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The first record of polystomes (Monogenea: Polystomatidae) from caecilian hosts (Amphibia: Gymnophiona), with the description of a new genus and two new species

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Abstract New taxa are proposed for *Nanopolystoma lynchi* n. g., n. sp. from the urinary bladder and phallodeum of the caecilian *Caecilia* cf. *pachynema* and *N. brayi*. n. sp. from the urinary bladder of *C. gracilis*, both from South America. These are the first species of polystomatids to be described from caecilian hosts. The parasites are small with a maximum body length of 2 mm. The two gut caeca are not confluent posteriorly and have neither diverticula nor anastomoses. The haptor bears six well-developed suckers and one pair of hamuli. The single follicular testis lies in the mid-body and the ovary is small. Vaginae are present. A single large, operculate egg lies in the short uterus, which leads to an armed genital bulb. Neither the nature of the oncomiracidium nor the phylogenetic position of these worms is known. *Nanopolystoma* n. g. shares various morphological features with other polystomatid genera; however, the non-confluent gut-caeca lacking diverticula and the presence of skeletal elements in the haptoral suckers place it with the chelonian polystomes of the subfamily Polystomoidinae. These findings suggest that the caecilian host species are probably semi-aquatic. More polystomatid species are expected to be found in association with other species of *Caecilia*.

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Introduction

The platyhelminth Class Monogenea includes at least 20,000 species (Rohde, 1996), mainly parasitising chondrichthyan and teleost fishes. One family, the Polystomatidae Carus, 1863, has radiated to a variety of aquatic and semi-aquatic tetrapod hosts and is currently represented by 20 genera. Of these, *Diplorchis* Ozaki, 1931, *Eupolystoma* Kaw, 1950, *Mesopolystoma* Vaucher, 1981, *Metapolystoma* Combes, 1976, *Neodiplorchis* Yamaguti, 1963, *Parapolystoma* Ozaki, 1935, *Parapseudopolystoma* Nasir & Fuentes Zambrano, 1983, *Polystoma* Zeder, 1800, *Protopolystoma* Bychowsky, 1957, *Pseudodiplorchis* Yamaguti, 1963, *Riojatrema* Lamothe-Argumento, 1964, *Sundapolystoma* Lim & Du Preez, 2001 and *Wetapolystoma* Gray, 1983 are known from anuran hosts, *Pseudopolystoma* Yamaguti, 1963 and *Sphyranura* Wright, 1879 from caudate hosts, *Neopolystoma* Price, 1939, *Polystomoidella* Price, 1939 and *Polystomoides* Ward, 1917 from chelonians, *Concinnocotyla* Pichelin, Whittington &

Pearson, 1991 from the Australian lungfish and *Oculotrema* Stunkard, 1924 from a mammal, the hippopotamus. Of the 153 known polystome species, no less than 61% are known from anuran hosts, while 3% infect caudate amphibians. Until now the third order of amphibians, namely the Gymnophiona, i.e. the caecilians, was not known to harbour any polystomes.

Two caecilian species were recently found to be infected with a distinct species of what appears to be a new genus of polystome. This paper reports the formal description of the first finding of polystomes from the urinary bladder and phallodeum of *Caecilia* cf. *pachynema* Günther and the urinary bladder of *C. gracilis* Shaw from South America.

Materials and methods

When caecilians archived in formalin in the herpetological collection of the National Museums of Scotland (NMSZ) were examined by one of us (MW), polystomes were retrieved from the urinary bladders and phallodeum (posterior part of male cloaca) of specimens of two species.

The parasites were stained using Mayer's paracarmine and mounted in Canada balsam by Dr Rodney Bray at the Natural History Museum, London (NHM). In order to study the genital spine arrangement and the structure of the haptoral suckers, mounted specimens were examined using a Nikon PCM2000 confocal-laser microscope fitted on a Nikon TE300 inverted microscope. An attempt to extract DNA from one specimen at the NHM proved unsuccessful as the DNA was too fragmented. All parasite measurements are in micrometres.

