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Proctocaecum blairi sp. nov. (Digenea, Cryptogonimidae) from the freshwater crocodile, *Crocodylus johnstoni*, in Northern Territory, Australia

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Abstract

Proctocaecum blairi sp. nov. is described from specimens found in the intestine of an Australian freshwater crocodile, *Crocodylus johnstoni*, from Northern Territory, Australia. The most important diagnostic features of the new species are the body proportions and size, the position of the pharynx (relative length of the prepharynx and oesophagus), the relative length and position of the vitelline fields, and the number, shape and size of the circumoral spines. The new species is morphologically most similar to *Proctocaecum atae*, *P. elongatum*, *P. crocodili*, *P. gairhei* and *Acanthostomum slusarskii*. It differs from all of these species in having a much longer prepharynx, and differs from both *P. atae* and *P. crocodili* in having a much longer body and posteriorly situated vitelline fields. *Proctocaecum blairi* sp. nov. differs from *P. elongatum* in having a shorter body, a greater forebody to hindbody ratio, a much smaller ventral sucker, and a higher number of circumoral spines (23 *vs* 21 in *P. elongatum*). The new species differs from *P. gairhei* in possessing a much larger body length:width ratio and an ovary separated from the anterior testis by a seminal receptacle. *Acanthostomum slusarskii* lacks a gonotyl and has fewer circumoral spines than the new species. *Proctocaecum blairi* sp. nov. is the third species of *Proctocaecum* and the fourth cryptogonimid species known from crocodiles in Australia.

Keywords

Platyhelminthes, Digenea, Cryptogonimidae, *Proctocaecum*, freshwater crocodile, *Crocodylus johnstoni*, Northern Territory, Australia

Introduction

The Cryptogonimidae Ward, 1917 is a large, globally distributed digenean family comprised of at least 64 genera (Miller and Cribb 2008) and over 200 species, the vast majority of which parasitize marine and freshwater fishes. Members of only four genera parasitize crocodilians, namely *Proctocaecum* Baugh, 1957; *Timoniella* Rebecq, 1960; *Caimanicola* Freitas et Lent, 1938 and *Acanthostomum* Looss, 1899 (for references see Brooks 1980, Miller and Cribb 2008). Two of these genera are represented by three species in Australian crocodiles: *Timoniella absita* Blair, Brooks, Purdie et Melville, 1988 from the saltwater crocodile, *Crocodylus porosus*, *Proctocaecum elongatum* (Tubangui et Masilungan, 1936) from *C. porosus*, and *Proctocaecum nicolli* Brooks, 1980 from the freshwater crocodile, *C. johnstoni* (for references see Nicoll 1918, Brooks and Blair 1978, Brooks 1980, Blair *et al*. 1988). Digeneans belonging to several different families were collected from juvenile freshwater crocodiles caught in the Victoria and Daly Rivers in the Northern Territory (NT), Australia. Two of these crocodiles harboured several digeneans that appeared to be a new species of *Proctocaecum* Baugh, 1957. This new species is the second cryptogonimid reported from *C. johnstoni* and is described herein.

Materials and methods

Five specimens of the new cryptogonimid species were collected from a single juvenile *C. johnstoni* collected by hand in June of 2004 from the Coolibah Crocodile Farm, Victoria River, NT. In May of 2006, additional specimens of the same digenean species were collected from one out of four juvenile *C. johnstoni* collected by hand from the Daly River near Ool-

