J. Parasitol., 95(1), 2009, pp. 204–207

HAPLORCHIS POPELKAE N. SP. (DIGENEA: HETEROPHYIDAE) FROM SHORT-NECKED TURTLES (CHELIDAE) IN NORTHERN AUSTRALIA

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ABSTRACT: *Haplorchis popelkae* n. sp. is described from the intestine of the short-necked turtles *Elseya dentata* and *Emydura victoriae* in Northern Territory, Australia. This slipper-shaped heterophyid digenean differs from the 9 previously known species of *Haplorchis* in its larger overall size, a forebody that is wider than the hindbody, comparatively short ceca, and a ventral sucker that is less heavily spined than in many other species in the genus. This is the first report of *Haplorchis* in turtles.

Haplorchis Looss, 1899 is comprised of 9 recognized species collected from birds and mammals in Africa, Asia, Australia, and Oceania (Pearson, 1964; Pearson and Ow-Yang, 1982). *Haplorchis pearsoni* Pande & Shukla was reported from a varanid lizard that regularly preyed upon fish at a Kenyan fish farm (Sommerville, 1982), making this the only report of *Haplorchi*s from a reptile. As part of an ongoing survey of parasites of Australian freshwater turtles, we recovered specimens of *Haplorchis* from the short-necked turtles *Elseya dentata* and *Emydura victoriae* in northern Northern Territory. These specimens did not represent any known species and, herein, we describe a new species of *Haplorchis*.

MATERIALS AND METHODS

In June 2004, 3 *Emydura victoriae* were collected in crab traps baited with tinned sardines from the Victoria River in Gregory National Park, Northern Territory. In May 2005, 6 *Elseya dentata* and 5 *Em. victoriae* were collected by hand from the Daly River, Northern Territory and in June 2006, 1 *El. dentata* was collected in a baited trap from the Victoria River at Coolibah Crocodile Farm, Northern Territory. Collection proceeded under permits from the Northern Territory Parks and Wildlife Commission. Numerous adult worms were recovered from *Em. victoriae* from the Victoria River and all *El. dentata*. Living worms were rinsed in saline, briefly examined prior to fixation, killed with hot water, and fixed in 70% ethanol. Specimens were stained with aqueous alum carmine, Harris hematoxylin, or Mayer's hematoxylin; dehydrated in a graded ethanol series; cleared in clove oil; and mounted permanently in Damar balsam.

Type and voucher specimens were examined from the collection of the United States National Parasite Collection (USNPC): *Haplorchis vanissimus* Africa, 1938 (060322–060323); *Haplorchis paravanissimus* Pearson and Ow-Yang, 1982 (076160–076161); *Haplorchis yokogawai* Katsuta, 1932 (008936, 060316, 060318, 068461, 073531); *Haplorchis pumilio* Looss, 1896 (060310–060312, 060950, 068435, 073525); *Haplorchis taichui* Nishigori, 1924 (060320, 068327, 073526); *Haplorchis sprenti* Pearson, 1964 (060325); *Haplorchis parataichui* Pearson, 1964 (060327); *Haplorchis wellsi* Pearson, 1964 (060329, 075172); *Haplorchis parapumilio* Pearson and Ow-Yang, 1982 (076316).

Specimens used for scanning electron microscopy (SEM) were fixed in 70% ethanol, dehydrated in a graded series of ethanol and dried with the use of hexamethyldisilazane (HMDS) (Ted Pella, Inc., Redding, California) as transition fluid. The specimens were mounted on stubs, coated with gold, and examined with the use of a Hitachi 4700 scanning electron microscope (Hitachi USA, Mountain View, California) at an accelerating voltage of 5–10 kV. Measurements were taken from a compound microscope with the use of digital imaging and Rincon measurement software (v. 7.1.2, Imaging Planet, Goleta, California) as well as with an ocular micrometer. Mean, standard deviation, and coefficient of variation (CV) were calculated according to Steel and Torrie (1980). The CV is a percentage value of the ratio of the standard deviation to the mean of a particular metric character. Characters with lower CV have values that are more stable around the mean than those with higher CV.

