PROUTERINA WESCOTTI N. GEN., N. SP. (TREMATODA: PROUTERINIDAE N. FAM.) FROM THE BRAIN, LUNGS, AND NASAL SINUSES OF A BLACK BEAR (URSUS AMERICANUS) FROM IDAHO

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PROUTERINA WESCOTTI N. GEN., N. SP. (TREMATODA: PROUTERINIDAE N. FAM.) FROM THE BRAIN, LUNGS, AND NASAL SINUSES OF A BLACK BEAR (*URSUS AMERICANUS*) FROM IDAHO

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ABSTRACT: *Prouterina wescotti* gen. n. and sp. n. (Trematoda: Prouterinidae N. Fam.) is described from a free-ranging black bear (*Ursus americanus*) which died in May 1995 in northern Idaho (USA). Adult digenetic trematodes were detected in brain, lungs, and nasal sinuses, and were likely responsible for the emaciated condition, copious nasal discharge, neurological signs, and death of the bear. Mature trematodes recovered from the bear were conical with small spines on the tegument. The anterior end was broad and tapered gradually toward the posterior. Mean (±SE) size of the mature trematodes was 3.67 (±0.08) by 2.14 (±0.04) mm (n = 80). Eggs are operculated, gold, and 68.2 (±0.42) by 41.4 (±0.41) µm (n = 75). Suckers are well developed and located in the anterior half of the body, with the genital pore just posterior to the ventral sucker. Testes are tandem and the ovary is lateral and slightly anterior to the anterior testes just posterior to the ventral sucker. The uterus is predominantly anterior to the ventral sucker and is the most distinctive feature of the trematode.

Key words: *Prouterina wescotti*, new species, trematode, brain, nasal sinus, lungs, *Ursus americanus*, black bear.

INTRODUCTION

Although numerous parasite surveys in black bears (*Ursus americanus*) have been reported, only two trematodes, *Heterobilharzia americana* and *Pharyngostomoides procyonis*, have been reported (Forrester, 1992). In each report, a single parasite of each species was detected in one bear. Knapp and Millemann (1970) experimentally infected black bears with the salmon poisoning fluke, *Nanophyetus salmincola*, but infections in free ranging bears have not been reported. In this report, we describe a new family, genus, and species of a digenetic trematode recovered from the brain, lungs, and nasal sinuses of a black bear from northern Idaho (USA). The trematode likely was the cause of its emaciated condition, copious nasal discharge, neurological signs, and death of the bear.

MATERIALS AND METHODS

In April 1995, a 2 to 3 yr-old female black bear south of Troy, Idaho (46°43′N, 116°45′W), was observed for several days next to the house of a local resident, was unafraid of humans, and appeared weak and thin. The local conservation warden from the Idaho Department of Fish and Game captured the bear on 19 April 1995, by placing a wire noose around the bear's neck and pulling the bear into a holding cage. The bear was then brought to Washington State University, Pullman, Washington (USA), for observation. The bear was thin, lethargic, with a copious thick reddish-brown nasal discharge, and had intermittent muscle tremors and seizures. Numerous ticks, including *Dermacentor andersoni* and *Dermacentor variabilis* were attached to the skin and many were partially or fully engorged with blood. Ticks were identified using the diagnostic keys of Furman and Catts (1982), and deposited in the H. W. Manter Laboratory, University of Nebraska State Museum, Lincoln, Nebraska (USA) as accession numbers HWML 38721 (*D. andersoni*) and HWML 38722 (*D. variabilis*). Respiration rate was 28 per minute, but coughing was not observed. Treatment included 200 mg of ivermectin (Ivomec, MSD Agvet, Rahway, New Jersey, USA) administered orally in sweet food, 6 million units of penicillin G benzathine and penicillin G procaine (Flo-Cillin, Fort Dodge Laboratories, Fort Dodge, Iowa, USA) given subcutaneously twice at a 48-hr interval, and 960 mg of a sulfadiazine combination (Tribrissen 960, Cooper's Animal Health, Kansas City, Kansas, USA) given orally in sweet food twice daily for 7 days. Condition of the bear became progressively worse, and on 5 May 1995, the bear was found dead and submitted to the Washington Animal Disease Diagnostic Labo-

ratory, Pullman, for necropsy. Because rabies was considered as a possible cause of death, half of the brain was sent to the State of Washington Department of Health, Olympia, Washington, for rabies evaluation by direct immunofluorescence (White and Fenner, 1994). Tissues including brain, lung, heart, spleen, liver, kidney, and pancreas were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