Results

The hosts

Caecilians are elongate, limbless, mostly soil-dwelling amphibians of the wet tropics. *Caecilia* L., the type-genus of the large and heterogeneous family Caeciliidae, includes some 33 Neotropical species, all of which are poorly known and subject to taxonomic uncertainty (Wilkinson & Nussbaum, 2006). Two specimens of *C.* cf. *pachynema* were examined, and one specimen, with a length of 703 mm, had eight polystomes in the urinary bladder and phallodeum. Of three specimens of *C. gracilis* examined, one with a length of 455 mm harboured four polystomes in the urinary bladder. Both host specimens are unregistered material from the collections of the National Museums of Scotland and lack any associated data except for the *C. gracilis*, which is from Demerara, Guyana. The specimens are old and were probably collected sometime in the 19th Century. *C.* cf. *pachynema* is found in old growth and disturbed forests and has been found along roads, streams and rivers (Azevedo-Ramos et al., 2004). *C. gracilis* occurs in old growth forests and savannah (Coloma et al., 2004).

Nanopolystoma n. g.

Diagnosis

Polystomoidinae: Small ovoid worms, *c.*1–2 mm in length. Body pyriform. Haptor with three pairs of suckers, two hamuli and 16 marginal hooklets, hooklets; 1 & 2 situated between hamuli; hooklets 3–5 at base of suckers; hooklets 6–8 posterior between third pair of suckers. Mouth subterminal, surrounded by false sucker. Pharynx large, muscular. Gut bifurcate; intestinal caeca simple, lacking diverticula, anastomoses and posterior confluence, extending to posterior extremity of body but not into haptor. Genito-intestinal canal present. Testis single, consists of many follicles, post-ovarian, intercaecal. Vas deferens extends antero-medially to copulatory organ (genital bulb). Copulatory organ armed with 10–19 spines, opens through prominent common genital pore. Ovary oval, median. Uterus short, with single egg. Vitelline follicles in two dense lateral fields; fields extend from pharyngeal region to posterior region of body where they are confluent. Two vaginae, lateral to ovary; left and right vaginal ducts connected to respective vitelline ducts. Egg operculate, with no indication of intra-uterine development of oncomiracidium. Adult parasitic in urinary bladder and phallodeum of caecilians in Neotropical region. Type-species *N. lynchi* n. sp.

Nanopolystoma lynchi n. sp.

Type-host: *Caecilia* cf. *pachynema* Günther.
Site: Urinary bladder and phallodeum.

Type-locality: South America (exact locality not known).

Type-material: Eight sexually mature worms. Holotype (2007.8.15.1) and 7 paratypes (2007.8.15.2-8) in the Parasitic Worms Collection, Natural History Museum, London.

Etymology: The generic name refers to the small size of the parasites. The species parasite is named for Dr John D. Lynch in acknowledgement of his tremendous contribution in furthering our knowledge of Neotropical amphibians.

Description (Figs. 1–3)

[Based on 6 specimens.] Adult pyriform (Fig. 1). Body length 1,706 (1,358–2,012); greatest width 690 (631–737); width at vagina 661 (630–698). Haptor 561 (504–592) long, 590 (552–630) wide. Eye-spots not observed in adult. Mouth subterminal, ventral, surrounded by false oral sucker; musculature of false oral sucker interspersed with gland-cells. Pharynx spherical, 152 (137–175) long, 184 (156–199) wide. Intestine bifurcates closely posterior to pharynx;

