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

Louis H. du Preez*

Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2520 South Africa

Received 4 June 2012. Accepted 18 March 2013

***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

Polystomatids of anurans are represented in Africa by the genera *Eupolystoma* Kaw, 1950, *Metapolystoma* Yamaguti, 1963, *Polystoma* Zeder, 1800 and *Protopolystoma* Bychowsky, 1957. The genus *Polystoma* has a widespread occurrence in all zoogeographical realms except the Australian realm and 32 of the 65 currently known species are from Africa. The species known from South Africa include *P. australe* Kok & Van Wyk, 1986 from *Kassina senegalensis* and *Semnodactylus wealii*, *P. claudecombesi* Du Preez & Kok, 1995 from *Amietia quecketti*, *P. dawiekoki* Du Preez, Vaucher & Mariaux, 2002 from *Ptychadena anchietae*, *P. marmorati* Van Niekerk, Kok & Seaman, 1992 from *Hyperolius marmoratus*, *P. natalense* Combes & Channing, 1979, from *Strongylopus grayii*, *P. sodwanense* Du Preez & Kok, 1992 from *Ptychadena porosissima*, *P. testimagna* Du Preez & Kok, 1993 from *Strongylopus fasciatus*, *P. umthakathi* Kok & Seaman, 1987 from *Natalobatrachus bonebergi* and *P. vernoni* Du Preez, 2011 from *Ptychadena oxyrhynchus*.

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.

*E-mail: louis.dupreez@nwu.ac.za

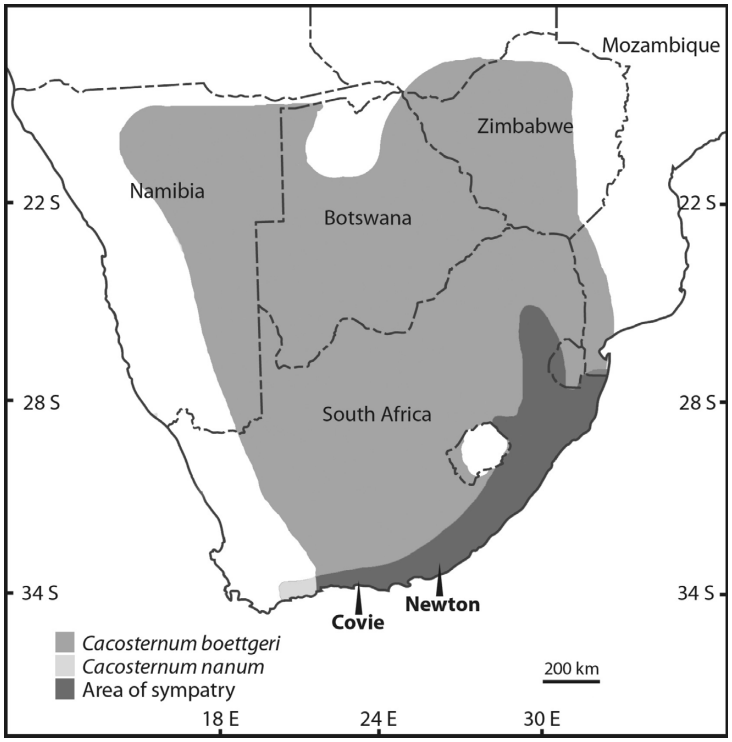

Fig. 1. Map of southern Africa with the known distribution for *Cacosternum boettgeri* and *C. nanum*. Localities where parasites were found are indicated.

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.