On dissection of the head, we observed numerous trematodes in the nasal sinuses. Two intact trematodes were also recovered from formalin fixed lung tissue. These trematodes were originally placed in saline solution, fixed in either 10% formalin, alcohol-formalin-acetic acid solution (AFA) or Bouin's fluid (Pritchard and Kruse, 1982) for later evaluation. Whole specimens were stained either with Gower's acetic carmine stain or Erlich's hematoxylin (Schell, 1970). Frontal, sagittal, and transverse sections were prepared and stained for examination of anatomical features. Other parasite specimens were embedded in paraffin, sectioned at 6 μm intervals and stained with hematoxylin and eosin. Additional specimens were processed for scanning electron microscopy as described by Bozzola and Russell (1992). After mounting, specimens were coated with gold and examined with a scanning electron microscope (Hitachi S570, Hitachi, Santa Clara, California, USA) at an acceleration rate of 20 kV.

RESULTS

Parasite description

Approximately 600 specimens of *Prouterina wescotti* n. gen., n. sp. (Trematoda: Prouterinidae n. fa.) were recovered from the nasal and paranasal sinuses of a black bear from northern Idaho. Two intact adult specimens were recovered from the lung, and additional adult specimens were observed in histological sections of lung and brain.

Prouterinidae fam. n.

Diagnosis: Parasites are small, conical and grayish-white, spinous tegument, with a broad rounded anterior end, tapering gradually toward the narrow posterior end, and widest at the level of the posterior sucker (acetabulum). Suckers well developed, located in anterior half of body; pharynx present, prepharynx absent, esophagus short, ceca long. Uterine folds confined to forebody; genital pore posterior to ventral sucker. Ovary and testes in hindbody, testes tandem, ovary lateral and just anterior and dorsal to the anterior testes. Vitelline follicles abundant, distributed in lateral areas of body from pharynx to posterior end of body.

Prouterina gen. n.

Diagnosis: With characters of the family Prouterinidae.

Type species: Prouterina wescotti. Spines on tegument, genital opening just posterior to acetabulum, uterus predominantly anterior to the acetabulum. Parasite of the nasal and paranasal sinuses of the black bear, *Ursus americanus,* also found in lung and brain.

Etymology: The generic name is from Greek (pro = before + uterine = womb or uterus) and refers to the anterior location of the uterus.

Prouterina wescotti sp. n.

Description: Body spinous, pyriform and conical, rounded at the wider anterior end, gradually tapering toward the posterior end (Figs. 1 to 3), 3.67 mm (range, 2.0 to 5.2 mm; $n = 80$) long by 2.14 mm (range, 1.5 to 3.0 mm; $n = 80$) wide at the level of the ventral sucker (greatest width). Body wall thick, tends to be impervious to viscous reagents. Oral and ventral suckers equal or subequal in diameter, oral sucker oriented toward anterior end of body, 390 to 403 μm in diameter; ventral sucker in anterior third of body, 374 to 402 μm in diameter; pharynx immediately posterior to oral sucker, 187 long by 156 μm wide, prepharynx absent, esophagus short; ceca extend to posterior end of body, end blindly. Ovary and testes in hindbody; testes tandem, irregular in shape, ovoid to lobed, equal or subequal, 498 to 502 μm long by 312 to 318 μm wide; ovary spherical or slightly ovoid, 350 to 360 μm in diameter, located anterior and to the right of the anterior testis or lateral to ventral sucker, ovary more dorsal in position than the tes-

