Polystomatidae (Monogenea) of southern African Anura: Polystoma channingi n.sp. parasitic in two closely related Cacosternum species

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Polystoma channingi is described as a new species of polystomatid flatworm (Monogenea) parasitic in the urinary bladder of Cacosternum nanum in the Eastern Cape Province, South Africa. In a locality where C. nanum and C. boettgeri occur sympatrically the parasite has been found in both species. This finding of a Polystoma sp. in two closely related host species questions the strict host-specificity generally ascribed to the genus, but supports the hypothesis that host-specificity is determined by the oncomiracidium’s ability to recognize the tadpole as a suitable host. This is the first polystome to be described from the genus Cacosternum and the ninth species of Polystoma from South Africa. The species is distinguished by its huge haptor and suckers relative to the body size. The haptor length/body length ratio of 0.38 is by far the largest for the known southern African polystomes. The prevalence of infection with P. channingi n.sp. was 25% for C. nanum and 40% for C. boettgeri, while the mean intensity of infections in adult frogs was 1.5 for C. boettgeri and 1.0 for C. nanum.

Key words: Monogenea, Polystomatidae, Polystoma channingi, Cacosternum nanum, Cacosternum boettgeri, South Africa.

INTRODUCTION

Cacosternum belongs to the Pyxicephalidae, with 12 species currently recognized (Frost 2011). Concentrated largely in southern Africa, this is a non-tropical group of frogs known from all southern African biomes. They are found in areas with relatively high rainfall in a variety of vegetation types: fynbos, savanna, grassland, thicket and forest. They breed mostly in small ponds, dams, vleis, streams, roadside pools or flooded grassland. Males often form very large choruses and may begin calling during the day, especially after rain. Some 400 eggs are laid in individual clusters of 8-50. Tadpoles may develop quickly to complete metamorphosis in 17 days (Du Preez & Carruthers 2009).

During continual studies of African polystomes a bronze caco, Cacosternum nanum, was found to be infected, a first record of a polystome species in the genus Cacosternum. Molecular analyses of this polystome confirmed it to be an undescribed species (see Bentz et al. 2001). Bentz et al. (2001) referred to this polystome, collected at the farm Newton near Kenton on Sea in South Africa, as ‘Polystoma sp. (1). Genbank reference no.: AJ310400’. During field investigations over several years both C. nanum and C. boettgeri (common caco) from the Eastern Cape province in South Africa have been found infected with a polystome, which proved to be the same as the one originally collected from C. nanum. The objective of this paper is to formally describe this new species.

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MATERIALS & METHODS

During December 1996 an adult specimen of Cacosternum nanum was collected by Alan Channing on the farm Newton between Grahamstown and Kenton on Sea (33.581298S, 26.665182E) (Fig. 1). Upon dissection a single polystome was found in the urinary bladder. The locality was visited again intermittently from January 1997 to July 2010. All frogs collected were screened for polystomes and both C. nanum and C. boettgeri were found to be infected with the same unknown polystome. In January 1997, 11 C. nanum metamorphs were collected. Four were dissected and the remainder were kept alive with the aim of obtaining a series of different developmental stages of the parasite. One was dissected in March, two in June, one in August, and two in November of 1997, and the remaining one in April 1998. Of the 11 metamorphs, nine were infected with between one and six parasites. No frogs were found in January 1999 as the site was dry. In October 2004 two C. boettgeri and one C. nanum were collected. The two C. boettgeri were, respectively, infected with one and two parasites and the C. nanum with a single parasite. In December 2007 the site was dry and in November 2009 two mature and 12 metamorphs of C. boettgeri were collected. The mature frogs were not infected but one of the metamorphs was infected with a single parasite. In July 2010 one C. boettgeri and three C. nanum were collected. None were infected. In Dec. 2010 three C. nanum were collected at Covie (33.951667S, 23.603056E) (Fig. 1) 40 km east of Plettenberg Bay, near Nature’s Valley. One was infected with a single parasite. No specimens of C. boettgeri were found at this locality.