Three specimens, 1 adult each from *El. dentata* from the Daly River, *El. dentata* from the Victoria River, and *Em. victoriae* from the Victoria River were used for each DNA extraction upon preliminary morphological identification. Ribosomal DNA fragments of 1,424 base pairs and spanning the internal transcribed spacer 2 (ITS2) region and 5′ end of the 28S gene (including variable domains D1–D3) were amplified and sequenced according to Tkach and Snyder (2007) and submitted to GenBank under accession numbers EU883584–EU883586. Sequences were manually aligned and compared with the use of the BioEdit program, version 7.0.1 (Hall, 1999). All measurements are in micrometers (μm) unless otherwise stated.

DESCRIPTION

Haplorchis popelkae n. sp.

(Figs. 1–2)

Diagnosis (based on 19 adult specimens; measurements of the holotype are given in the text and measurements of the entire type series are given in Table I): Body slipper shaped, forebody elliptoid, hindbody ovoid. Body length 1,021, forebody length (anterior end to anterior margin of ventral sucker) 571, forebody width 222, hindbody length (anterior margin of ventral sucker to posterior end) 450, hindbody width 223, body width at level of ventral sucker 120. Forebody 56% of body length. Tegument spinose, forebody densely spined, minute spines decreasing in density from level of cecal bifurcation to posterior end. Each spine situated in square rhomboid tegumental depression. Spines absent around opening of ventrogenital complex. Oral sucker rounded, subterminal, 48 long × 54 wide. Prepharynx short, 37; muscular pharynx, 31 × 30. Esophagus very long, 294; esophagous:prepharynx ratio 8:1. Ceca simple, end of ceca from level of seminal vesicle to level of ovary, left cecum length 266, right cecum length 260. Ventrogenital sac at mid-body, well posterior of cecal bifurcation, contains ventral sucker and genital pore. Ventral sucker 66 long × 63 wide, situated on antero-sinistral axis, containing 1 antero-ventral row of larger spines and 3 small fields of minute spines situated on lateral and antero-ventral lobes.

Testis singular, spherical, postovarian, ventral to ceca in posterior third of body, midway between ventral sucker and posterior body end, often obscured by eggs in the uterus. Testis 82 wide × 88 long. Seminal vesicle bipartite, sinistral, anterior portion 70 × 53, much larger than posterior portion, 13 × 26. Ejaculatory duct well developed, thick-walled, with well pronounced circular musculature taking tortuous path leading to and opening at bottom of common genital atrium. Short, apparently non-muscular protrusion present near base of genital atrium. Male genital pore opens on top of short, aspinose protrusion. Genital atrium tubular with strong circular muscles, ending in sphincter surrounding genital pore. Genital atrium surrounded by thick-walled conical structure.

Ovary median to markedly dextral, spherical, 85 long × 85 wide. Ovary positioned somewhat posterior to seminal vesicle and ventral to seminal vesicle and testis. Seminal receptacle spherical to elliptical, 62 × 76, slightly dextral, dorsal to and overlapping both ovary and testis. Uterus fills most of hindbody posterior to ovary. Very short, thick-walled metraterm opens at bottom of common genital atrium. Vitellarium consists of large, irregularly shaped follicles, scattered mostly near the dorsal surface between the anterior margin of the ovary and the

Received 31 March 2008; revised 10 July 2008; accepted 18 July 2008.

