

Polystoma okomuensis n. sp. (Monogenea: Polystomatidae) from Boulenger's striped frog, *Phlyctimantis boulengeri* (Perret, 1986) in Nigeria

M.S.O. Aisien^{1*}, L.H. Du Preez² and A.A. Imasuen¹

¹Laboratory of Parasitology Research, Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria: ²School of Environmental Sciences and Development, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

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Abstract

Polystoma okomuensis is described as a new species of the Polystomatidae parasitic in the urinary bladder of Boulenger's striped frog *Phlyctimantis boulengeri* in the Okomu National Park, Edo State, Nigeria. Although other African polystomes have been reported from Nigeria, this is the first to be described from the country and the first from *Phlyctimantis*. It is distinguished from other African *Polystoma* species by a combination of characters, including the body size, size and shape of the hamuli, size and shape of marginal hooklets and intestinal location. *Phlyctimantis boulengeri* was found to be infected in two of four seasonal lakes where specimens were caught with prevalences and mean intensities ranging from 14.3 to 22% and 1.0 to 1.5, respectively. Of the total number of 45 frogs examined, the prevalence was 15.6% and mean intensity 1.4.

Introduction

Polystomatidae of amphibians in Nigeria have, until recently, been poorly studied. According to Thurston (1970) and Avery (1971) the first polystome reported from Nigeria was *Protopolystoma xenopodis* (Price, 1943). Tinsley & Jackson (1998) reported another polystome from this region, namely *Protopolystoma occidentalis* from *Xenopus muelleri*. Recently Aisien *et al.* (2003, 2004a, b, 2009) reported on other polystomes from Nigeria. Apart from *Protopolystoma*, other polystomes known from Nigeria include *Polystoma prudhoei* Saoud, 1967 from *Amietophrynus regularis*, *P. galamensis* Euzet, Bourgat & Salami-Cadoux, 1974 from *Rana galamensis*, *Eupolystoma alluaudi* (de Beauchamp, 1913) from *Amietophrynus regularis* and *A. maculatus*, *Metapolystoma cachani* Gallien, 1956 from *Ptychadena longirostris*, and unidentified *Polystoma*

spp. from *Amietophrynus* sp. and *Hyperolius sylvaticus*. *Polystoma prudhoei* reported from *A. regularis* was a mis-identification as this parasite has now been identified as *Polystoma africanum* (Szidat 1932) and recently re-described by Aisien & Du Preez (2009).

Amphibian diversity in Nigeria has been the focus of several studies (Schjötz, 1963, 1964, 1966, 1967, 1969; Reid *et al.*, 1990; Oldham, 2000). Opinions vary on the official number of amphibian species represented in Nigeria. Schjötz (1963) reported 69 species while a later study by Oldham (2000) reported more than 90 species. Reid *et al.* (1990), referring to unpublished work, listed 75 species from the Calabar-Oban area alone. The most recent information by IUCN *et al.* (2006) put the number of species in the country at 103. In the Okomu National Park, which is a protected lowland rainforest habitat, we have recorded 40 species of amphibians, representing 10 families. Despite the rich amphibian diversity in Nigeria, very little information is available on the polystome diversity in the country. Our count to date shows that as

*E-mail: aisien@uniben.edu

many as 11 previously described African polystome species do occur in the country, while a number of undescribed species are still being investigated. With the high amphibian diversity present in Nigeria, we presume that there will be correspondingly rich polystome diversity.

The polystome that we describe herein was recovered from the urinary bladder of the Hyperolid frog, *Phlyctimantis bouleengeri* Perret, 1986 collected at two seasonal lakes in the Okomu National Park, Nigeria. Apart from Nigeria, the frog occurs in Cameroon, Ivory Coast, Equatorial Guinea, Ghana, Guinea and Liberia (Schj tzt, 1999). The frog is found in subtropical or tropical moist lowland forests, intermittent freshwater marshes, and heavily degraded former forested areas where males tend to aggregate in vast numbers.