Prior to dissection, frogs were euthanased with benzocaine (4-aminobenzoate). They were dissected and the complete urinary bladder of infected frogs was removed and transferred to a Petri dish with 0.03% saline. Following fixation for two hours in 10% neutral buffered formalin (NBF), while under coverslip pressure, parasites were removed and preserved in 10% NBF. Prior to staining, parasites were rinsed in tap water for one hour, agitating the Petri dish every 10 minutes and replacing the water after 30 minutes, then stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene and mounted in Canada balsam. Genital spines were photographed using a Nikon DXM1200 camera fitted on
a Nikon E800 compound microscope. Two of the specimens retrieved from *C. nanum* were fixed in 96% ethanol for future molecular studies. Parasite eggs were harvested from infected hosts prior to dissection. Eggs were collected by sieving water through plankton netting with a mesh size of 112 µm and incubated in 10 ml of aged tap water in glass Petri dishes. Oncomiracidia were mounted in ammonium picrate glycerine. Marginal hooklet pairs were numbered one to eight with pair one being the posterior-most pair closest to the median (see Murith 1981a). All measurements are in micrometres and are given with the mean followed by the range in brackets.

**RESULTS**

**Levels of infection**

For *Cacosternum boettgeri* the prevalence was 40.0% for mature frogs and 8.3% for metamorphs. The mean intensity was 1.5 for mature frogs and 1.0 for metamorphs. For *C. nanum* the prevalence was 25.0% for mature frogs and 81.8% for metamorphs. The mean intensity was 1.0 for mature frogs and 2.7 for metamorphs.

**Polystoma channingi** n.sp., Figs 2–4, Table 1

*Specimens studied.* Six sexually mature worms and eight immature worms. Holotype (Fig. 2) from urinary bladder of *Cacosternum nanum* from the farm Newton near Kenton on Sea, South Africa (33.581298S, 26.665182E), 5 October 2004, coll. L.H. Du Preez (NMB P344) and three paratypes (NMB P345–347) deposited in the Parasitic Worm Collection, National Museum, Bloemfontein, South Africa; remaining specimens in the collection of the author. DNA extraction and sequencing were done on one specimen (see Bentz *et al.* 2001). A recent attempt to extract DNA from the remaining specimen failed.

*Type host.* *Cacosternum nanum* Boulenger, 1887, sexually mature male (AACRG 2210) deposited in the Amphibian Collection, African Amphibian Conservation Research Group, North-West University, Potchefstroom, South Africa.

*Site.* Urinary bladder.

*Type locality.* Farm Newton, Kenton on Sea area, Eastern Cape Province South Africa (33.581298S, 26.665182E).

*Etymology.* The species is named after Professor Alan Channing of the University of the Western Cape who collected the first specimen.

**Fig. 2. Polystoma channingi** n.sp. Ventral view of holotype from *Cacosternum nanum* collected near Kenton on Sea, South Africa (33.581298S, 26.665182E) (NMB P344). *Abbreviations:* eg, egg; gb, genital bulb; gi, genito-intestinal canal; ha, hamulus; hp, haptor; ic, intestinal caecum; mg, Mehlis gland; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis distribution; vd, vas deferens; vg, vagina; vi, vitellarium distribution; vt, vaginal duct. Scale bar = 1 mm.

*Description.* General characteristics of mature, egg-producing parasite (Fig. 2) typical of *Polystoma.* Body elongate; total body length 4960 (3789–6397); greatest width 1750 (375–3795); width at vagina 1299 (702–1542); haptor length 1884 (1634–2289); haptor width 2335 (1284–3114); haptor length to body length ratio 0.39 (0.35–0.49); 6 haptoral suckers, mean diameter 501 (266–657) (n = 67); hamulus length to tip of handle 360 (327–385) (n = 11); hamulus length to tip of guard 274 (228–305), handle longer than guard and x/y ratio 1.30 (1.23–1.35) (Fig. 3A); hamulus hook length 57 (51–60), Mouth subterminal, ventral. False oral sucker 343 (277–389) wide; pharynx length 252 (200–314); pharynx width 236 (170–304). Intestine bifurcate, caeca confluent posteriorly extend-
ing into haptor. Of 14 worms three had a single pre-haptoral intestinal anastomosis.