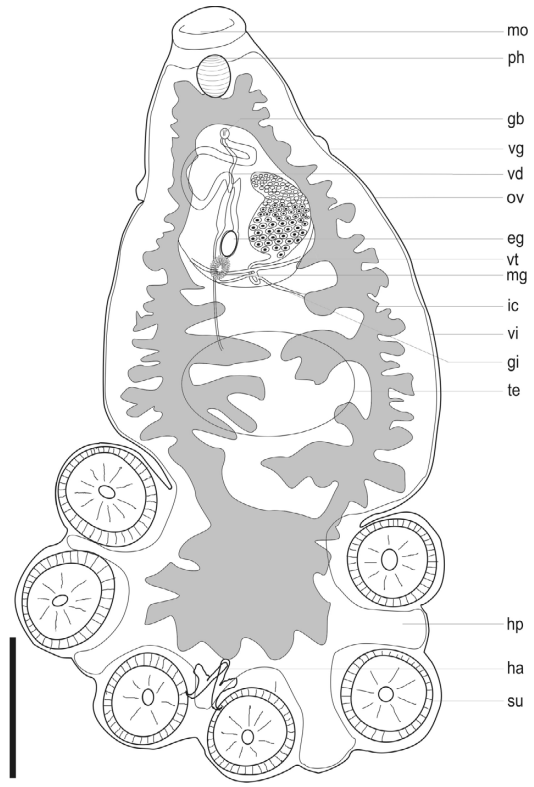

Fig. 2. *Polystoma channingi* n.sp. Ventral view of holotype from *Cacosternum nanum* collected near Kenton on Sea, South Africa (33.581298S, 26.665182E) (NMB P344). *Abbreviations:* eg, egg; gb, genital bulb; gi, genito-intestinal canal; ha, hamulus; hp, haptor; ic, intestinal caecum; mg, Mehlis gland; 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 (884–2427); 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-

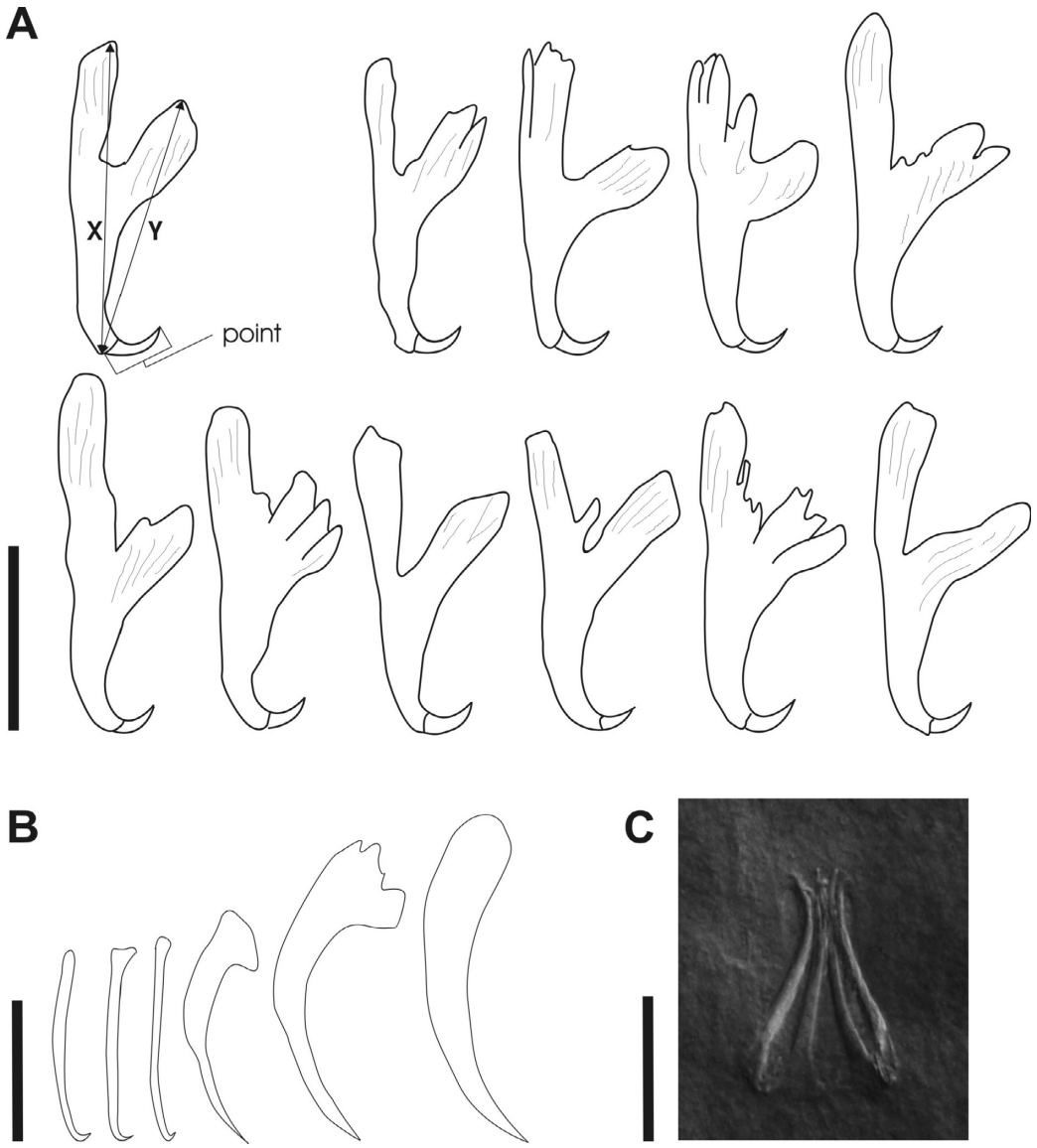

Fig. 3. *Polystoma channingi* n.sp. **A**, hamuli from holotype and paratypes; **B**, hamulus primordia and developing hamuli; **C**, crown of genital spines. *Abbreviations:* X, distance from hook to tip of handle; Y, distance from hook to tip of guard. Scale bars: A = 100 µm; B and C = 20 µm.