Materials and methods

Parasites were recovered from the urinary bladder of adult *P. bouleengeri* collected in August 2007 and May 2009 in Okomu National Park (which lies between latitudes 6°15'N and 6°25'N; longitudes 5°9'E and 5°23'E) located in Edo State of Nigeria. The park, which lies in the lowland rainforest belt of south-western Nigeria, is a protected sanctuary for flora and fauna (fig. 1). The total area of the park is 19,712 hectares. Mean annual rainfall is about 2100 mm with most of it falling between April and October. The dry season lasts from November to March.

The frog specimens examined were collected from four seasonal lakes within the park which are designated as sites 1 to 4. The coordinates for these sites are as follows: site 1 (6°23'N, 5°20'E); site 2 (6°22'N, 5°21'E); site 3 (6°22'N, 5°20'E) and site 4 (6°17'N, 5°18'E). Of the four sites indicated, only site 4 retains some water in the months of the dry season.

Frogs were anaesthetized by immersion in benzocaine solution. The parasites were recovered from the urinary bladder, fixed in 5% formol-saline under cover-slip pressure. Fixed specimens were carefully removed after 1 h and transferred to vials containing 5% formol-saline. Parasites were washed in several changes of tap water and then stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene and permanent mounts made using Canada balsam. One specimen was fixed in 96% ethanol for molecular studies. The morphometrics of the marginal hooks were determined according to the protocols of Du Preez & Maritz (2006).

Results

Levels of infection

Phlyctimantis bouleengeri specimens were collected from four different seasonal lakes within the Okomu National Park. Of the eight specimens collected from site 1 between October 2007 and January 2008, none was infected.

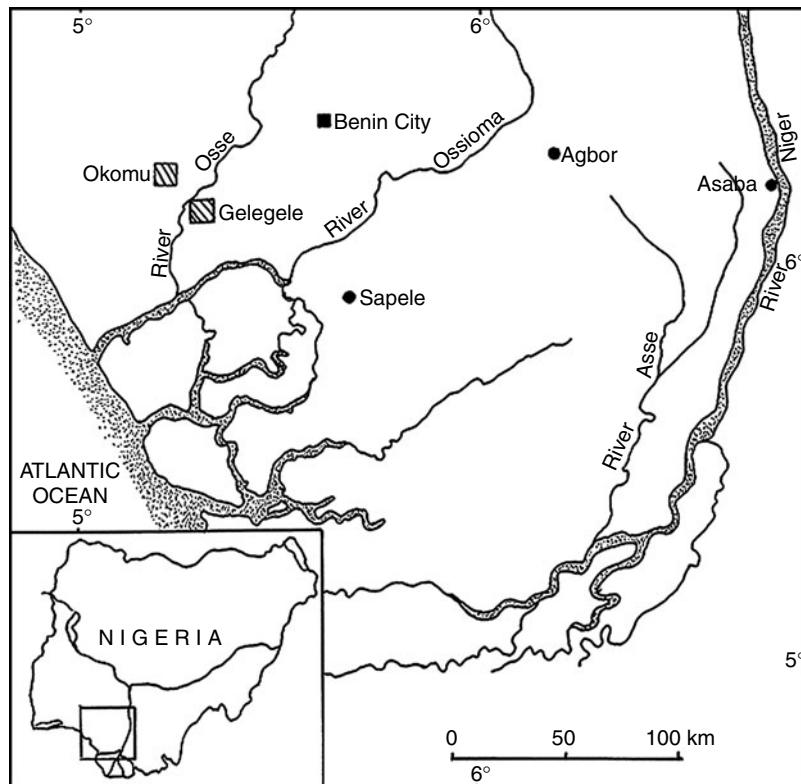


Fig. 1. Map of Nigeria showing the location of Okomu National Park, Edo State, Nigeria.