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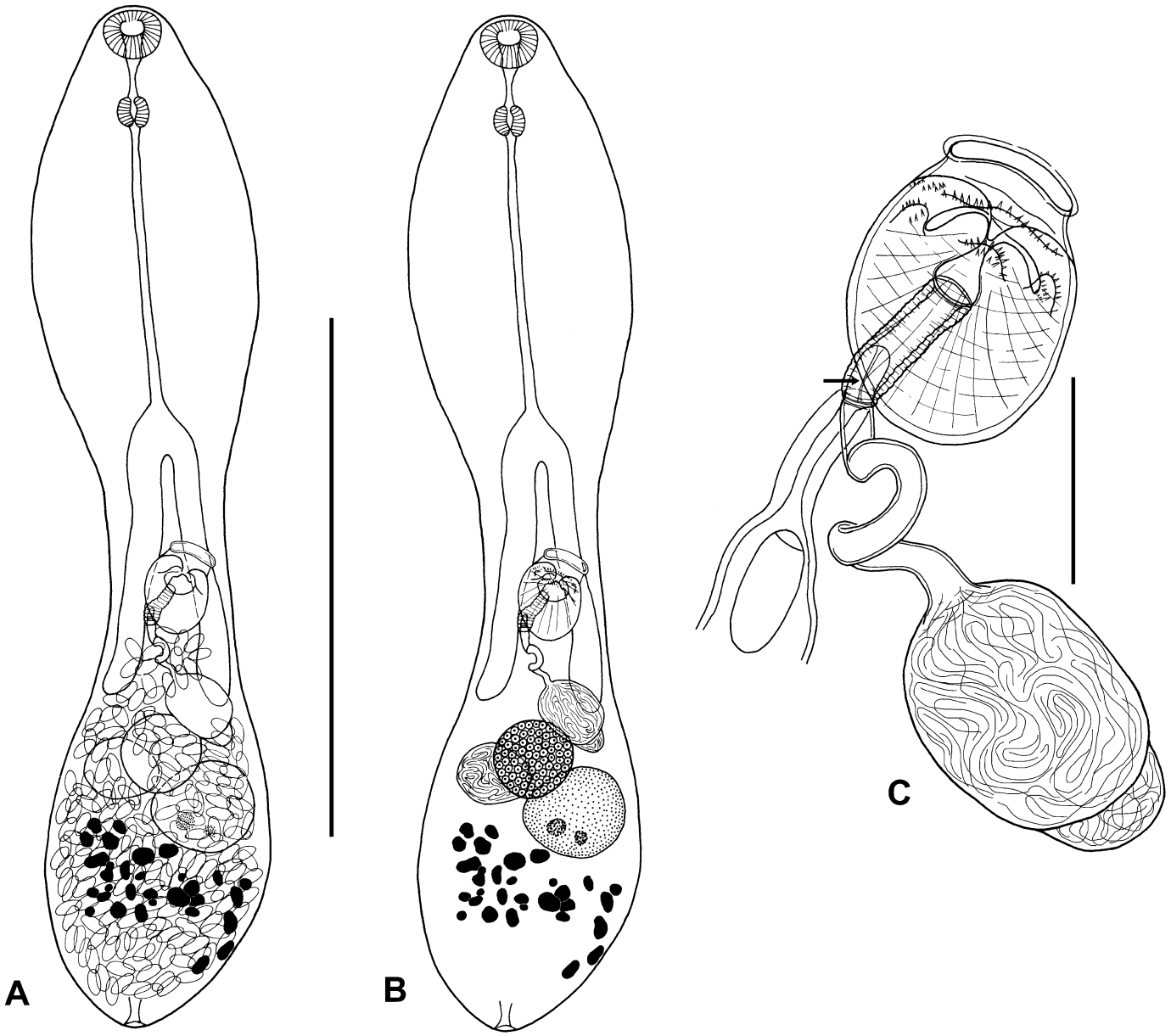

FIGURE 1. Holotype of *Haplorchis popelkae* n. sp. (**A**) General view showing distribution of eggs. (**B**) General view showing distribution of internal organs without eggs. (**C**) Ventrogenital sac region showing distal parts of male and female reproductive systems. Arrow indicates nonmuscular protrusion near genital atrium. Scale bars: A, B = 1 mm; C = 500 μm.

posterior end of body. Eggs operculate, 32 long. Excretory pore terminal; excretory vesicle reaches the level of ovary.

