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A redescription of *Polystoma africanum* Szidat, 1932 (Monogenea: Polystomatidae)

MARTINS S.O. AISIEN[1] & LOUIS H. DU PREEZ[2]

[1]*Laboratory of Parasitology Research, Department of Animal and Environmental Biology, University of Benin, P.M.B. 1154, Benin City, Nigeria. E-mail: aisien@uniben.edu*

[2]*School of Environmental Sciences and Development, Potchefstroom Campus, North West University, Private Bag X6001, Potchefstroom 2520, South Africa. E-mail: Louis.duPreez@nwu.ac.za*

Abstract

Polystoma africanum was originally described from a single specimen recovered from the common African toad, *Amietophrynus regularis*. The parasite has also been reported from the Angola river frog, *Amietia angolensis*, in Ethiopia; the Mascarene ridged frog, *Ptychadena mascareniensis*, in Uganda; and *Ptychadena sp*. in Zaire. One of the characters ascribed to this species was the lack of caecal anastomoses. Based on this and other characteristics, other polystomes from frog species like *Pty. mascareniensis* have been identified as *P. africanum*. A large collection of parasite specimens retrieved from *A. regularis* collected in Nigeria allowed a thorough re-examination of the species. A good proportion of the specimens (37%) correspond closely with the type in lacking intercaecal anastomoses. Other specimens showed considerable variation in this regard by possessing one to four intercaecal anastomoses. An unusual feature observed in several specimens is where two adjacent medial diverticula on one side join to form a loop. The type specimen also has a loop. The handle and guard of the hamuli are well separated, as in the type specimen from Liberia, and in other specimens from *A. regularis* (syn. *Bufo regularis*) in Uganda. The mean hamuli length of 388µm is close to the 370µm recorded for the type species, but this value is considerably smaller than the 459µm recorded for the specimens from *Pty. mascareniensis* in Uganda. On the basis of the aforementioned and invoking strict host specificity, we conclude that *P. africanum* is exclusively parasitic in *A. regularis*, and that the polystomes retrieved from *Pty. mascareniensis* from Uganda and a *Ptychadena* sp. from Zaire are most likely *Polystoma pricei*. The materials from *A. angolensis* most likely represent another species.

Key words: Monogenea, Polystomatidae, *Polystoma africanum*, *Amietophrynus regularis*, Nigeria

Introduction

Polystoma africanum Szidat, 1932 was described by Szidat (1932) from a single sub-adult parasite found in the urinary bladder of the Common African toad, *Amietophrynus regularis* (Reuss) (formerly known as *Bufo regularis* Reuss), in Liberia. This toad has a very wide distribution in the savannah and farm bush through sub-Saharan West Africa to the oases of Djanet in Algeria and Gat in Libya, along the Nile to Cairo, western Ethiopia southward to north-western Angola, Uganda, north-eastern Democratic Republic of Congo and southern Kenya. It was introduced to Cape Verde Island (Schleich 1987). *Polystoma africanum* has also been reported from *A. regularis* in Ivory Coast (Euzet *et al*. 1969), Sierra Leone (Williams 1969), Ethiopia and Uganda (Tinsley 1974a) and Togo (Salami-Cadoux 1978). According to Tinsley (1974b), this monogenean also parasitizes the Angola river frog, *Amietia angolensis* (Bocage) (formerly known as *Rana angolensis* Bocage) in Ethiopia; the Mascarene ridged frog, *Ptychadena mascareniensis* Duméril & Bibron in Uganda and Ethiopia; and *Ptychadena* sp. Boulenger in Zaire.

Considerable morphological variation among polystome parasites is well documented and intraspecific variation may exceed interspecies variation (Tinsley 1973). This complicates identification. The two principal

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systematic characters that appear to be the most informative for species closely related to *P. africanum* are the disposition of the intestine and the shape of the hamuli. Szidat (1932) described the intestine of the single specimen on which the species description is based as having branched medial diverticula extending over the midline but not forming any anastomoses. The illustration provided showed the two ceca fusing in the haptor with a diverticulated large dilation, lobed lateral diverticula and the intensely branched medial diverticula crossing the midline. Although there is no prehaptoral intercaecal anastomosis, two medial diverticula on one side join to form a loop. Although details of the shape of the hamuli were not provided, the illustration shows a division between the handle and the guard. The length of the hamulus was given as 370 μm. Whereas Tinsley (1974a) reported that the three specimens recovered from *A. regularis* in Uganda, (housed in the Natural History Museum in London), are adult parasites and that one of the specimens contained 10 eggs *in utero*, the precise disposition of the intestine was impossible to determine. Tinsley noted that the caeca were relatively heavily branched. Although the hamuli were obliquely oriented, making it impossible to observe the precise shape, it was possible to conclude that the straight handles were well separated from the wing-like guard.