The three specimens caught between February and July 2008 from site 2 were also not infected. In site 3, one of the seven frogs caught between June and August 2007 was infected, giving a prevalence of 14.3% and a mean intensity of 1. At site 4, six of the 27 frogs caught in May 2009 harboured a total of nine polystomes, giving a prevalence rate of 22.2% and a mean intensity of 1.5. Of the total number of 45 frogs examined the prevalence was 15.5% and the mean intensity 1.4. Mean intensity of infection is defined as the average number of parasites/infected host.

Class: Monogenea Carus, 1863
Order: Polystomatidea Lebedev, 1988
Family: Polystomatidae Gamble, 1896

Polystoma okomuensis n. sp

Specimen studied. Nine adult specimens. Holotype LPRM15-0707-01 and two paratypes (LPRM15-0905-03 and LPRM15-0905-05) are deposited in the Parasitic Worm Collection of Professor A.B.M. Egborge Museum, University of Benin, Benin City, Nigeria; two paratype specimens (NHM 2010.1.28.1-2) in the Parasitic Worms Collection of the Natural History Museum, London; and the remaining specimens in the collection of the senior author.

Type host. *Phlyctimantis boulengeri* Perret, 1986 sexually mature male and female specimens (CT069002 and CT055413) are deposited in the Amphibian Collection of the Professor A.B.M. Egborge Museum, University of Benin, Benin City, Nigeria.

Type locality. Okomu National Park, Edo State, Nigeria (lies between latitudes 6°15'N, and 6°25'N; longitudes 5°9'E and 5°23'E).

Site. Urinary bladder.

Etymology. The specific name *okomuensis* relates to the Okomu National Park, where the host was collected.

Description. Based on adult parasites ($n = 9$); measurements given in micrometres. The average measurement is followed by the range in parentheses. Measurement of larval sclerites based on adult parasite specimens.

Adult. General characteristics of mature egg-producing parasite (fig. 2) typical of *Polystoma*. Body elongate, total length 6513 (5570–7597); greatest width 2334 (1882–3207); width at vagina 1565 (1188–1853). Haptor 1857 (1141–2153) long, 2459 (1871–2765) wide, haptor length to body length ratio 0.28 (0.20–0.31). Haptor with six suckers 403 (324–592) diameter; hamuli 369 (325–456) long, handle longer than guard and mean x/y ratio 1.43 (fig. 3); hamulus point 56 (51–67) long. Mouth subterminal, ventral. Oral sucker 472 (324–592) wide; pharynx 242 (218–277) long, 225 (204–253) wide. Intestine bifurcate with lobed lateral diverticula averaging 29.3 (20–38) in number per parasite, slender intensely branched medial diverticula numbering 17.3 (15–21) per parasite. Intestinal anastomoses infrequent, occurring in 1 (11.1%) of 9 specimens examined (fig. 4B). In two (22.2%) of the specimens two medial diverticula on one side join to form loops (fig. 4A). Caeca