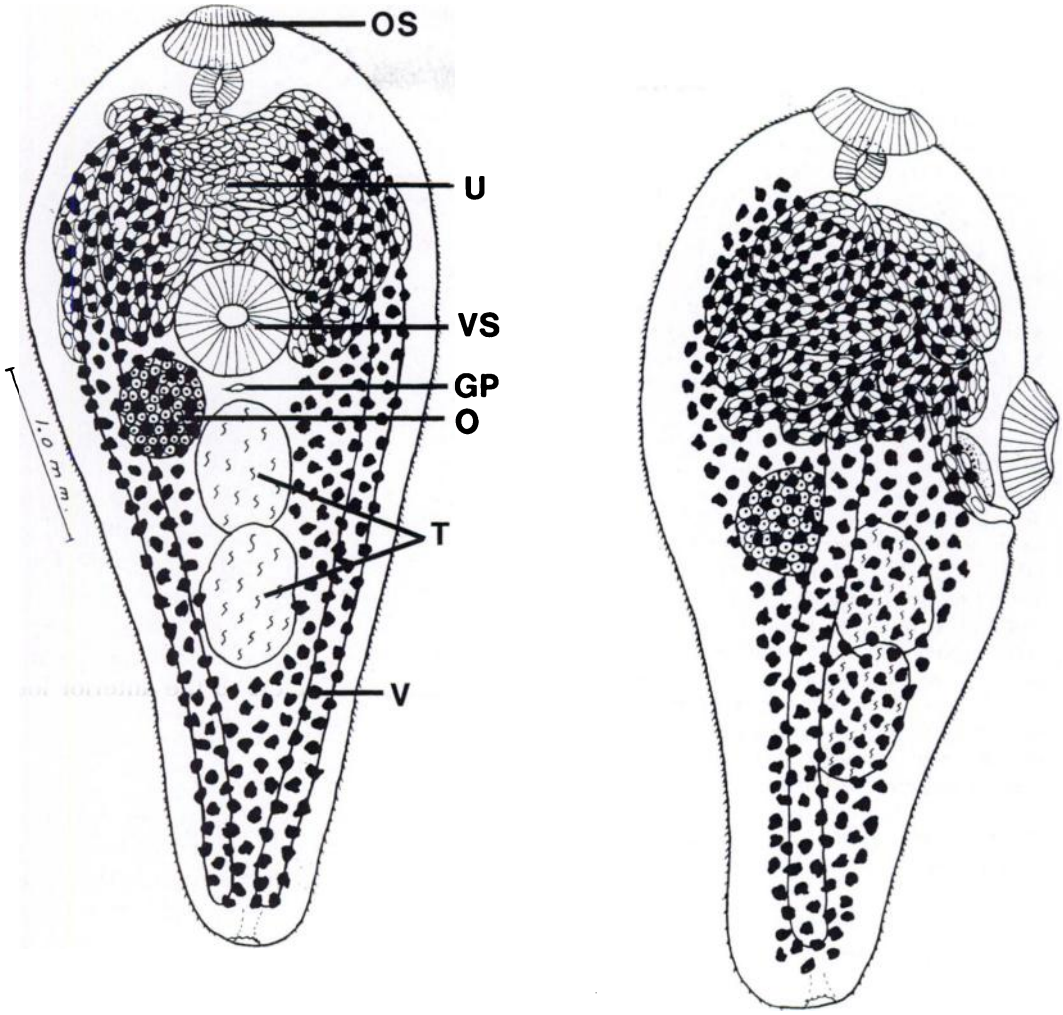

FIGURE 1. Ventral (left) and lateral (right) views of *Prouterina wescotti* n. sp. from the black bear (*Ursus americanus*). OS, oral sucker; U, uterus; VS, ventral sucker; GP, genital pore; O, ovary; T, testes; V, vitellaria. Scale bar = 1 mm.

tes (Fig. 4). Uterine coils confined predominantly to forebody, occupying much of forebody, terminus of uterus thickened to form metraterm which fuses with the cirrus sac; genital pore located immediately posterior to ventral sucker. Cirrus sac ovoid, located dorsal to ventral sucker, contains looped prostate canal, cirrus, and some prostate cells. Eggs operculated, gold, and 68 by 41 μm (range, 60 to 75 μm by 33 to 55 μm) (n = 75), contain developing embryos. Vitelline follicles abundant, distributed in lateral regions of the body from level of pharynx to posterior ends of ceca, follicles contiguous in dorsal part of body posterior to testes and dorsal to testes, transverse vitelline duct passes dorsal to anterior testis. Excretory system, seminal receptacle and Mehlis gland not observed.