Taxonomic summary

Type host: Elseya dentata.

Type locality: Daly River, near town of Daly River, Northern Territory, Australia, 13°44.33′S, 130°41.12′E.

Other localities: Daly River, near Oolloo Crossing, Northern Territory, Australia, 14°00.31′S, 131°14.46′E; Victoria River at Victoria Highway, Gregory National Park, Northern Territory, Australia, 15°36.85′S, 131°07.86′E; Victoria River at Coolibah Crocodile Farm, Northern Territory, Australia, 15°33.75′S, 130°56.78′E.

Site of infection: Small intestine.

Prevalence and intensity of infection: Three of 3 *Em. victoriae* and 1 of 1 *El. dentata* from the Victoria River and 6 of 6 *El. dentata* from the Daly River were infected. Intensities ranged from 3 to over 50 worms.

Specimens deposited: The type series consists of 19 fully mature specimens from the Daly River. Holotype: Queensland Museum (QM) No. G23049; paratypes: QM Nos. G230350–230558, Harold W. Manter Laboratory Nos. HWML48900–48908.

Etymology: The specific epithet honors Emil and Evie Popelka, maternal grandparents of the first author, for their contributions to his education and career in parasitology. Additionally, Popelka is the Czech name for the fairy tale character Cinderella, and the new species is slipper shaped.

REMARKS

Based on general morphology, the new species belongs to *Haplorchis* Looss, 1899 as interpreted by Pearson (1964), although it differs most dramatically from the 9 recognized (Pearson and Ow-Yang, 1982) *Haplorchis* species in size. *Haplorchis*

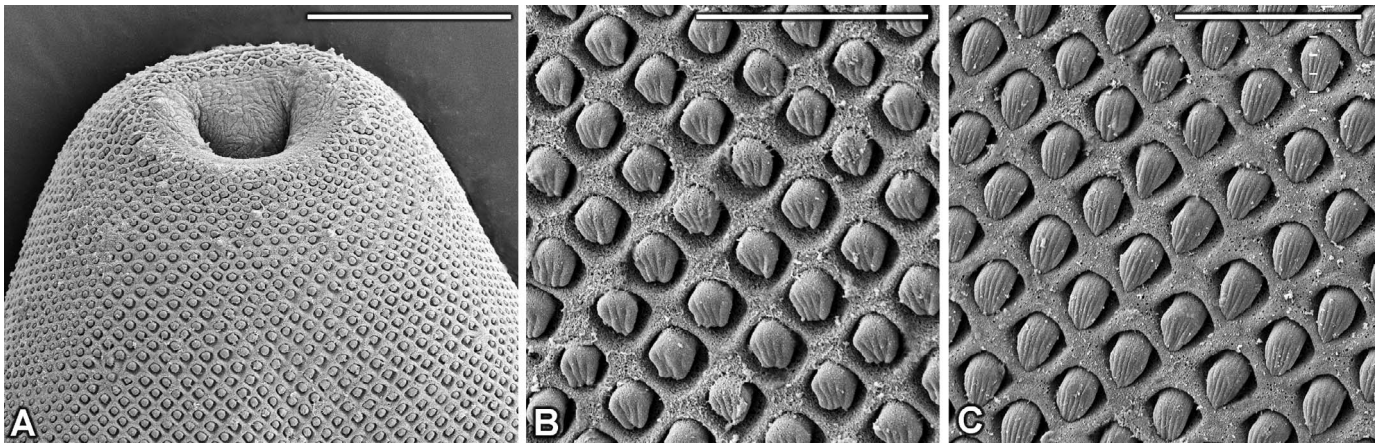

FIGURE 2. Scanning electron micrographs of *Haplorchis popelkae* n. sp. (**A**) Ventral surface surrounding the oral sucker. Scale bar = 500 μm. (**B**) Tegumental spination on ventral surface between oral and ventral suckers. Scale bar = 10 μm. (**C**) Tegumental spination in the anterior third of dorsal surface. Scale bar = 10 μm.