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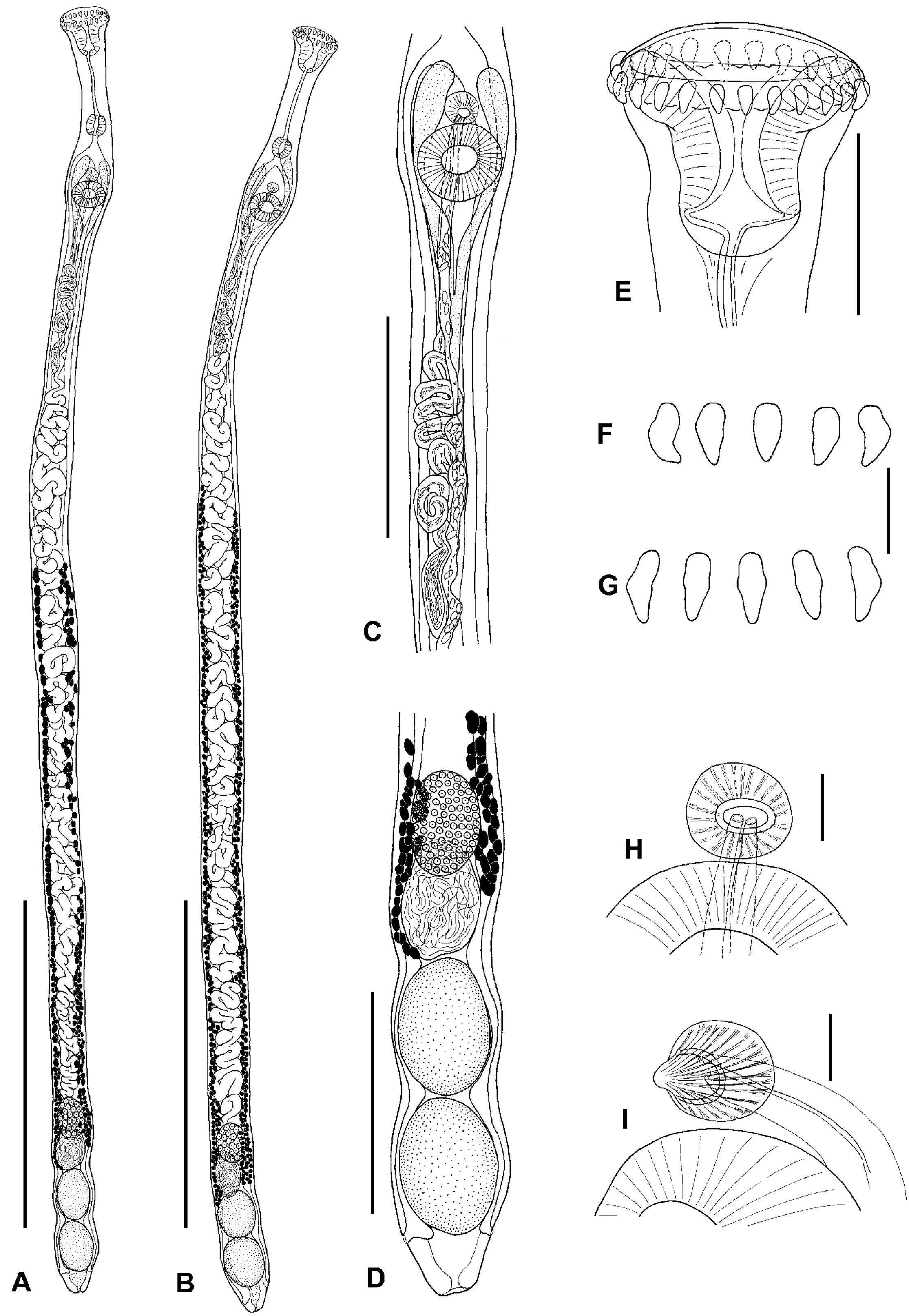

Fig.1. *Proctocaecum blairi* sp. nov. from *Crocodylus johnstoni*. **A** – overall view of holotype. **B** – overall view of a paratype. **C** – region of seminal vesicle and ventral sucker of the holotype. **D** – posterior end of the holotype. **E** – oral sucker and circumoral spines of the holotype. **F** and **G** – **v**entral and lateral circumoral spines of holotype (**F**) and paratype (**G**). **H** and **I** – gonotyl in holotype (**H**) and paratype (**I**). Scale bars = 2 mm (A, B), 500 μm (C, D), 200 μm (E), 50 μm (F, G), 50 μm (H, I)

loo Crossing, NT. Crocodiles were collected under permits issued by the Northern Territory Parks and Wildlife Commission.

Living worms were rinsed in saline, briefly examined prior to fixation, heat-killed and fixed in 70% ethanol. Digeneans were stained with aqueous alum carmine or Mayer's hematoxyline, dehydrated in a graded ethanol series, cleared in clove oil after carmine or methyl salicylate after hematoxyline, and mounted permanently in Damar balsam. Measurements were taken from a compound microscope using digital imaging and Rincon measurement software (v. 7.1.2, Imaging Planet, Goleta, California). All measurements are given in micrometers unless otherwise stated.

Results

Proctocaecum blairi **sp. nov.** (Fig. 1)

Measurements of holotype given in text; measurements of entire type series given in Table I.

Body very elongate with almost parallel lateral margins; length 7.72 mm; width at level of ventral sucker 282; body length/width ratio 27.4:1. Tegument armed with densely arranged small spines gradually diminishing in size and decreasing in number posteriorly, usually disappearing short distance anterior to ovary. Oral sucker (237 × 270) larger than ventral sucker (173 × 195), terminal, funnel-shaped, possessing pronounced musculature; its opening surrounded by single uninterrupted row of 23 spines. Spines rounded at proximal end, bluntly pointed at distal end, unequal in size (Fig. 1E-G); lateral spines somewhat longer than ventral and dorsal spines that measure 34–35 × 17–18. Ventral sucker round or slightly elongated transversally; forebody is 13.3% of body length. Oral/ventral sucker width ratio 1.4:1. Ventral sucker submerged in tegument or elevated above tegument surface, depending on state of worm at fixation. Prepharynx thin, long, 375. Pharynx, 130 × 121. Oesophagus very short, 23, surrounded by cluster of numerous glandular cells. Intestinal bifurcation somewhat anterior to ventral sucker. Each caecum opens via separate lateral anus at posterior end of body (Fig. 1A-D), at level of posterior margin of posterior testis.