Type host and locality: Black bear (*Ursus americanus* Pallas, 1780), south of Troy, Idaho (46°43′N, 116°45′W) at an elevation of approximately 780 m.

Type specimens: Holotypes (USNPC no. 85269), Paratypes (USNPC no. 85270), voucher specimens (USNPC no. 85271).

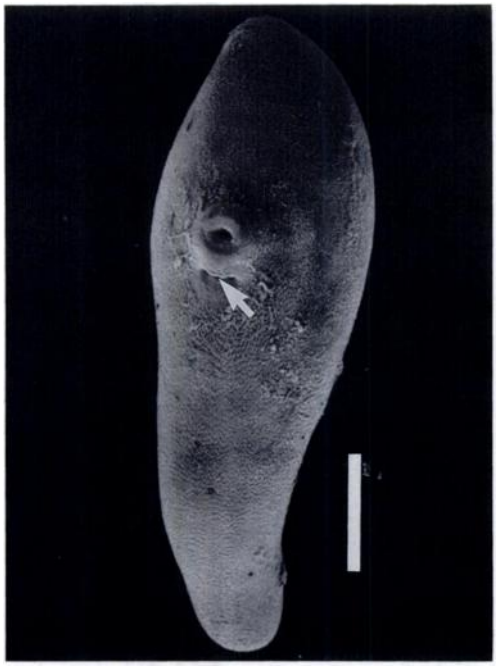

FIGURE 2. Scanning electron micrograph of *Prouterina wescotti* showing the shape of the trematode and the position of the genital opening (arrow) just posterior to the ventral sucker. Scale bar = 1 mm.

FIGURE 3. Scanning electron micrograph of the small spines covering the entire tegument of *Prouterina wescotti*. Scale bar = 15 μm.

Etymology: The specific epithet is named for the late Richard B. Wescott, friend and colleague.

Remarks: The family Prouterinidae resembles the family Renicolidae in body shape. In the prouterinids the uterus occupies the forebody; suckers are well developed, genital pore is posterior to the ventral sucker, testes are tandem, ceca long, cirrus sac present and the vitelline follicles fill most of lateral regions of the body and thus far, prouterinids are parasitic of the brain, lungs, and nasal sinuses of mammals. In contracted specimens the ovary and testes are in a more anterior position and testes may appear to be slightly oblique. The anterior testis is dorsal to the acetabulum, and the ovary is on the right side of the body. Testes can be separated by one and a half lengths of a testis. Eggs do not float well in sugar flotation solution, specific gravity 1.27.

The renicolids have a more extensive uterus which occupies both fore- and hindbody but not the body extremities. In the renicolids the ventral sucker is vestigial or absent, the testes are opposite or oblique, the genital pore is in the forebody, ceca are short, the cirrus sac is absent, the vitelline follicles are less numerous, occupying the middle or posterior third of the body. Renicolids are parasitic in the kidneys and uterus of birds, and found frequently in pairs.

Pathology

At necropsy, the bear weighed 36 kg and was in poor body condition with little subcutaneous fat. A porcupine (*Erethizon dorsatum*) quill was in the subcutaneous tissue to the right of midline at the level of the larynx. There was mild swelling and reddening of the subcutaneous tissue associated with the quill. Approximately 20 to 30 ml of viscous reddish-brown fluid-filled the entire frontal nasal sinus and approximately 10 ml drained from the nose