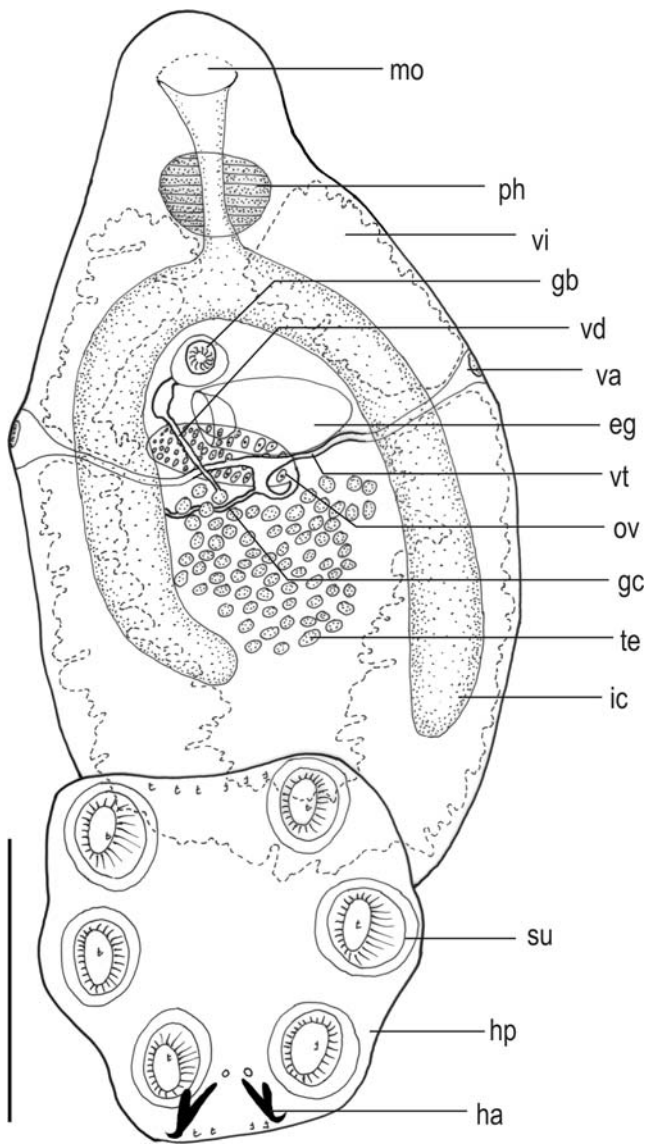

Fig. 1 *Nanopolystoma lynchi* n. sp. Ventral view of holotype. Genital system is a composite drawing based on all specimens examined. *Abbreviations:* eg, egg; gb, genital bulb; gc, genito-intestinal canal; ha, hamulus; hp, haptor; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; va, vagina; vd, vas deferens; vi, vitellarium; vt, vitelline duct. *Scale-bar*: 500 μm

intestinal caeca blind, with neither diverticula nor anastomoses; left and right caeca of almost equal length, extend along 80% of body.

Testis single, follicular, post-ovarian, median in mid-body. Vas deferens widens slightly anteriorly forming seminal vesicle, narrows at genital bulb to open at common genital opening. Genital bulb 78 (74–84), armed with 10–12 genital spines. Genital spines 19 (18–19) in length, curved, with branched root proximally and sharp point distally, arranged in circle located anterior to ovary and posterior to intestinal bifurcation (Figs. 1, 2C, 3A). Two vaginae on lateral margins in middle of body at level of ovary; vaginal ducts descend to respective vitelline ducts. Main left and right vitelline ducts unite medially to form vitelline reservoir, with posterior duct connecting with oviduct. Vitellarium follicular, dense, co-extensive with caeca; main left and right vitelline fields confluent posteriorly just anterior to haptor. Ovary 173 (132–204) long, 83 (65–96) wide, medial, in middle of body, oval (Fig. 1). Oviduct leaves ovary, ascends and receives duct from vitelline reservoir, forms oötype, which is surrounded by Mehlis' gland. Uterus short, anterior to ovary, holds single egg. Genito-intestinal canal arises just before entry of posterior vitelline duct, connects oviduct with caecum. Uterine eggs 218 (151–262) × 123 (110–173), yellowish-tan, operculate, oval, with operculate end flattened giving flask-shaped appearance; no indication of intra-uterine development. Oncomiracidium not known.

Haptor with 3 pairs of laterally located suckers, with diameter of 162 (139–182), 2 hamuli and 16 marginal hooklets. Placement of marginal hooklets as for other polystomes: pairs 1 and 2 posterior-most, between suckers; pairs 3, 4 and 5 at base of suckers; pairs 6–8 anterior in haptor between anterior-most suckers. Hooklet 1 longest and largest, with length 18 (17–19) (Fig. 2B); hooklets 2–8 measure 16 (15–18). Marginal hooklet guard tending to curve away from blade (Fig. 2B). Hamulus size and shape variable in adult specimens; hamulus shaft robust with deep V between 2 roots (Fig. 2A); inner length (Y in Fig. 2A) 80 (72–89); outer length (X in Fig. 2A) 104 (81–121); recurved point (Z in Fig. 2A) 28(26–29). Spherical sclerotised droplets associated with hamuli, distributed between posterior-most suckers (Fig. 3B), 3 (2–5) in number, 8 (2–16) in diameter.

