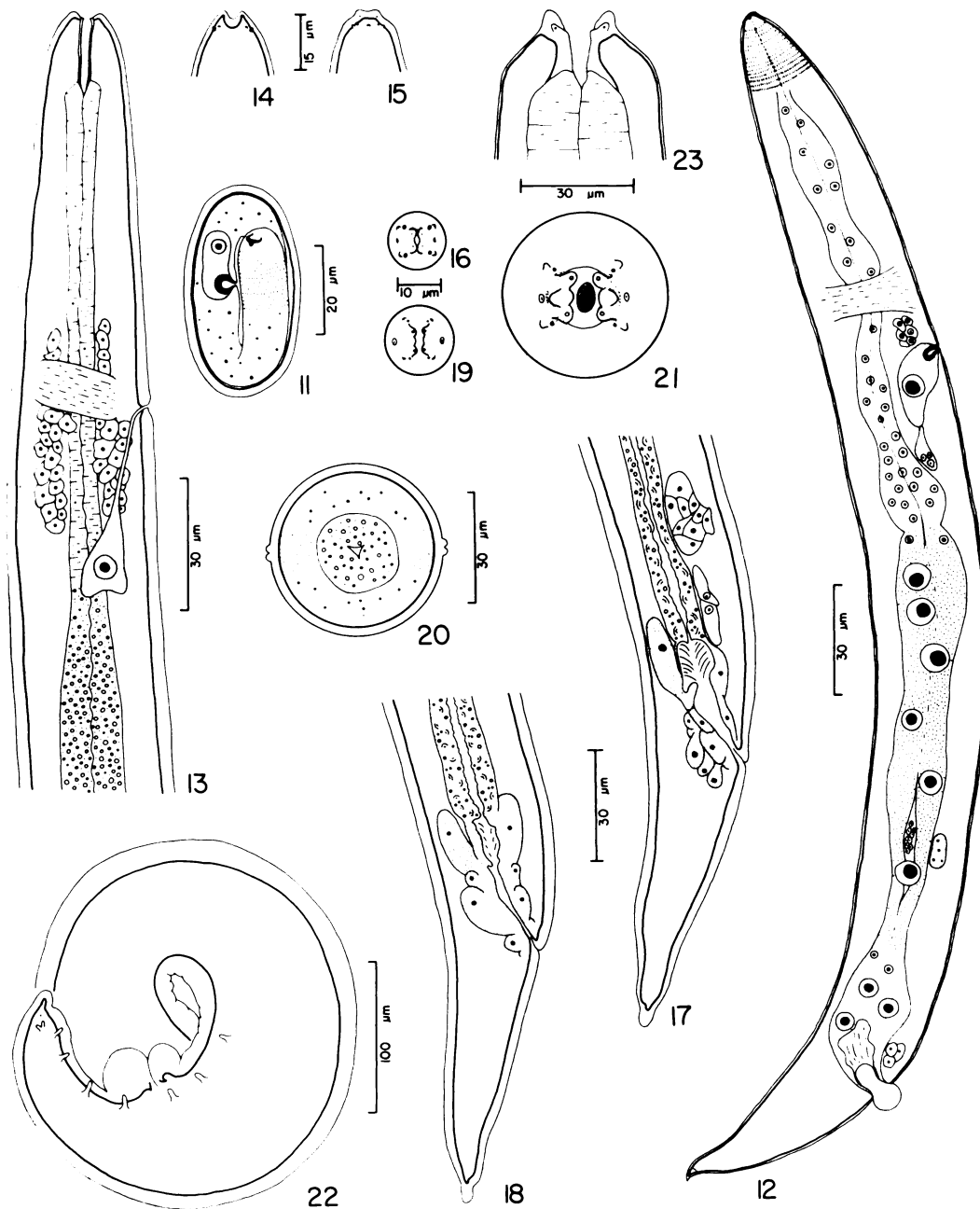


FIGURE 10. Dead *Geopetitia aspiculata* (D) surrounded by eosinophilic material (E) and giant cells (G) in white-lined tanager. Bar = 300 μ m.

- 6) No. 78134. Mature adults from *Salinator coerulescens*.
- 7) No. 78135. Mature adults from *Pitangus sulphuratus*.
- 8) No. 78136. Mature adults from *Hoplopterus armatus*.

DISCUSSION

Transmission of *G. aspiculata*, like that of other nematodes in the order Spirurida, involves an arthropod intermediate host. Although *G. aspiculata* developed to the infective third stage only in experimentally infected crickets, the possibility that cockroaches are suitable intermediate hosts should not be ruled out. Eggs used in this study were from worms from dead birds and 3–5 days elapsed between the time birds died and eggs were fed to invertebrates. Viability of eggs may have decreased during this time. Furthermore, many eggs in female worms fed to inver-



FIGURES 11-23. *Geopetitia aspiculata*. 11. Larvated egg from uterus of female worm from fairy bluebird. 12. First-stage larva from cricket, lateral view. 13. Anterior end of third-stage larva from cricket, lateral view. 14. Anterior extremity of third-stage larva from cricket, dorsal-ventral surface view. 15. Anterior extremity of third-stage larva from cricket, lateral surface view. 16. Anterior extremity of third-stage larva from cricket, en face view. 17. Tail of female third-stage larva from cricket, lateral view. 18. Tail of male third-stage larva from cricket, lateral view. 19. Anterior extremity of third-stage larva from white-lined tanager, en face view. 20. Cross-section of third-stage larva from white-lined tanager. 21. Anterior extremity of young female adult from blacksmith plover, en face view. 22. Tail of young male adult from cutthroat finch, lateral view. 23. Anterior extremity of mature male adult from blacksmith plover, dorsal-ventral view.

tebrates were not larvated; only larvated eggs would be expected to be infective.