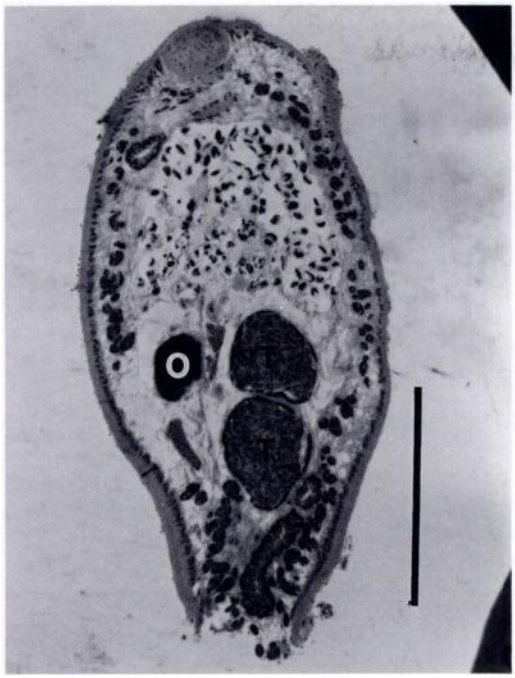

FIGURE 4. *Prouterina wescotti* sectioned longitudinally. The position of the tandem testes (T) and ovary (O) is shown. H&E stain. Scale bar = 1 mm.

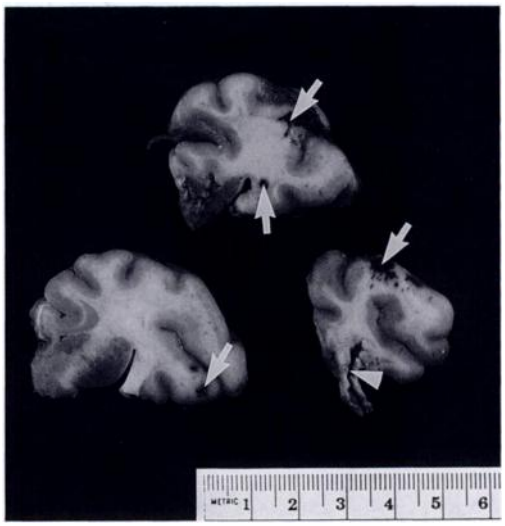

FIGURE 5. Three cross sections of cerebral frontal lobe, including dark lesions (arrows) associated with migratory tracts of *Prouterina wescotti*. Note hemorrhage and malacia predominantly in the superficial gray matter adjacent to the meninges and extending into the deeper white matter. Tract in the smallest section (arrowhead) is a portion of the first ventricle. Numbers on scale refer to cm.

when the bear was moved during necropsy. The entire nasal sinus contained proliferative friable, reddened tissue. A reddened pedunculated mass 3 × 1 cm, composed of proliferative nasal mucosa extended from the frontal sinus and was suspended into the left nasal cavity, and a similar mass 2 × 1 cm was in the right nasal cavity. The cribriform plate was friable and reddened. The olfactory lobes of the brain were grey, soft, and gelatinous. The cerebral hemispheres had several focal, 0.5 cm reddened areas which extended into the underlying gray matter. A brown, friable 0.5 cm area of malacia was within the right frontal lobe. In sections of cerebral frontal lobe, parasite migration tracts appeared as darkened areas of hemorrhage and malacia (Fig. 5). In the lung, three firm, 0.5 to 1 cm, discrete yellow nodules were in the parenchyma of the left cranial lung lobe. On section, the nodules were firm and walled off from the adjacent parenchyma. Grossly normal tissues included heart, liver, kidney, stomach, intestines, uterus, ovaries, thyroid glands, pancreas, trachea, esophagus, adrenal glands, and urinary bladder. Evaluation of the brain for rabies was negative.

Parasites were not observed grossly during the necropsy, but on later evaluation of the hemisected skull, we observed approximately 600 trematodes. Eggs were observed in about 90% of them. Trematodes were most prevalent in the nasal and paranasal sinuses (Figs. 6 and 7) and were mixed with the copious reddish-brown mucus. Gold, operculated eggs were abundant in direct smears of the mucus (Fig. 8).