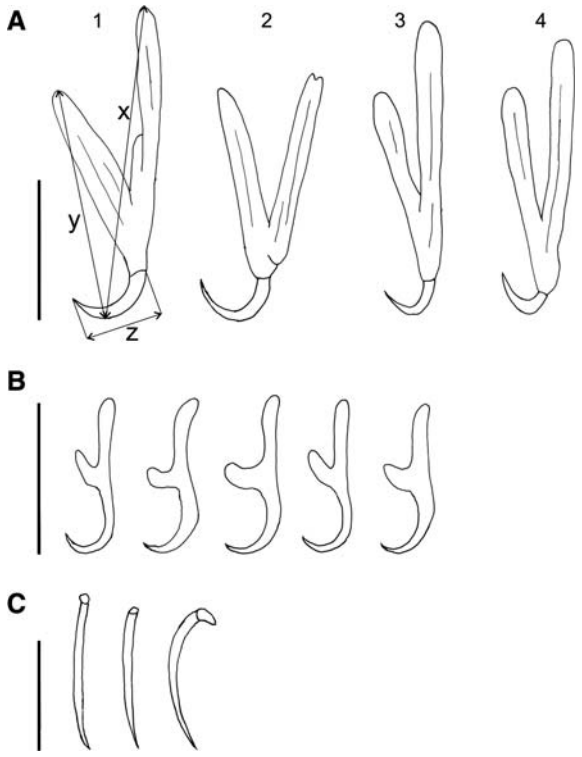

Fig. 2 *Nanopolystoma lynchi* n. sp. A. hamulus from holotype (1) and paratypes (2–3), X – outer length, Y – inner length, Z – point length; B. marginal hooklets 1 from holotype and paratypes; C. genital spines from holotype and paratypes. *Scale-bars*: A, 50 μm; B, C, 10 μm

Nanopolystoma brayi n. sp.

Type-host: *Caecilia gracilis* Shaw.
Site: Urinary bladder.
Type-locality: Demerara, Guyana.
Type-material: Four sexually mature worms. Holotype (2007.8.15.9) and 3 paratypes (2007.8.15.10-13) in the Parasitic Worms Collection, Natural History Museum, London.
Etymology: This species is named for Dr Rodney Bray in acknowledgement of his contribution to furthering our knowledge on amphibian parasites.

Description (Figs. 4–5)

Adult pyriform (Fig. 4). Body length 1,237 (1,091–1,407); greatest width 555 (543–572); width at vagina 529 (514–534). Haptor 415 (398–446) long, 457 (388–529) wide. Eye-spots not observed in adult.