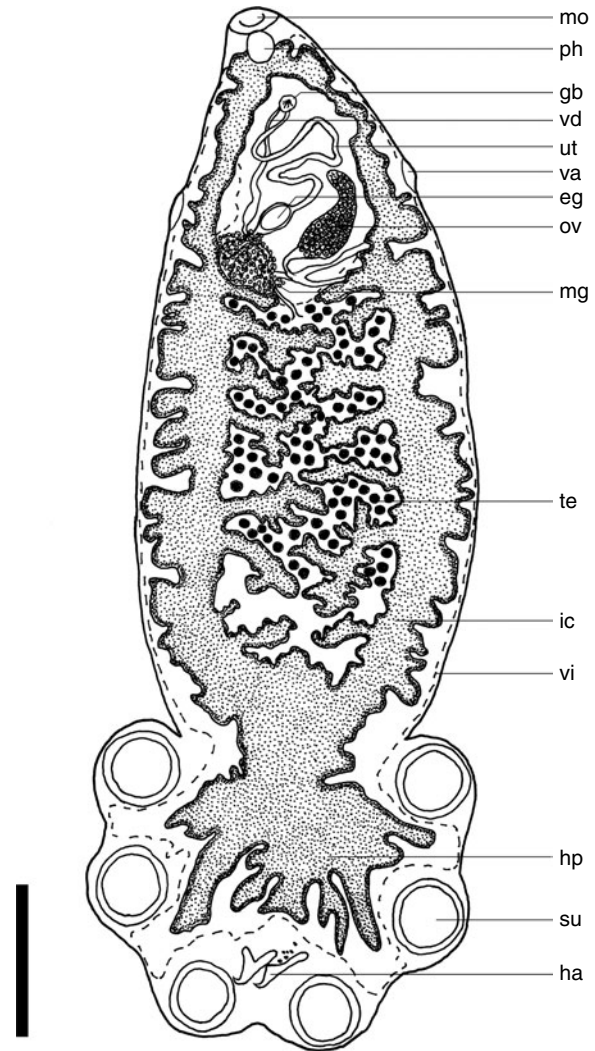


Fig. 2. *Polystoma okomuensis* n. sp. Ventral view of holotype; the dashed lines indicates the outline of the vitelline system. eg, egg; gb, genital bulb; ha, hamulus; hp, haptor; ic, intestinal caecum; mg, Mehlis gland; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; ut, uterus; va, vagina; vd, vas deferens; vi, vitelline distribution. Scale bar = 1 mm.

confluent posteriorly, extending into haptor forming haptoral caecal diverticula.

Testis follicular post-ovarian, ventral, median (fig. 2) occupying 70% of pre-haptoral region. Lots of sperm present among testis follicles. Seminal vesicle prominent and packed with sperm. Genital atrium median, ventral. Genital bulb 79 (62–97) in diameter, posterior to intestinal bifurcation, with 7–8 genital spines 29.7 (28.3–32.0) long. Ovary very prominent, sinistral, submedian, 775 (675–1040) long, 267 (169–402) wide and packed with oocytes. Ootype well developed. Genito-intestinal canal present on same side as ovary, joining intestinal caecum posterior to ovary. Uterus confined to area anterior to ovary, holding one egg in six polystomes and