Histologically there was encephalitis with necrosis and chronic fibrosing bronchiolitis. Multiple sections of the frontal lobe of the cerebral cortex contained focally extensive areas of inflammation and necrosis (Fig. 9), primarily within the superficial lamina, but occasionally extending into the deeper gray and white matter. Lesions were most severe within the olfactory

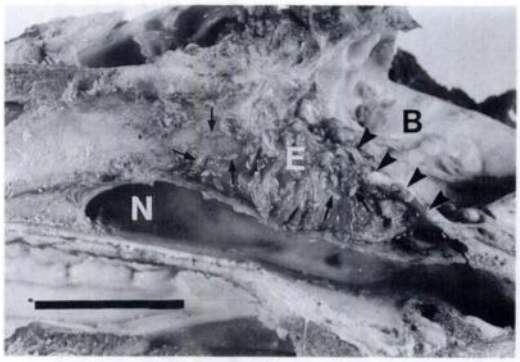

FIGURE 6. Cross section of skull from rostral to left, exposing the nasopharynx (N), ethmoid turbinates (E) and braincase (B). Numerous *Prouterina wescotti* (arrows) are on the osseous septum, ethmoid turbinates and within the rostral portion of the braincase. Note the severe erosion of the cribriform plate (arrowheads) adjacent to the ethmoid bone. Scale bar = 2 cm.

FIGURE 7. Enlarged photo of Figure 6. *Prouterina wescotti* indicted at arrows. Scale bar = 5 mm.

lobes and decreased in severity in caudal sections of brain. Inflammation was composed predominantly of dense accumulations of neutrophils with admixtures of lymphocytes, plasma cells, eosinophils, and glial cells. The inflammatory cells were mixed with necrotic debris and proliferating glial cells that replaced focal areas of white and gray matter. Several cross sections of the trematode, up to 1 mm long, and numerous eggs were within the areas of inflammation and necrosis (Figs. 9, 10, and 11). Meninges and subarachnoid space were densely infiltrated with inflammatory cells and fibrin, and contained numerous trematode eggs. The subarachnoid space overlying the olfactory bulb was filled with granulation tissue, fibrin, and inflammatory cells. Sections of lung nodules were examined, and both contained cross sections of the trematodes within bronchioles (Figs. 12 and 13). Numerous inflammatory cells, including eosinophils, neutrophils, and lymphocytes were within the airways adjacent to the parasites. The wall of the bronchioles were infiltrated and segmentally effaced by dense accumulations of inflammatory cells. Moderate fibroplasia and collagen deposition were associated with the mural inflammation. The inflammation and fibrosis extended into the adjacent parenchyma compressing and collapsing the alveolar spaces. Trematode eggs were scattered throughout the proliferative fibrous tissue. No significant lesions were observed in heart, liver, spleen, kidney, and pancreas.

The neurological signs demonstrated by this bear while alive, and death of the bear were attributed to intracranial and intracerebral trematode migration and egg de-

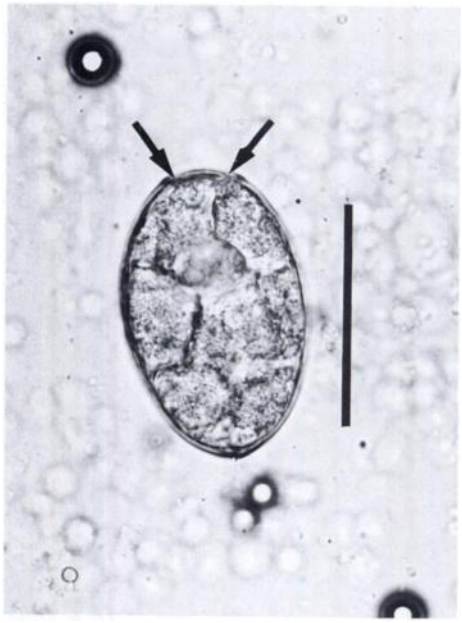

FIGURE 8. Egg of *Prouterina wescotti* indicating the oval shape and operculum (arrows). Scale bar = 50 μm.