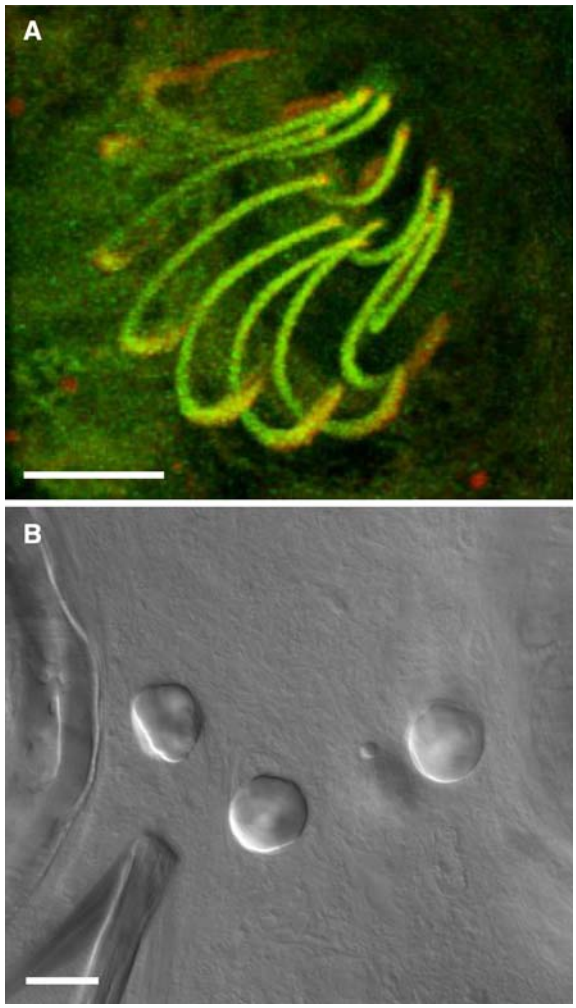

Fig. 3 *Nanopolystoma lynchi* n. sp. A. confocal image of genital spines; B. micrograph of sclerotised droplets using DIC optics. *Scale-bars*: 10 μm

Mouth subterminal, surrounded by false oral sucker; musculature around mouth interspersed with gland-cells. Pharynx spherical, 68 (60–86) long, 106 (101–108) wide. Intestine bifurcates just posterior to pharynx; caeca blind, with neither diverticula nor anastomoses; left and right caeca almost of equal length, extending full length of body but not into haptor. Genito-intestinal canal connects oviduct to caecum.

Testis single, follicular, median in mid-body, intercaecal. Vas deferens widens slightly anteriorly forming seminal vesicle, narrows at genital bulb to open at common genital opening. Genital bulb prominent, with diameter 97 (91–108), armed with 16–19 genital spines. Genital spines 21 (20–22) in length, curved, with branched root on proximal end and sharp point distally (Fig. 5C), arranged in circle, located anterior to ovary and posterior to intestinal bifurcation (Fig. 4). Two vaginae, on lateral margins in middle of body at level of ovary; vaginal ducts descend to respective vitelline ducts. Main left and right vitelline ducts unite medially to form vitelline reservoir, with posterior duct connecting to oviduct. Vitellarium follicular, dense, co-extensive with caeca; main left and right vitelline fields confluent posteriorly just anterior to haptor. Ovary 119 (106–130) long, 62 (55–67) wide, in middle of body, oval (Fig. 4). Uterus short, anterior to ovary, holding single egg. Uterine egg 221 (182–238) × 114 (110–115), yellowish-tan, operculate, with no indication of intra-uterine development. Oncomiracidium not known.

Haptor with 3 pairs of laterally located suckers 129 (122–139) diameter, 2 hamuli and 16 marginal hooklets. Placement of marginal hooklets as for other polystomes; hooklet pairs 1 and 2 posterior-most between suckers; pairs 3, 4 and 5 at bases of suckers; pairs 6–8 anterior in haptor between anterior-most suckers; hooklet 1 longest and largest, with length 20 (19–20); hooklets 2–8 measure 19 (17–21) (Fig. 5B). Marginal hooklet guard at right-angle to blade (Fig. 5B). Hamulus size and shape variable in adults; fully-developed hamulus robust with deep notch between 2 roots (Fig. 5A); inner length (Y in Fig. 5A) 63 (53–71); outer length (X in Fig. 5A) 95 (74–110); recurved point (Z in Fig. 5A) 26 (only 1 point measurable). Spherical sclerotised droplets number 6 (5–7), measuring 12 (3–21) in diameter, present at base of hamuli.

Remarks. No hamuli for *N. brayi* n. sp. were oriented perfectly flat in any of the specimens studied. The reported root lengths and point lengths could, however, be measured with confidence.