popelkae ranges in length from 996 to 1,323 μm; the next largest species, *H. taichui*, ranges from 450 to 890 μm (Pearson, 1964). In addition, *H. popelkae* is comprised of a forebody that is distinctly wider than the hindbody (1.34:1), a trait that the new species shares with only 2 congeners. *Haplorchis vanissimus* has a similar forebody:hindbody width ratio (1.29:1) but is considerably smaller (470–570 μm body length; data from Pearson, 1964) than *H. popelkae* and has ceca that run nearly to the posterior end instead of terminating at the level of the seminal vesicle as in the new species. The ventral sucker of *H. vanissimus* is also considerably more lobate and spinose than that of *H. popelkae*. *Haplorchis paravanissimus* is also described and illustrated as having a wider forebody than hindbody (Pearson and Ow-Yang, 1982) but relevant measurements

TABLE I. Metric data for *Haplorchis popelkae* n. sp.

Characters	n	Min–max	Mean	SD	CV*
Body length	19	963.6–1323.6	1,117.4	103.9	9.3
Forebody length	19	536.0–709.8	610.9	52.7	8.6
Forebody width	19	209.5–293.3	250.9	33.2	16.4
Hindbody length	19	430.6–609.2	507.8	57.0	11.2
Hindbody width	19	156.4–261.5	202.4	33.2	16.4
Midbody width	19	110.1–185.6	142.4	22.6	15.9
Forebody length as percent of body length	19	51.2–57.5	54.6	17.0	3.1
Oral sucker length	19	48.0–65.4	57.3	4.2	7.3
Oral sucker width	19	54.0–66.5	61.2	3.4	5.5
Prepharynx length	19	25.0–71.4	49.8	10.9	21.9
Pharynx length	19	31.0–41.6	35.8	2.9	8.1
Pharynx width	19	24.2–35.5	30.7	3.0	9.9
Esophagus	19	264.1–362.5	312.0	29.3	9.4
Esophagous:prepharynx ratio	19	4.5–11.4:1	6.6:1	1.6	25.1
Left cecum length	19	218.9–420.7	303.0	49.6	16.4
Right cecum length	19	232.0–437.9	311.1	51.1	16.4
Ventral sucker length	19	53.3–96.5	73.3	10.0	13.6
Ventral sucker width	19	49.6–81.4	66.1	9.3	14.0
Testis length	17	53.6–100.3	82.2	13.7	16.6
Testis width	18	57.5–121.2	87.0	17.0	19.5
Anterior seminal vesicle length	19	54.8–117.8	85.3	18.0	21.2
Anterior seminal vesicle width	19	42.0–149.2	77.7	30.7	39.5
Posterior seminal vesicle length	17	13.0–45.0	25.4	10.6	41.8
Posterior seminal vesicle width	17	21.0–60.4	36.3	11.6	32.0
Ovary length	19	47.0–97.0	67.3	13.7	20.4
Ovary width	19	46.3–92.7	68.7	15.7	22.9
Seminal receptacle length	17	29.0–80.0	60.1	13.7	22.9
Seminal receptacle width	19	40.3–98.7	62.9	16.0	25.4
Egg length	95	31.0–35.6	33.4	1.1	3.4

* Coefficient of variation.

from Pearson (1964) provide a forebody:hindbody width ratio of 0.99:1 and the paratype available from USNPC has a ratio of 0.79:1. The ceca of *H. paravanissimus* also approach the posterior end, the ventral sucker is heavily spinose, and eggs average 25 μm in length (Pearson, 1964) compared to 33 μm in *H. popelkae*. The above morphological differences allow for the reliable differentiation of *H. popelkae* from previously described species of *Haplorchis*. Among the most stable characters in the new species (as indicated by a low coefficient of variation) were egg length and the ratio of forebody length to total body length (Table I).