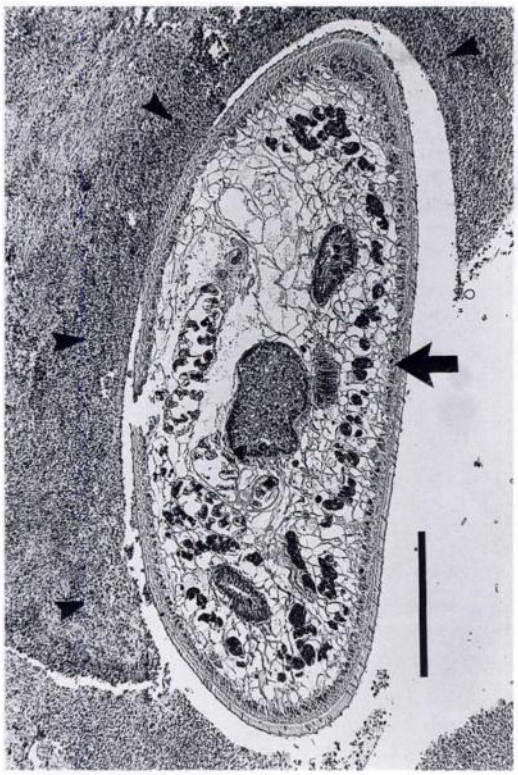

FIGURE 9. Histologic section from the frontal lobe of the brain. Gray matter adjacent to *Prouterina wescotti* (arrow) is malacic and infiltrated with a mixed population of inflammatory cells (arrowheads). H&E stain. Scale bar = 500 μm.

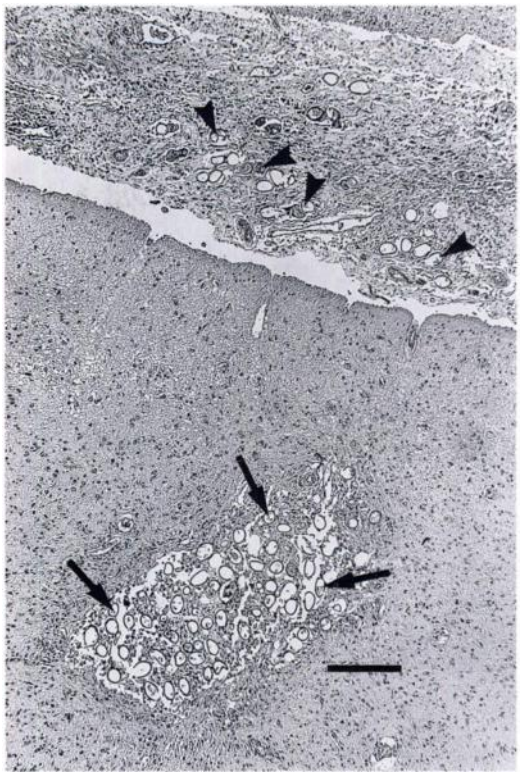

FIGURE 10. Histologic section of brain with focal malacia, inflammation and glial cell proliferation associated with trematode eggs (arrows). Note dense accumulations of inflammatory cells and fibrin associated with the eggs (arrowheads) that widen adjacent meanings. H&E stain. Scale bar = 500 μm.

position. Large numbers of flukes were found within nasal sinuses, and entry into the cranial vault was suspected to be through the cribriform plate.

DISCUSSION

In this unusual case, the trematodes had penetrated brain tissue and were responsible for the clinical signs and death of the bear. Entry into the brain was likely through the cribriform plate from the nasal sinuses. Trematodes were not observed during the gross necropsy procedure, but were first observed by microscopic examination of the tissues. At that time, only the head was still available for further evaluation, and the trematodes in the nasal and paranasal sinuses were detected grossly by one of the investigators (WJF). We were not able to thoroughly evaluate all the organs for *P. wescotti* because the rest of the carcass had been discarded. However, based on the numerous trematodes in the head, this may be the normal location or one of the usual locations of the trematode in black bears. Adult trematodes were also observed in lung and brain, but it is possible that these are aberrant locations for the trematode. Trematode eggs of *Campula* spp. and possibly *Hunterotrema* spp. and *Zalophotrema* spp. have been observed in the brain tissue of cetaceans (Ridgeway and Dailey, 1972), but adults and eggs of *P. wescotti* were not compatible with descriptions of those parasites (Schell, 1985).