Testis single and follicular, situated post-ovarian, ventral, halfway towards the haptor (Fig. 2). Seminal vesicle prominent. Genital atrium median, ventral; genital bulb 70 (53–81) in diameter posterior to intestinal bifurcation, seven genital spines 30 (29–31) long (Fig. 3C). Ovary well developed, dextral, submedian; anterior in body, ovary length 713 (466–952); ovary width 274 (98–441). Oötype well developed. Genito-intestinal canal present on same side as ovary, joining intestinal caecum posterior to ovary. Uterus tubiform. Maximum of one egg observed in uterus. Vitellarium distributed throughout the body excluding oral region, region around ovary and uterus and haptoral suckers (Fig. 2).

Oncomiracidium. Ciliated oncomiracidium has narrow cylindrical body with circular cup-shaped opisthaptor and resembles a typical polystomatid
oncomiracidium. Opisthaptor bears 16 marginal hooklets, which are retained in adult parasites. Marginal hooklet one (postero-medial), 34 (32–36) in length (Fig. 4A), hooklets two to seven 21 (20–23) in length (Fig. 4B), and hooklet eight 31 (30–32) in length (Fig. 4C). Hamulus primordia 31 (25–34) in length (Fig. 3B). Measurements based on 10 specimens.

**Diagnosis**

*Polystoma channingi* n.sp. differs from all other *Polystoma* species known from southern Africa by a combination of characters: relative to the size of the body the haptor is large with a haptor length/body length ratio of 0.38 (Table 1). This ratio is by far the greatest with the nearest value of 0.33 for *P. natalense*. The average diameter of the suckers as percentage of the total length of the parasite is 10.1%. This is by far the largest ratio for all known South African polystome species. Other known *Polystoma* species have ratios of 5.6% for *P. australe*, 5.2% for *P. claudecombesi*, 6.4% for *P. dawiekkoki*, 7.8% for *P. marmorati*, 8.1% for *P. natalense*, 6.9% or *P. sodwanense*, 7.0% for *P. testimagna*, 6.5% for *P. umthakathi* and 4.9% for *P. vernoni*.

**DISCUSSION**

In polystome parasites the oncomiracidium, which hatches from the egg, does not directly infect the bladder of the post-metamorphosis frog but establishes itself in the gills of a tadpole. Here it develops into either an egg-producing neotenic
form or, depending on the age of the tadpole, into a non-egg-producing form that migrates to the bladder during metamorphosis. The bladder parasite grows into the adult parasite whereas the neotenic parasite dies during tadpole metamorphosis.


The discovery of Polystoma channingi n.sp. in both Cacosternum boettgeri and C. nanum is in contrast with the notion that anuran polystomatids are strictly host-specific. A similar observation of two closely related host species Kassina senegalensis and Semnodactylus wealii harboring the same polystome namely Polystoma australe at a locality near Ladybrand in the eastern Free State, South Africa has been reported by Kok & Du Preez (1987). The discovery of Polystoma channingi n.sp. in both Cacosternum boettgeri and C. nanum is in contrast with the notion that anuran polystomatids are strictly host-specific. A similar observation of two closely related host species Kassina senegalensis and Semnodactylus wealii harboring the same polystome namely Polystoma australe at a locality near Ladybrand in the eastern Free State, South Africa has been reported by Kok & Du Preez (1987).