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 utero. 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

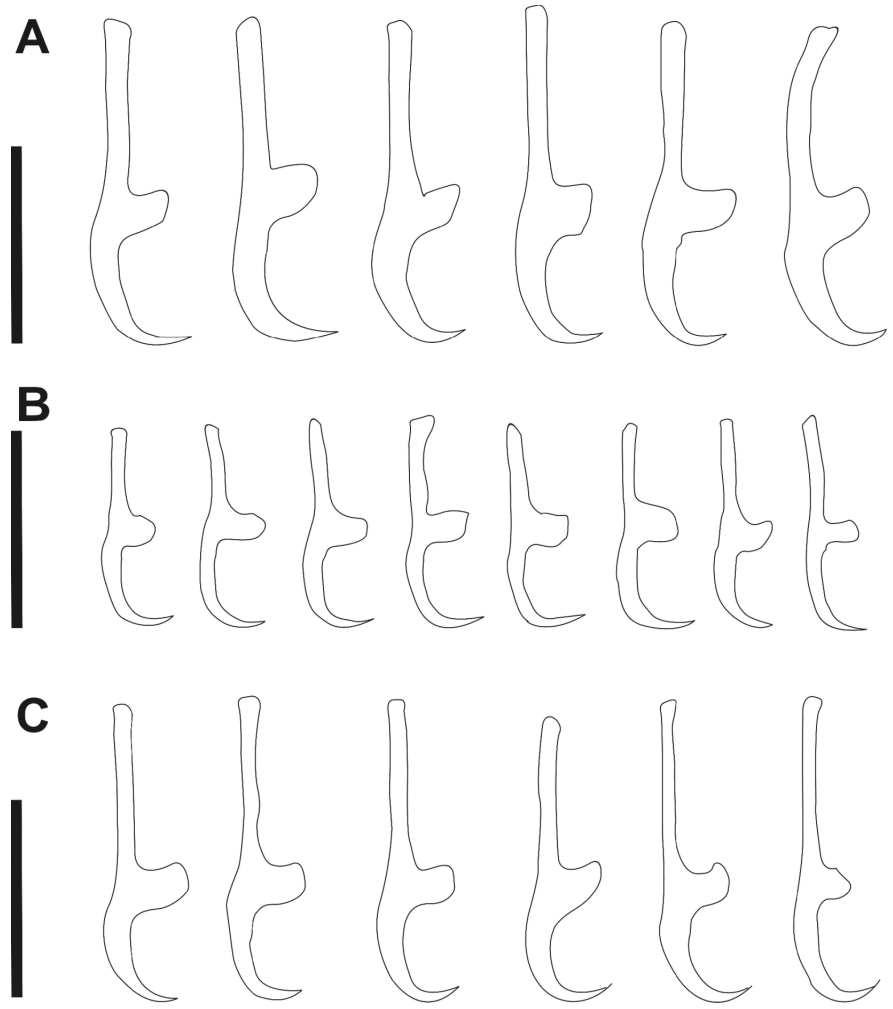

Fig. 4. *Polystoma channingi* n.sp. Marginal hooklets from holotype, paratypes and juvenile parasites; **A**,marginal hooklet 1; **B**, marginal hooklets 27; **C**, marginal hooklet 8. Scale bars: A, B and C = 20 µm.

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. dawiekoki,* 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

Table 1. Measurements of sexually mature, egg-producing *Polystoma channingi* n.sp. compared to the relevant measurements for *P. australe* (*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).