Testes 2, post-ovarian, usually slightly elliptical, with smooth margins, situated in tandem at posterior end of body. Anterior testis 311 × 306, always slightly smaller and less elongated than posterior testis, 298 × 216. Seminal vesicle free in parenchyma, long, with strongly coiled proximal region and slightly coiled, sinuous distal region. Proximal end of seminal vesicle at mid-distance between ventral sucker and anterior margin of vitelline fields. Pars prostatica not observed. Geni-

Table I. Metric data for *Proctocaecum blairi* sp. nov.

Characters	N	Min.-Max.	Mean	StD	CV*
Body length	9	5767–8000	6714.2	798.5	11.9
Body width	9	246–340	295.2	32.4	11.0
Body length/width	9	18.6–27.4	22.9	3.3	14.2
Oral sucker length	9	220–289	260.2	24.8	9.5
Oral sucker width	9	242–306	269.1	18.1	6.7
Number of oral spines	9	23	23.0	0.0	0.0
Oral spine length	15	31–41	36.0	3.42	9.5
Oral spine width	15	14–18	16.47	1.51	9.1
Prepharynx length	9	253–426	315.1	63.5	20.2
Pharynx length	9	114–148	129.3	11.1	8.6
Pharynx width	9	99–139	120.6	15.4	12.7
Oesophagus length	9	21–45	27.8	7.8	28.0
Ventral sucker length	9	162–203	183.4	12.2	6.6
Ventral sucker width	9	171–210	197.3	14.1	7.1
Anterior vitelline field-ventral sucker	9	1263–2160	1615.8	347.0	21.5
Anterior vitelline field-anterior end	9	2179–3450	2697.4	447.4	16.6
Posterior vitelline field-posterior end	8	432–740	592.8	107.5	18.1
Anterior testis length	8	206–311	256.3	38.8	15.1
Anterior testis width	8	192–272	231.0	28.1	12.2
Posterior testis length	8	237–326	283.6	30.9	10.9
Posterior testis width	8	200–274	232.3	23.2	10.0
Gonotyl length	8	52–98	79.8	13.2	16.5
Gonotyl width	8	56–102	84.4	13.8	16.4
Ovary length	7	194–254	228.1	21.1	9.2
Ovary width	7	158–193	175.7	13.4	7.6
Seminal receptacle length	8	126–272	224.6	54.1	24.1
Seminal receptacle width	8	129–214	162.0	26.6	16.4
Egg length	27	30–33	31.6	0.6	2.0
Egg width	27	14–16	14.7	0.7	4.4

*Coefficient of variation.

tal atrium median, immediately anterior to ventral sucker. Muscular sucker-like gonotyl, 81 × 80, longer than wide, median, at anterior margin of ventral sucker.

Ovary slightly elliptical, pretesticular, entire, 235 × 158, separated from anterior testis by seminal receptacle. Seminal receptacle well developed, sac-like, elliptical, 256 × 170, between ovary and anterior testis, overlaying posterior portion of ovary dorsally.

Vitellarium well developed, consisting of 2 narrow fields of small vitelline follicles on either side of body; vitelline fields do not merge at any point. Vitelline fields supra- and extracaecal. Anterior limits of vitelline fields 2134 from posterior margin of ventral sucker and substantially separate (960) from distal end of seminal vesicle. Posterior limit of vitelline fields 740 from posterior end of body, usually at level of middle of seminal receptacle or anterior margin of anterior testis. Right vitelline field usually extends farther posteriorly than left field.

Ootype and Mehlis' gland situated antero-dorsal to ovary. Laurer's canal present. Uterine loops occupy intercaecal space between ovary and ventral sucker; metraterm thin-walled, weakly developed. Fully-developed eggs operculate, 30–31 × 14–15.

Excretory bladder Y-shaped with long stem which bifurcates almost immediately posterior to ventral sucker, and relatively short arms which extend to level of intestinal bifurcation. Excretory pore terminal.

Taxonomic summary

Type host: Freshwater crocodile, *Crocodylus johnstoni* (Krefft, 1873) (Crocodilia: Crocodylidae Cuvier, 1807).

Site of infection: Small intestine.

Type locality: Coolibah Crocodile Farm, Victoria River, Northern Territory, Australia, 15°33.75′S, 130°56.78′E.

Other localities: Daly River near Oolloo Crossing, Northern Territory, Australia, 14°00.31′S, 131°14.46′E.