Based on experimental studies Du Preez & Kok (1997) postulated that the basis of host-specificity in Polystoma species lies with the oncomiracidium’s recognition of the host species tadpole. They found that when an oncomiracidium made contact with its host tadpole, it either remained stationary on the tadpole, looped over the surface or performed a gliding motion over the surface of the tadpole. When an oncomiracidium made contact with a non-host tadpole it performed the same motions but frequently broke contact with the tadpole to swim. In a small container where an oncomiracidium is forced to frequently make contact with a non-host tadpole it may eventually enter the gill chamber and establish on the gills. If an oncomiracidium should break contact with a tadpole in a big natural pond the chance of a 200 micrometre oncomiracidium with a life span of only a few hours making contact with another tadpole is extremely small. In the present study the pond where both Cacosternum species were collected sympatrically was only a few metres in diameter and very shallow. Tadpoles of both species thus shared the same micro-habitat. It would appear that it may be possible that where closely related species occur sympatrically, the oncomiracidia may recognize tadpoles of closely related species as potential hosts. More comprehensive studies of host-specificity are evidently required.

The discovery of a 5-mm-long polystome in a 18 mm host was unexpected, even more so as up to three parasites were found in a single adult frog and up to six in a subadult frog. The haptoral suckers are quite large and secure a firm grip on the bladder wall. The huge ovary, packed with oocytes, indicates that this parasite can produce a large number of eggs in a relatively short time. This would fit in

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**Table 1.** Measurements of sexually mature, egg-producing Polystoma channingi n.sp. compared to the relevant measurements for Polystoma australis (P.a), P. claudecombesi (P.c), P. dawiekoki (P.d), P. marmorati (P.m), P. natalense (P.n), P. sodwanense (P.s), P. testimagna (P.t), P. umthakathi (P.u) and P. vernoni (P.v) (see Kok & Van Wyk 1986; Van Niekerk et al. 1992; Combes & Channing 1979; Du Preez & Kok 1992a, 1993, 1995; Kok & Seaman 1987; Du Preez et al. 2002; Du Preez 2011, respectively).

<table>
<thead>
<tr>
<th>Body measurement or ratio</th>
<th>P. channingi n.sp.</th>
<th>P.a</th>
<th>P.c</th>
<th>P.d</th>
<th>P.m</th>
<th>P.n</th>
<th>P.s</th>
<th>P.t</th>
<th>P.u</th>
<th>P.v</th>
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<tr>
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<td>6730</td>
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<td>7096</td>
<td>6250</td>
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<td>Width at vagina</td>
<td>1299</td>
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<td>2060</td>
<td>–</td>
<td>1510</td>
<td>–</td>
<td>1710</td>
<td>1680</td>
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<td>–</td>
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<td>Haptor length (HL)</td>
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<td>1740</td>
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<td>1582</td>
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<td>1250</td>
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<td>2050</td>
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<td>2432</td>
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<td>HL/BL ratio</td>
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<td>0.34</td>
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<td>332</td>
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<td>305</td>
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<td>482</td>
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<td>474</td>
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<td>5.2</td>
<td>6.4</td>
<td>7.8</td>
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<td>6.9</td>
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<td>429</td>
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with the host’s reproductive strategy. Species of Cacosternum are opportunistic and explosive breeders with rapid tadpole development. Both C. boettgeri and C. nanum complete metamorphosis in 17 to 21 days (Du Preez & Kok 1998). This leaves very little time for the neotenic parasite to have a significant impact on the parasite population. Of the 24 different polystomatid genera only Polystoma and Metapolydnoma have been documented to have a neotenic phase infecting the gills of tadpoles (Du Preez & Kok 1992b). The neotenic phase does not play an equally important role in the life cycles of all polystome species. In species with slow-developing tadpoles, for example Natalobatrachus bonebergi, the neotenic form may play a key role in maintaining an infection (Du Preez & Kok 1998). The role of the neotenic parasite warrants further investigation especially when infecting rapid developers such as C. boettgeri and C. nanum.

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REFERENCES


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