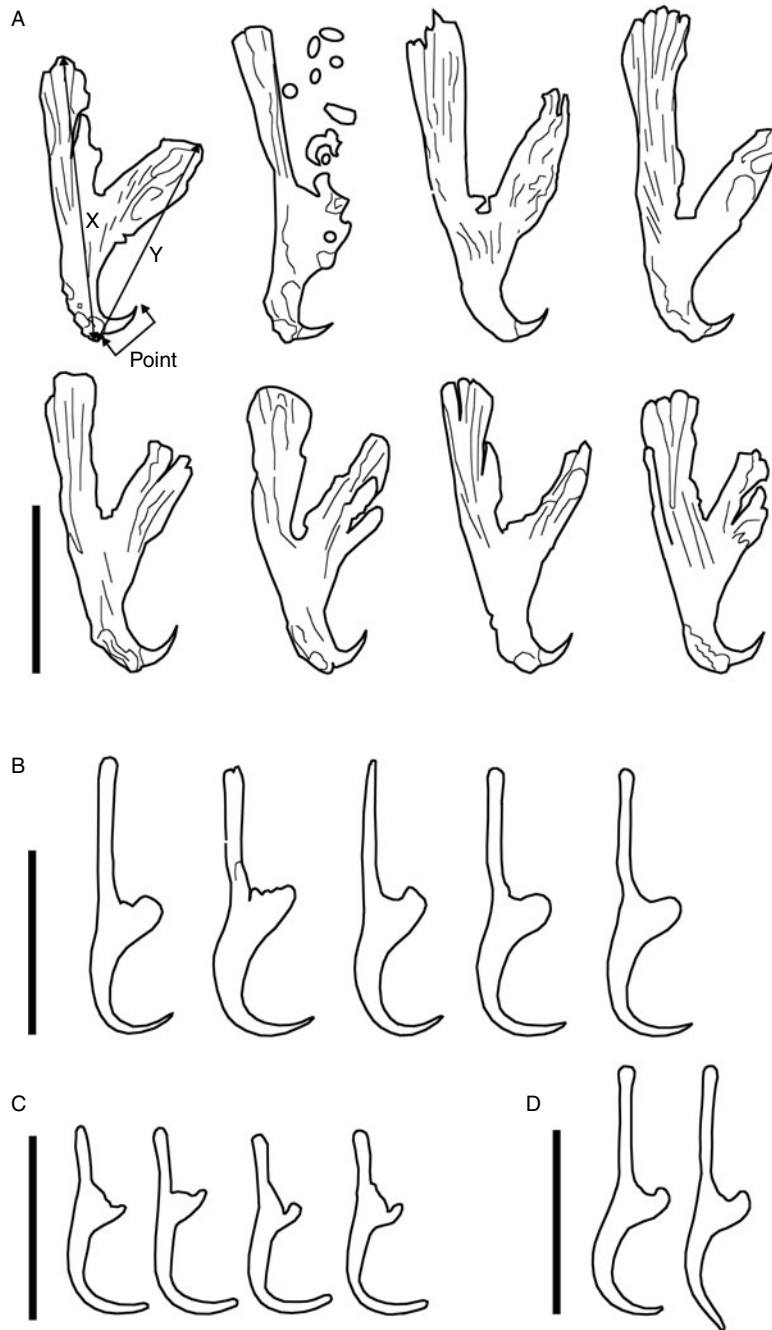


Fig. 3. *Polystoma okomuensis* n. sp. (A) Hamuli from holotype and paratypes; (B) marginal hooklets 1 from adult specimens; (C) marginal hooklets 2–7; (D) marginal hooklet 8. Scale bars: (A) 200 μm ; (B–D) 30 μm .

three polystomes with respectively two, three and seven eggs. Eggs operculate. Egg capsule length 249 (256–297), width 167 (145–192). No intrauterine development of eggs observed. Vitellaria distributed throughout most of the body (fig. 2).

Larval sclerites. Marginal hooklet 1 43 (39.8–44.7) long; marginal hooklets 2–7 28 (21–33) long, marginal hooklet 8 38.5 (37–40) long.

Discussion

In Africa, the genus *Polystoma* is at the moment represented by 33 described and several undescribed species. Of these, six species have been reported from Nigeria while information on undescribed species is emerging. *Polystoma okomuensis* n. sp. is the first species to be described from the country. The separation of

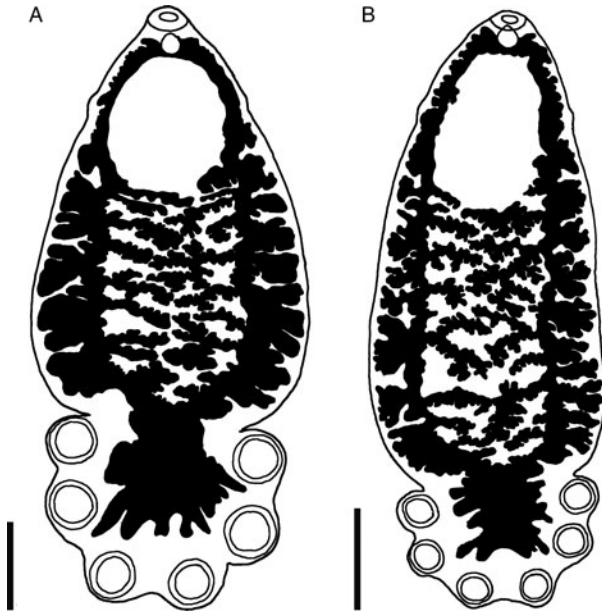


Fig. 4. *Polystoma okomuensis* n. sp. ventral view showing only the pharynx, intestine and suckers. Variation in intestinal arrangement: (A) specimen with loop; (B) specimen with caecal anastomosis. Scale bar = 1 mm.