Body measurement or ratio	*P. channingi* n.sp.	*P.a*	*P.c*	*P.d*	*P.m*	*P.n*	*P.s*	*P.t*	*P.u*	*P.v*
Body length (BL)	4960	6730	9390	7096	6250	4050	6960	7850	7290	7244
Greatest width	1750	2530	3090	2031	2260	1420	2190	2580	2450	1816
Width at vagina	1299	1580	2060	–	1510	–	1710	1680	1770	–
Haptor length (HL)	1884	1740	1950	1582	1460	1250	1600	2050	2060	1407
Haptor width	2335	2490	3140	2432	2230	1720	2370	2860	2740	1796
HL/BL ratio	0.38	0.26	0.21	0.34	0.23	0.33	0.23	0.26	0.28	0.19
Pharynx width	236	238	387	245	303	–	270	294	300	223
Pharynx length	252	251	381	270	332	–	305	293	295	279
Ovary length	713	784	1127	760	723	481	675	836	862	719
Sucker diameter	501	376	487	455	490	329	482	553	474	356
Sucker as % of body	10.1	5.6	5.2	6.4	7.8	8.1	6.9	7.0	6.5	4.9
Hamulus length	360	326	458	440	323	345	429	411	408	395
Intestinal anastomoses	0	>1	0	0-2	0	<1	0	<1	<1	0

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.

A high degree of host-specificity has been documented for several African polystomes (Combes 1966, 1968; Tinsley 1973, 1974, 1981; Euzet *et al.* 1974a,b; Bourgat & Salami-Cadoux 1976; Combes & Channing 1979; Kok & Van Wyk 1986; Kok & Du Preez 1987; Murith 1981a,b, 1982; Du Preez & Kok 1992b, 1993, 1997; Tinsley & Jackson 1998). 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

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 & Carruthers 2009). 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 *Metapolystoma* 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*.

ACKNOWLEDGEMENTS

I am indebted to the South African National Research Foundation (NRF) for financial support; Alan Channing, Roger Bills, Ché Weldon, and postgraduate students for assisting in collection of frogs; Olivier Verneau and his team for conducting the molecular analyses. Any opinion, findings and conclusions or recommendations expressed in this material are those of the author and therefor the NRF does not accept any liability in regard thereto.

REFERENCES

BENTZ, S., LEROY, S., DU PREEZ, L.H., MARIAUX, J. & VAUCHER, C & VERNEAU, O. 2001. Origin and evolution of African *Polystoma* (Monogenea : Polystomatidae) assessed by molecular methods. *International Journal for Parasitology* **31**: 697–705.

BOURGAT, R. & SALAMI-CADOUX, M.L. 1976. Recherches expérimentales sur la spécificité parasitaire des polystomes de *Bufo regularis*, *Rana galamensis* et *Hylarana albolabris* au Togo. *Revue des Sciences Médicales et Biologiques du Togo* **1**: 41–42.

COMBES, C. & CHANNING, A. 1979. Polystomatidae (Monogenea) d'amphibiens d'Afrique du Sud: *Polystoma natalensis* n. sp., Parasite de *Strongylopus grayii* (Smith 1849). *Vie Milieu*, Série C **28–29**: 61–68.

COMBES, C. 1966. Recherches expérimentales sur la spécificité parasitaire des polystomes de *Rana temporaria* et de *Pelobates cultripes* (Cuv.). *Bulletin de la Société Zoologique de France* **91**: 439–444.

COMBES, C. 1968. Biologie, écologie des cycles et biogéographie de digènes et monogènes d'amphibiens dans l'est des Pyrénées. *Mémoire du Muséum National d'Histoire Naturelle Série A Zoologie* **51**: 1–195.

DU PREEZ, L.H. 2011. *Polystoma vernoni* n. sp. (Monogenea: Polystomatidae) from the sharp nosed grass frog *Ptychadena oxyrhynchus* (Smith, 1849) in South Africa. *Journal of Helminthology* **85**: 294–299.

DU PREEZ, L.H. & CARRUTHERS, V.C. 2009. *A Complete Guide to the Frogs of Southern Africa.* Random House Struik, Cape Town.

DU PREEZ, L.H. & KOK, D.J. 1992a. The frog genus *Ptychadena* as host for polystomatid (Monogenea) parasites in Africa. *African Journal of Herpetology* **40**: 47–50.

DU PREEZ, L.H. & KOK, D.J. 1992b. Syntopic occurrence of new species of *Polystoma* and *Metapolystoma* (Monogenea: Polystomatidae) in *Ptychadena porosissima* in South Africa. *Systematic Parasitology* **22**: 141–150.

DU PREEZ, L.H. & KOK, D.J. 1993. Polystomatidae (Monogenea) of Anura in southern Africa: *Polystoma testimagna* n. sp. parasitic in *Strongylopus f. fasciatus* (Smith, 1849). *Systematic Parasitology* **25**: 213–219.