Discussion

The newly described species of *Nanopolystoma* n. g. have a combination of characteristics that place them in the monogenean family Polystomatidae, but not within any of the previously described genera. The non-confluent gut caeca is a characteristic shared only with the anuran polystomatids *Parapolystoma, Sundapolystoma,*

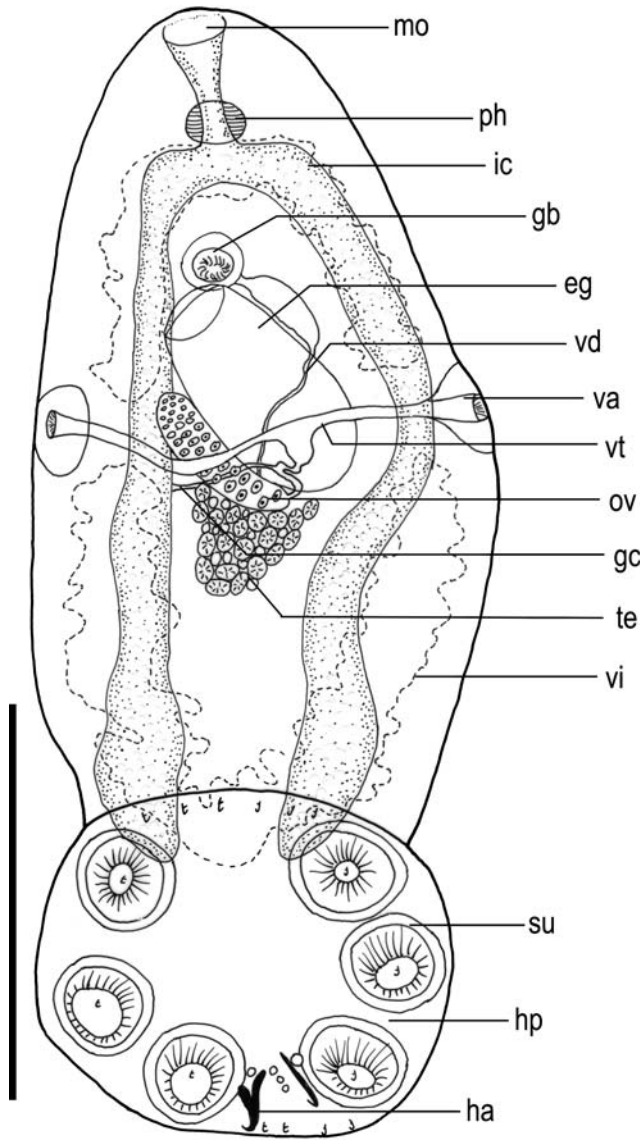

Fig. 4 *Nanopolystoma brayi* n. sp. Ventral view of holotype. Genital system is a composite drawing based on all specimens examined. *Abbreviations:* eg, egg; gb, genital bulb; gc, genito-intestinal canal; ha, hamulus; hp, haptor; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; va, vagina; vd, vas deferens; vi, vitellarium; vt, vitelline duct. *Scale-bar*: 500 μm

Diplorchis and some individuals of *Protopolystoma*, the chelonian polystomes *Polystomoides, Polystomoidella* and *Neopolystoma*, and the mammalian polystome *Oculotrema.* The presence of one pair of hamuli also separates *Nanopolystoma* from *Protopolystoma*, *Polystomoides, Neopolystoma* and *Oculotrema. Protopoly stoma* and *Polystomoides* both have two pairs of hamuli, while *Neopolystoma* and *Oculotrema* lack hamuli altogether. The distribution of the vitellarium, confined to lateral fields and the posterior margin of the body, distinguishes *Nanopolystoma* from *Parapolystoma*, which has extensive vitelline follicles extending over most of the body. Also, *Nanopolystoma* has a single testis, while *Parapolystoma* has multiple testes. *Diplorchis* and *Sundapolystoma* are separated from *Nanopolystoma* by the presence of an extensive tubular uterus rather than a very short uterus holding a single egg. The greatest morphological similarity is with the chelonian polystome *Polystomoidella*, to which *Nanopolystoma* exhibits a very close resemblance. However, these genera are clearly separated by the well-defined, posteriorly confluent, lateral vitelline fields in *Nanopolystoma*, as opposed to the diffuse vitellarium of *Polystomoidella.*