polystomes based on morphological characters is complicated because of the limited interspecific morphological variation and large intraspecific variation. Murith (1981) has, however, shown that marginal hooklets of polystomes display little intraspecific variation and assist species separation. A plot of the products of the total length (a in fig. 5) and the width at the level of the guard (c in fig. 5) versus the product of the total length versus the length of a tangent between the tip of the blade to the guard (b in fig. 5) of marginal hooklet 1 (Du Preez & Maritz, 2006) showed an overlap of *P. okomuensis*

n. sp. with *P. dorsalis* Maeder, Euzet & Combes, 1970 (fig. 5). *Polystoma perreti*, which infests *Hylarana albolabris* (Hallowell, 1856) in the same environment, occupied a distinct position separated from *P. okomuensis* and *P. dorsalis* in the plot.

Despite the overlap of *P. okomuensis* n. sp. and *P. dorsalis*, a number of features differ between the two parasites. With a mean total body length of 6513 (5570–7597) *P. okomuensis* n. sp. is larger than *P. dorsalis* described by Maeder *et al.* (1970) from Liberia (4.0–5.5) and much larger than those specimens recovered from the Ivory Coast (1.7–2.7) by Maeder (1973). In *P. okomuensis* n. sp. the medial intestinal diverticula are highly branched (figs 2, 4A and B) while those of *P. dorsalis* have fewer branches with a higher frequency of forming pre-haptoran anastomoses. The presence of a loop formed by two adjacent medial diverticula (fig. 4A) in *P. okomuensis* n. sp., which is absent in *P. dorsalis*, is another distinguishing difference between the two parasites. The shape of the hamuli, which is an important systematic character for separating polystome species further differentiates *P. okomuensis* n. sp. from *P. dorsalis*. Whereas the handle and guard of the hamulus in *P. okomuensis* n. sp. are well separated, those of *P. dorsalis* are, in most cases, poorly divided and, in some others, lacking a division between handle and guard.

Another factor that must be taken into consideration in separating *P. okomuensis* n. sp. from *P. dorsalis* is the host specificity of the two parasites. *Polystoma okomuensis* parasitizes *P. bouleengeri* while *P. dorsalis* is parasitic in *Afrivalus dorsalis* (Peters, 1875). Host specificity is a well-documented phenomenon for the genus *Polystoma* (see Combes, 1966, 1968; Tinsley, 1973, 1974; Euzet *et al.*, 1974a, b; Bourgat & Salami-Cadoux, 1976; Combes & Channing, 1979; Murith, 1981, 1982; Kok & Van Wyk, 1986; Kok & Du Preez, 1987; Du Preez & Kok, 1992, 1993, 1997). Invoking strict host-specificity along with some key morphological characteristics of African polystomes, Aisien & Du Preez (2009) recently concluded that *P. africanum*, which was thought to be a multi-host parasite, was exclusively parasitic in *A. regularis* and that polystomes retrieved