Specimens deposited: The type series consists of 9 fully mature specimens. Holotype: Queensland Museum (QM) no. G232133; labeled ex. *Crocodylus johnstoni*, Victoria River, Coolibah Crocodile Farm, Northern Territory, Australia, 12 June 2004, coll. S.D. Snyder. Paratypes: Queensland Museum (QM) nos. G232134, G232135; labeled: ex. *Crocodylus johnstoni*, Victoria River, Coolibah Crocodile Farm, Northern Territory, Australia, 12 June 2004, coll. S.D. Snyder. Paratypes: U.S. National Parasite Collection, Beltsville, Maryland, USNPC 102779 (2 slides) labeled: *Crocodylus johnstoni*, Victoria River, Coolibah Crocodile Farm, Northern Territory, Australia, 12 June 2004, coll. S.D. Snyder. and USNPC 102780 (3 slides) labeled: ex. *Crocodylus johnstoni*, Daly River near Oolloo Crossing, Northern Territory, 23 May 2006, Coll. V.V. Tkach, S.D. Snyder.

Etymology: The species is named for Dr. David Blair, James Cook University, Townsville, Queensland, Australia for his support for our work, his contributions to helminthology, and his contribution to our knowledge of Australian digeneans in particular.

Differential diagnosis

A combination of characters indicates that the new species belongs to the cryptogonimid genus *Proctocaecum*. These characters include the presence of a single row of circumoral spines, a funnel-shaped oral sucker, a post-ovarian seminal receptacle, a well developed muscular gonotyl, vitellarium that is arranged in narrow lateral fields and intestinal branches with independent anal openings near the posterior end of the body. Among the most stable characters in the new species (as indicated by a low coefficient of variation) were egg size, ovary size, sucker size and number of circumoral spines (Table I).

Proctocaecum blairi sp. nov. is morphologically most similar to *P. elongatum*, *P. atae* (Tubangui et Masiluñgan, 1936), *P. crocodylii* (Yamaguti, 1954), *P. gairhei* (Junker, Brooks et Boomker, 2008) and *Acanthostomum slusarskii* Kalyankar, 1977, all parasites of crocodilians in Australia and southeastern and southern Asia (Tubangui and Masiluñgan1936, Yamaguti 1954, Kalyankar 1977, Brooks 1980, Junker *et al*. 2008).

Among these species, *P. elongatum*, *P. atae* and *P. crocodilii* have anal openings at the posterior end of the body, whereas *P. blairi* sp. nov. has lateral anal openings near the posterior margin of the posterior testis. The new species also differs from *P. elongatum* in possessing vitellaria that extend posteriorly to the ovary (in *P. elongatum* the vitelline fields do not reach the ovary), having a ventral sucker that is relatively more posterior on the body, possessing a relatively longer prepharyx, and having a ventral sucker that is nearly half the size of *P. elongatum*. In addition to these qualitative characters, the new species possesses 23 circumoral spines *vs* 21 found in *P. elongatum*, and the spines in the former are more than a third shorter than the spines in the latter.

In addition to the position of anal openings, *P. blairi* sp. nov. differs from *P. atae* and *P. crocodylii* in possessing a longer prepharynx, smaller circumoral spines (34–41 µm in the new species *vs* 50–58 µm in *P. atae* and 63–80 µm in *P. crocodylii*), and an anterior vitelline field margin at a substantial distance posterior from the seminal vesicle. In both *P. atae* and *P. crocodylii*, the vitelline fields reach the proximal end of seminal vesicle. *Proctocaecum atae* also has 26 circumoral spines *vs* 23 in *P. blairi* sp. nov.

Lateral anal openings are found in both *P. blairi* sp. nov. and *P. gairhei* but the new species differs from *P. gairhei* in having a very long prepharynx (*P. gairhei* does not have a prepharynx), a much higher body length:width ratio (19–27 *vs* 8–12 in *P. gairhei*) and an ovary separated from the anterior testis by a seminal receptacle. In *P. gairhei* these organs are not separated. *Acanthostomum slusarskii* bears a general resemblance to the new species, but lacks a gonotyl and can also be distinguished from *P. blairi* sp. nov. by having fewer circum-oral spines (18–19 in *A. slusarskii*).

All these differences strongly support the status of *P. blairi* sp. nov. as a new species, now the third member of *Proctocaecum* and the fourth known cryptogonimid from crocodiles in Australia. The presence of this new species in a crocodilian that has been previously examined for parasites indicates that helminths of crocodilians are insufficiently studied. A thorough parasitological survey of crocodilians will likely reveal a diversity of parasites that is thus far unknown. Understanding the biodiversity, morphology and host specificity of this parasite fauna is critical to understanding evolutionary relationships and host associations among parasites of this ancient group of reptiles.

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