Geopetitia aspiculata developed in the fat body of the arthropod, as do some other Habronematoidea (see Quentin and Barre, 1976; Quentin et al., 1983), and melanin likely caused the amber color in capsules containing larvae. Melanin deposition often accompanies defense responses in arthropods (Salt, 1970; Nappi, 1973). Its occurrence in the present study suggests that *A. domesticus* may not be the most suitable intermediate host of *G. aspiculata*.

Eggs of *G. aspiculata* are passed presumably in the feces of infected birds; the vulva of the female worm is near the posterior extremity of the body (Fig. 4) and eggs would be deposited in the lumen of the proventriculus. In the confined environment of the Tropical House at the Assiniboine Park Zoo, feral invertebrates probably encountered and ingested bird feces containing eggs or food contaminated with egg-laden feces. The parasite probably then spread among those birds feeding opportunistically on feral invertebrates. *Geopetitia aspiculata* apparently shows little host specificity, as 14 species of birds representing three orders were infected. The predominance of Passeriformes among those infected may reflect their tendency to eat arthropods.

It is unlikely that *G. aspiculata* was introduced to Assiniboine Park Zoo by local birds since there are no reports of *G. aspiculata* in wild birds in North America or elsewhere. The report by Barus (1971) of *G. aspiculata* in wild Piciformes in Cuba is herein considered incorrect since he illustrates the presence of spicules in his specimens. It is the absence of spicules in the male of *G. aspiculata* that readily distinguishes this species from others in the genus (Webster, 1971).

Geopetitia aspiculata was likely introduced to the Zoo by infected birds obtained from other zoos or wild-caught. *Geopetitia aspiculata* has been reported from one other zoo—the species descrip-

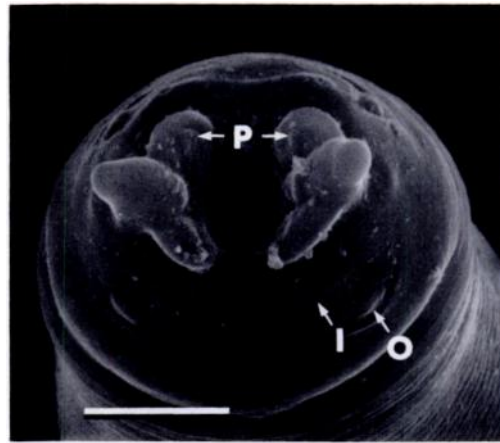


FIGURE 24. En face of mature male *Geopetitia aspiculata* from kiskadee. Note papillae (P) on pseudolabia; also inner (I) and outer (O) cephalic papillae. Bar = 10 μ m.

tion by Webster (1971) was based on specimens from exotic birds in the National Zoological Park in Washington, D.C. As in the present study, most infected birds were Passeriformes. Although it is not known what wild birds are natural hosts of *G. aspiculata* tropical birds would be suspected because they were the host group at both zoos. Seven other nominal species of *Geopetitia* are known; they occur in wild Falconiformes, Cuculiformes, Coraciiformes, Piciformes, or Passeriformes in France, the Soviet Union, Taiwan, Australia, India, Madagascar, Ghana, the Congo, and Cuba (Chabaud, 1951, 1960; Rasheed, 1960; Shumilo and Borgarenko, 1963; Vuylsteke, 1963; Bain and Chabaud, 1965; Mawson, 1966; Barus, 1968, 1969a, b, 1971; Schmidt and Kuntz, 1971; Webster, 1971; Sonin and Larchenko, 1974; Barus et al., 1978).

Geopetitia aspiculata certainly contributed to or caused the death of some infected birds at the Assiniboine Park Zoo; postmortem results showed that some infected birds had an associated peritonitis, were dehydrated, and in poor body condition. However, some infected birds died from drowning or other trauma. *Geopetitia* infections are not mentioned in any

books on zoo animal medicine or pathology although some of the "periproventricular Filaridae" reported by Fox (1923) in birds at the Philadelphia Zoo might have been *Geopetitia* sp. Species of *Geopetitia* apparently do, however, have epizootic and pathogenic potential in zoo environments. To prevent transmission of *G. aspiculata* at the Assiniboine Park Zoo, the feeding of "farm-raised" crickets was stopped in 1982 and efforts were made to reduce the numbers of feral crickets and cockroaches. Decreasing numbers of dead, infected birds may reflect some success in these efforts. In 1981 nine infected birds were found, in 1982 there were five, and in 1983 there were three. In view of the apparent lack of host specificity of *G. aspiculata*, efforts should be made to prevent the spread of the parasite to the local avifauna. Infected birds should not be allowed access to outdoor flight cages.

Larvated eggs of *G. aspiculata* from birds at the Assiniboine Park Zoo were 48 by 26 μm whereas Webster (1971) stated that eggs were 30 by 15 μm . We found, however, that eggs from a paratype female were 45 by 27 μm and conclude that Webster's measurements are in error. Eggs might not be present in the feces of all infected birds. Dead worms were observed in some birds and in other birds eggs were apparently deposited in the cyst, rather than in the lumen of the proventriculus.

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