A short, apparently nonmuscular, protrusion was observed near the base of the genital atrium of all specimens. This protrusion represents the terminal portion of the ejaculatory canal and has a lumen that is normally too thin to see, but can be observed when sperm are present in it. We are hesitant to term this structure a cirrus, as it is clearly not eversible. This structure is not mentioned in previous works on *Haplorchis* (Pearson, 1964; Pearson and Ow-Yang, 1982) and was not visible in museum specimens of other species examined. Although not universally poor, the overall quality of the museum specimens would probably preclude observation of this feature if it were present. This structure does not correspond with the gonotyl of Pearson (1964): ''...usually muscular and sometimes armed, of the wall of the genital sac or ventrogenital sac, either adjacent to or surrounding the genital pore.'' The protrusion found in the new species is neither muscular nor armed, and is present in the posterior portion of the genital atrium, and thus is relatively distant from the genital pore.

The arrangement and shape of tegumental spines in the new species is of interest. The spines are situated in rhomboidal depressions of the tegument, forming a honeycomb pattern. Each spine resembles a human hand (Fig. 2) because of the presence of distal striations. Spines on the ventral surface have 4 striations, whereas dorsal spines have 6 striations.

The fragments of DNA sequence from 3 specimens of *H. popelkae* were 1,424 base pairs long and were identical for all specimens across 131 bases of the 5.8S, 291 bases of the ITS2 and 1,002 bases of the LSU ribosomal regions. Specimens recovered from the single *El. dentata* from the Victoria River were more robust in appearance than all other specimens examined, being wider on average than specimens from the type series. Forebody width of 3 specimens of this form averaged 308.9 μm versus an average of 250.9 μm in the type series and midbody width averaged 173.4 μm versus 142.4 μm. All other measurements are within the range of those found in the type series. The identity of the DNA sequence of this robust form with the more gracile forms supports the conspecific status of the 2 forms and suggests that this phenotypic variation may be host induced.

Haplorchis popelkae is the first species in the genus reported from turtles; all other species are reported from avian and/or mammalian definitive hosts, with *H. pumilio* also reported from a varanid lizard (Pearson, 1964; Pearson and Ow-Yang, 1982; Sommerville, 1982). The ability of 4 *Haplorchis* species to develop in both birds and mammals (*H. pumilio*, *H. sprenti*, *H. taichui*, *H. yokogawai*) is indicative of limited definitive host specificity and suggests that *H. popelkae* might be found in other chelonian and non-chelonian hosts. Although this new parasite was recovered from both *El. dentata* and *Em. victoriae* in the Victoria River, and in 6 of 6 *El. dentata* in the Daly River, it was not found in 5 *Em. victoriae* or 4 *Emydura tanybaraga* in the Daly River, or in 6 syntopic *Chelodina rugosa* or 4 *Carettochelys insculpta*. The absence of this new species in *C. rugosa* is somewhat surprising, given that all known life cycles of *Haplorchis* species involve fish intermediate hosts (Pearson and Ow-Kang, 1982), and fish are a prominent part of the *C. rugosa* diet (Kennett and Tory, 1996). All *El. dentata* examined were infected with *H. popelkae*, yet fish were not found to be part of the diet of this turtle in nature (Kennett and Tory, 1996). This information suggests that *H. popelkae* is either transmitted through a non-fish intermediate host or that it is specific to short-necked (*Elseya* and *Emydura*) Australian turtles.

ACKNOWLEDGMENTS

The authors thank Sean Doody, Dave Rhind, and Maggie Snyder for collection assistance; Patricia Pillet for making available USNPC specimens; and Donna Laturnus for providing her expert technical assistance during SEM observations at the Imaging Center of the Medical School, University of North Dakota. This material is based upon work supported by the National Science Foundation under Grants NSF 0515460, 0132289, and 0515492. Support was also received from the University Committee on Research of the University of Nebraska at Omaha (S.D.S.).

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