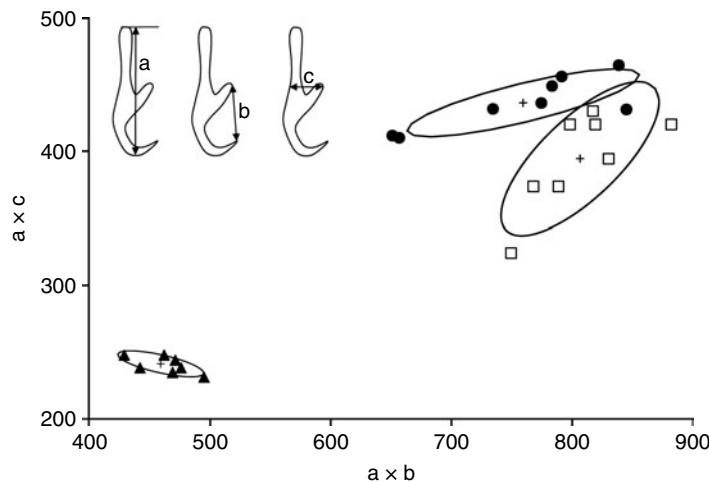


Fig. 5. Scatter diagram of $a \times b$ plotted against $a \times c$ for *Polystoma okomuensis* n. sp. (●), *P. dorsalis* (□) and *P. perreti* (▲). Measurements for *P. dorsalis* and *P. perreti* were obtained from Murith (1981).

from *Ptychadena mascareniensis* (Duméril & Bibron, 1841) hitherto described as *P. africanum* were most likely *P. pricei* Vercammen-Grandjean, 1960 and those from *Amietia angolensis* (Bocage, 1866) most likely represented another species. In the same vein, we conclude that *P. okomuensis* n. sp. is a different species from *P. dorsalis* and should be admitted as a separate species parasitic in *P. boulengeri*. We hypothesize that Nigerian polystomes will display the same degree of host-specificity displayed by other anuran polystomes; however, experimental infection and cross-infection experiments are needed to confirm this.

Unfortunately, the dimension of the testis in *P. dorsalis* was not discussed by Maeder *et al.* (1970) nor by Maeder (1973). The testis of *P. okomuensis* n. sp. is rather large, occupying about 70% of the pre-haptoral space (fig. 2). The only other African polystome known with such a large testis is *P. testimagna* (Du Preez & Kok, 1993) described from *Stongylopus fasciatus* (Smith, 1849). Whereas the testis follicles are restricted to the area between the intestinal caeca in *P. okomuensis* n. sp., the testis follicles in *P. testimagna* extend beyond the intestinal caeca, reaching as far as the body wall and occupying three-quarters of the pre-haptoral body. The huge testis, vast amount of sperm among testis follicles, prominent ovary and relatively few eggs in the uterus reflect on the mode of reproduction. These features are typical of a polystome that can produce a large number of eggs per day over a long period. This fits in with the reproductive strategy of the host, which has an extended breeding season.

Polystoma okomuensis n. sp. is further distinguished from other African polystomes by the length of its marginal hook 1. A length of 43 µm in this regard separates it from *P. africanum* Szidat, 1932 (38), *P. sodwanensis* Du Preez & Kok, 1992 (38), *P. togoensis* Bourgat, 1977 (40), *P. baeri* Maeder *et al.*, 1970 (41), *P. manganoti* Gallien, 1956 (42). While marginal hooklet length does overlap with that of *P. prudhoei*, the hosts belong to different families.

The mean length of marginal hooklets 2–7 in *P. okomuensis* n. sp. is of the same size as those reported for *Polystoma claudecombesi* Du Preez & Kok, 1995 (28 µm) but they are longer than those of *P. testimagna* (24), *P. sodwanensis* (23), *P. dawiekoki* Du Preez, Vaucher & Mariaux, 2002 (20) and *P. africanum* (19). Irrespective of the size similarity to those of *P. claudecombesi* and their disparity with those of the other listed African polystomes, a peculiar feature of marginal hooklets 2–7 in *P. okomuensis* n. sp. that separates them from those of other African polystomes is the length and shape of hooklet tips. While the tips of these hooklets are elongated and rounded in *P. okomuensis* n. sp. (fig. 3C), in other African polystomes, the tips of these marginals are short and pointed.

Although the general body features and morphometrics remain the fundamental basis of polystome species identification and description, there is a need for a comprehensive molecular study resolving taxonomic complexes and relationships among African polystomes.

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