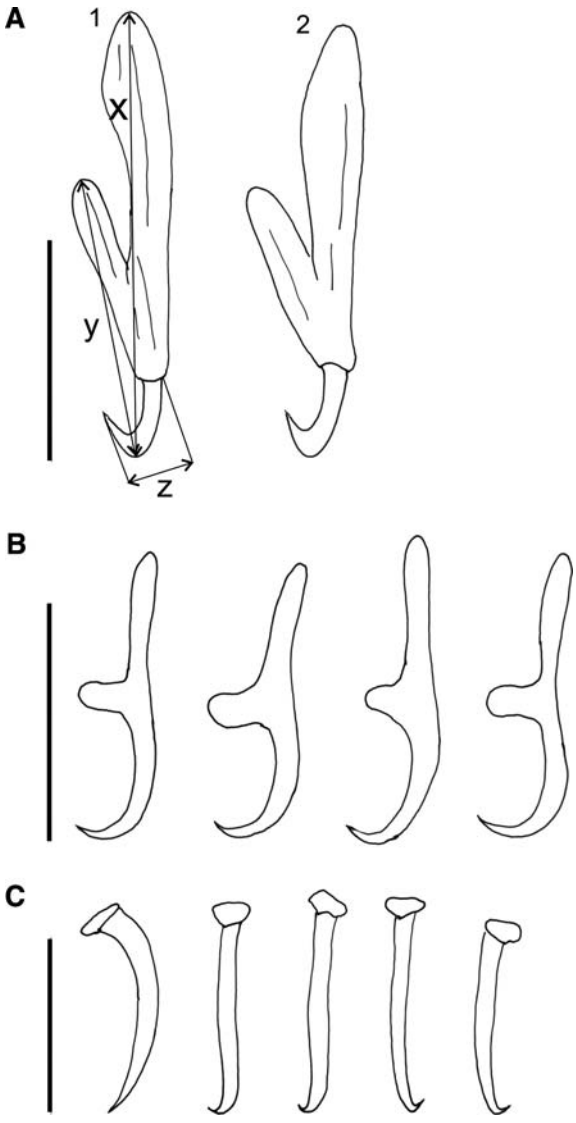

Fig. 5 *Nanopolystoma brayi* n. sp. A. hamulus from holotype (1) and a paratype (2), X – outer length, Y – inner length, Z – point length; B. marginal hooklets 1 from holotype and paratypes; C. genital spines drawn from holotype and paratypes. *Scale-bars*: A, 50 μm; B, C, 10 μm

The general body ratios and placement of the organs of *Nanopolystoma* are very similar to the chelonian polystomes. The prominent genital bulb armed with a large number of genital spines is also reminiscent of the equivalent structures in the chelonian polystomes *Neopolystoma* and *Polystomoides* (see Pichelin, 1995). In anuran polystomes, the genital bulb is relatively small in relation to the body size, whereas it is quite large in relation to the body size for *Neopolystoma* (see Pichelin, 1995) and *Nanopolystoma*. The testis of anuran polystomes is usually not distinctly visible in whole-mounts, and authors often serially section specimens to determine its position and extent (see Du Preez & Kok, 1993). In *Nanopolystoma*, the testis is a distinct spherical structure in the middle of the body. This is also the case for the chelonian polystomes *Polystomoides, Polystomoidella* and *Neopolystoma* (see Pichelin, 1995) and for the mammalian polystome *Oculotrema* (see Du Preez & Moeng, 2004). The single large egg reported herein for *Nanopolystoma* shows a striking similarity with some chelonian polystomes, for example *Neopolystoma cribbi* Pichelin, 1995, *N. spratti* Pichelin, 1995 and *N. kreffti* Rohde, 1980 (see Pichelin, 1995). No traces of haematin were observed in the gut of *Nanopolystoma lynchi* n. sp. or *N. brayi* n. sp., suggesting that these parasites are not sanguinivorous. The gut resembles that of *Polystomoides, Polystomoidella* and *Neopolystoma*, which are reported to feed on mucus and epithelial cells of their hosts (Allen & Tinsley, 1989).

Nanopolystoma lynchi n. sp. and *N. brayi* n. sp. are distinguished on the basis of the number of genital spines and the angle of the guard of the marginal hooklets. For *N. lynchi* we found a rosette consisting of 10–12 genital spines, whereas in *N. brayi* we found 16–19 genital spines. In *N. lynchi* the guard is obliquely angled towards the handle and away from the blade, while it is at a right-angle in *N. brayi*. Although the marginal hooklets could be measured in preparations of mature parasites, no such information is available for the oncomiracidia, the morphology of which needs further investigation.