Because of the morphology of this trem-

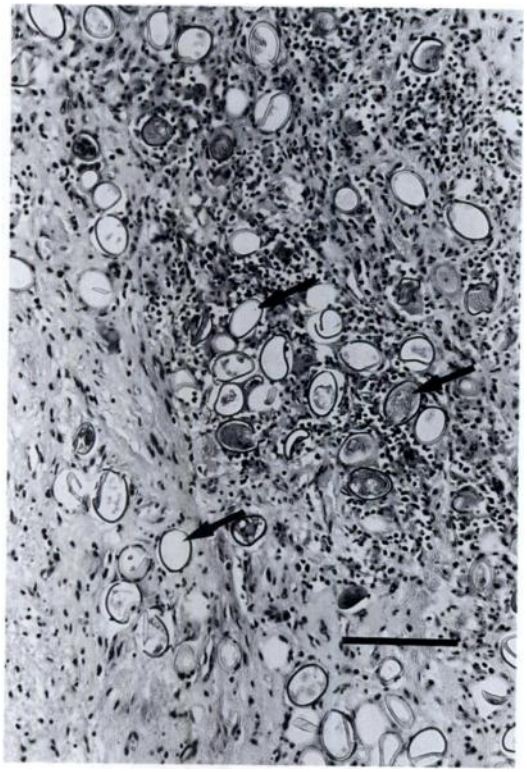

FIGURE 11. Enlarged view of Figure 10. Trematode eggs at arrows. H&E stain. Scale bar = 250 μm.

atode from the black bear, primarily the anterior location of the uterus, a new family, genus, and species was described. The family Renicolidae also has an anterior uterus, but is found primarily in the kidney of birds, and differs in several morphological features (Yamaguti, 1958; Schell, 1970). The functional significance of an anterior-positioned uterus is unknown. Trematodes of the family Troglotrematidae are found in the nasal sinuses of carnivores (Schell, 1985), but differ morphologically from *P. wescotti*.

The most obvious clinical signs in the bear included emaciation, neural signs probably related to the trematodes in the brain, and the massive nasal discharge. Future investigators working with bears, especially bears with nasal discharges should evaluate the mucus for trematode eggs, and the nasal sinuses should be examined carefully for the presence of trematodes.

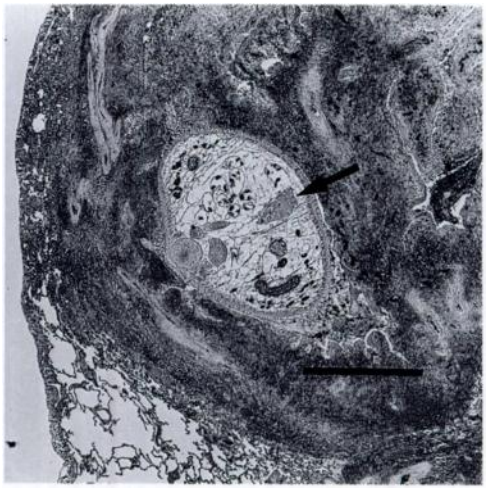

FIGURE 12. Histologic section of lungs with cross section of *Prouterina wescotti* (arrow) and associated chronic proliferative bronchiolitis and intraluminal inflammatory exudates. H&E stain. Scale bar = 1 mm.

Because eggs of *P. wescotti* did not float well in sugar flotation solution at a specific gravity of 1.27, direct smears from nasal mucus, and sedimentation methods for evaluation from feces should be used for detection of eggs in live animals. Such information will be valuable to document the distribution and prevalence of the parasite in bears, and perhaps other mammals

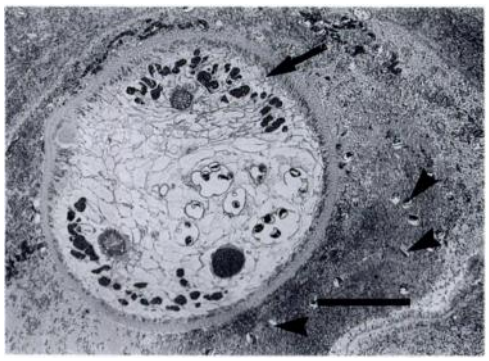

FIGURE 13. Histologic section of lung with *Prouterina wescotti* (arrow) and eggs (arrowheads) within the lumen of a bronchiole with severe bronchiolitis. Dense accumulation of fibrin and inflammatory cells, predominantly eosinophils and neutrophils, surround the trematode. H&E stain. Scale bar = 500 μm.

which could function as definitive hosts for *P. wescotti*.

ACKNOWLEDGEMENTS

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