DU PREEZ, L.H. & KOK, D.J. 1995. Polystomatidae (Monogenea) of southern African Anura: *Polystoma claudecombesi* n.sp. parasitic in *Rana angolensis* Bocage, 1866. *Systematic Parasitology* **30**: 223–231.

DU PREEZ, L.H. & KOK, D.J. 1997. Supporting evidence of host-specificity among southern African polystomes (Polystomatidae: Monogenea). *Parasitology Research* **83**: 558–562.

DU PREEZ, L.H. & KOK, D.J. 1998. The relative importance of bladder versus neotenic stages of *Polystoma marmorati* and *P. umthakathi* in natural frog populations in South Africa. *Journal of Helminthology* **72**: 117–121.

DU PREEZ, L.H., VAUCHER, C. & MARIAX, J.P. 2002. Polystomatidae (Monogenea) of southern African Anura: *Polystoma dawiekoki* n. sp. parasitic in *Ptychadena anchietae* (Bocage, 1867). *Systematic Parasitology* **52**: 35–41.

EUZET, L., BOURGAT, R. & SALAMI-CADOUX, M-L. 1974a. *Polystoma galamensis* (Monogenea) parasite de *Rana galamensis* Duméril et Bibron, 1841, au Togo. *Annales de Parasitologie Humaine et Comparée* **49**: 63–68.

EUZET, L., COMBES, C. & KNOEPFFLER, L. PH. 1974b. Parasites d'amphibiens de la République Centrafricaine. Polystomatidae (Monogenea). *Vie Milieu*, Série C **24**: 141–150.

FROST, D. 2011. Amphibian Species of the World: an Online Reference. Version 5.5 (31 January 2011). Electronic database online at http://research.amnh.org/vz/herpetology/amphibia/ American Museum of Natural History, New York, USA.

KOK, D.J. & DU PREEZ, L.H. 1987. *Polystoma australis* (Monogenea): life cycle studies in experimental and natural infections of normal and substitute hosts. *Journal of Zoology, London* **212**: 235–243.

KOK, D.J. & SEAMAN, M.T. 1987. Polystomatidae (Monogenea) parasitic in the anuran genus *Natalobatrachus* in South Africa. *South African Journal of Zoology* **21**: 189–263.

KOK, D.J. & VAN WYK, J.H. 1986. Polystomatidae (Monogenea) parasitic in the anuran genus *Kassina* in South Africa. *South African Journal of Zoology* **21**: 189–196.

MURITH, D. 1981a. Contribution à l'étude de la biologie du développement des polystomes (Monogenea) parasites d'amphibiens anoures de basse Côte-d'Ivoire.

Bulletin de la Société Neuchâteloize des Sciences Naturelles **104**: 5–33.

MURITH, D. 1981b. Contribution à l'étude de la systématique des polystomes (Monogènes, Polystomatidae) parasites d'amphibiens anoures de basse Côte-d'Ivoire. *Revue Suisse de Zoologie* **88**: 475–503.

MURITH, D. 1982. Etude *in vivo* de la nature des relations hôte-parasite dans le complexe amphibien-polystome (Monogenea). *Revue Suisse de Zoologie* **89**: 957–965.

TINSLEY, R.C. 1973. Observations on Polystomatidae (Monogenoidea) from East Africa with a description of *Polystoma makereri* n. sp. *Zeitschrift für Parasitenkunde* **42**: 251–263.

TINSLEY, R.C. 1974. Observations on *Polystoma africanum* Szidat with a review of the inter-relationships of *Polystoma* species in Africa. *Journal of Natural History* **8**: 355–367.

TINSLEY, R.C. 1981. The evidence from parasite relationships for the evolutionary status of *Xenopus* (Anura: Pipidae). *Monitore zoologico italiano* **19**: 367–385.

TINSLEY, R.C. & JACKSON, J.A. 1998. Speciation of *Protopolystoma* Bychowsky, 1957 (Monogenea: Polystomatidae) in hosts of the genus *Xenopus* (Anura: Pipidae). *Systematic Parasitology* **40**: 93–141.

VAN NIEKERK, S., KOK, D.J. & SEAMAN, M.T.S. 1992. A new species of *Polystoma* (Monogenea: Polystomatidae) parasitic in *Hyperolius marmoratus* (Anura: Hyperoliidae) in South Africa. *Systematic Parasitology* **25**: 73–80.

Responsible Editor. N.J. Smit