All specimens examined had small spherical, sclerotised droplets associated with the hamuli. *N. lynchi* specimens had between two and five of these structures, averaging 8 μm in diameter. *N. brayi*, on the other hand, had between five and seven, averaging 12 μm in diameter. Similar structures have been presented in drawings of hamuli of *Polystoma gabonense* Euzet, Combes & Knoepffler, 1966 (emend.), *P. prudhoei* Saoud, 1967, *P. batchvarovi* Euzet, Combes & Knoepffler, 1974 and *P. llewellyni* Euzet, Combes & Knoepffler, 1974 (see Euzet et al., 1974); *P. praecox* Combes & Laurent, 1978 and *P. andinum* Combes & Laurent, 1978 (see Combes & Laurent, 1978); and *P. natalense* Combes & Channing, 1979 (emend.) (see Combes & Channing, 1978). They have

also been observed in *Protopolystoma* and are referred to as "sclerotised nodules" (Tinsley & Jackson, 1998) and have been observed by the first author in, as yet undescribed, polystomes from Africa. These structures appear to be waste products from the formation of the hamuli.

Polystomes are currently known from all continents except Antarctica. No less than 13 of the 20 currently-known polystomatid genera occur in the urinary bladder of anurans. Only two genera are known from caudates and, until now, no polystomes have been reported from caecilians. The subterranean terrestrial life of most caecilians makes them unsuitable as hosts for polystomatids. As far as we are aware, polystomes can only infect a host in an aquatic environment. When relatively terrestrial amphibian hosts harbour polystomes, the parasite usually has a large uterus filled with eggs that are released *en masse* when the host enters the water to breed, as in the case of *Eupolystoma* (see Du Preez et al., 2003) and *Pseudodiplorchis* (see Tinsley, 1982, 1995; Tinsley & Jackson, 1988). On the other hand, *Protopolystoma*, which infects the aquatic hosts *Xenopus* spp., lacks a uterus altogether and eggs are released more or less constantly. The single egg in the uterus of *Nanopolystoma* suggests that its host species spends a considerable time in water.

Very little is known of the biology of any species of *Caecilia*. Whereas some terrestrial caecilians have an aquatic larval stage that might provide the opportunity for infection by polystomatids, aquatic larvae are unknown for *Caecilia* or any of its closest relatives (Wilkinson & Nussbaum, 1998). It seems likely that development is direct from eggs laid in terrestrial nest sites, as suggested by Funk et al. (2006) for *C. occidentalis*, who reported finding adults and a clutch of eggs of this species in a very wet site, several metres from the nearest stream. Lynch & Acosta (2004) reported finding the closely related *Oscaecilia polyzona* in very wet, boggy soil. The discovery of two species of *Nanopolystoma* in two species of *Caecilia* suggests that these infections are not accidental. It also suggests that adults of these caecilian species spend sufficient time in aquatic habitats to be infected by polystomes and may best be considered semi-aquatic. Interestingly, the sister group of the *Caecilia–Oscaecilia* clade, the typhlonectids, includes the only caecilians that are fully aquatic as adults and other species with a more ancestral semi-aquatic adult lifestyle. It may be that a semi-aquatic lifestyle is ancestral for both the typhlonectids and for the *Caecilia–Oscaecilia* clade. Alternatively, it may have evolved independently in these groups. We anticipate that other polystomatid species will be found in association with other species of *Caecilia* and that typhlonectids will also prove to be polystomatid hosts. No information is available on the biology of *Nanopolystoma* and there is a need to study these parasites in their natural environment.

According to Verneau et al. (2002), the first polystomatids evolved 425 million years ago during the evolutionary transition between actinopterygians and sarcopterygians. Indications are that the first polystomes of amphibians evolved 250 mya during the diversification of the lissamphibians. Chelonian polystomatids evolved around 191 mya, following a switch from an aquatic amniote (presumed to be extinct) to turtles (Verneau et al., 2002). The close resemblance of *Nanopolystoma* with the chelonian parasites suggests a close relationship and that host-switching across classes cannot be ruled out.

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