After re-examining the types from *Polystoma pricei* Vercammen-Grandjean, 1960 and *Polystoma aethiopiense* Meskal, 1970, Tinsley (1974a) concluded that these two species were conspecific but that the name *P. pricei* takes precedence. In a second paper, Tinsley (1974b) evaluated polystomes recovered from *Pty. mascareniensis* from Uganda and concluded that *P. pricei* cannot be adequately separated from *P. africanum* but that the latter designation takes precedence. He then concluded that *P. africanum* has a wide distribution, infecting several host, namely *A. regularis* from Liberia, Uganda, and Ethiopia, *Pty. mascareniensis* in Uganda and Ethiopia, *Ptychadena* sp. in Zaire and *A. angolensis* in Ethiopia.

Materials and methods

Adult *A. regularis* were collected from the following locations in Edo State of Nigeria: 222 specimens from Ugbovbighan-Erah (6°49′N, 6°06′E) between June and September, 2002; 114 specimens from Ogbonna (7°7′N, 6°27′E) between June 1999 and September 2003, and 107 specimens from Agbede (6°86′N, 6°25′E) between August 2007 and January 2008.

Prior to examination for polystome infection, toads were killed by exposure to chloroform vapour or immersion in Benzocaine solution. The toads were dissected and the urinary bladder removed and placed in a Petri dish containing 0.72% NaCl solution. Parasites were isolated and flattened under cover slip weight on a microscope slide and fixed with 10% formol-saline for approximately 1h. Fixed specimens were preserved in 10% formol-saline. Parasites were washed free of fixative and stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene and mounted using Canada balsam. Some of the specimens collected were fixed in 96% ethanol for future molecular studies. All measurements were in micrometers and are given with the mean followed by the range in parentheses. Marginal hooklets are numbered C1 to C8 with C1 being the most posterior (see Murith 1981)

Results

Monogenea

Polystomatidae

***Polystoma africanum* Szidat, 1932**
(Figs. 1–4)

Specimens studied: Four specimens (LPRM2-0710-8a-d) deposited in the Invertebrate Collection of the

Professor A.B.M. Egborge Museum, University of Benin, Benin City, Nigeria and six specimens (NHM 2008.11.18.1-6) in the Parasitic Worm Collection of the Natural History Museum London; remaining specimens in the collection of the senior author.

Levels of infection: Numbers of hosts and parasites collected are presented in Table 1. For the total sample of 443 toads examined the prevalence was 18.7% and the mean intensity (average number of parasites per infected host) was 4.6. The maximum number of parasites recovered from a single host was 20.

TABLE 1. Numbers and levels of infection of *Polystoma africanum* in *A. regularis* collected.

Month/Year	n Hosts infected	Prevalence (%)	Mean intensity
Ugbovbighan-Erah			
06/2002	6/44	13.6	3.0
07/2002	20/97	20.6	2.5
08/2002	0/18	0	0
09/2002	11/63	11.5	5.7
Total	37/222	16.7	2.9
Ogbonna			
06/2002-09/2003	1/114	0.90	4.0
Agbede			
08/2007	11/21	52.4	4.9
09/2007	19/23	82.6	5.9
10/2007	7/20	35.0	6.9
11/2007	1/10	10.0	1.0
12/2007	6/18	33.3	7.2
01/2008	1/15	6.7	11
Total	45/107	42.1	6.0
Grand total	83/443	18.7	4.6

Description: Based on egg-producing adults (n = 20). Larval sclerite characters based on oncomiracidia (n = 13) hatched from eggs released by specimens collected.

Adult: General characteristics of mature, egg-producing parasite (Figure 1) typical of *Polystoma*. Body elongate, total length 6,015 (5,162–7,481), greatest width 2,219 (1,835–2,586), haptor length 1,572 (1,352-1,887), width 2,245 (1,908–2,713); haptor length to body length ratio 0,26 (0,22–0,28); haptoral suckers 6, mean diameter 407 (315–491); hamulus length 388 (335–452); hamulus hook (Figure 4A) 85 (72–93). Mouth subterminal, ventral. False oral sucker 431 (288–577) wide, pharynx length 244 (204–300), width 232 (194–325). Intestine bifurcate with lobed lateral diverticula averaging 45 (31–53) in number per parasite and large intensely branched medial diverticula numbering 15.3 (12–20) per parasite. In 9.2% of specimens two neighboring medial diverticula on one side join to form loop (Figure 2 F&G). Of 65 specimens examined (for frequency of intercaecal anastomoses only) 41 (63%) have intercaecal anastomoses (37% with 0, 34% with 1, 21.5% with 2; 6% with 3 and 1.5% with 4). Posterior pre-haptoral medial anastomosis spanning position of joining two intestinal caeca (Figure 2 A-D) is present in 22% of specimens examined. Caeca confluent posteriorly, extending into haptor. Haptoral caecal diverticula present. Variation in gut arrangement shown in Figure 3.

Testis follicular, ventral, medial and posterior to ovary (Figure 2). Seminal vesicle prominent, packed with sperm. Genital atrium median, ventral, posterior to intestinal bifurcation; genital bulb with 7–8 spines, 34 (28 – 38) long. Ovary sinistral, 23% from anterior end, ovary length 628 (447 – 766), width 273 (154 – 371). Tubular uterus confined to area anterior to ovary, 90% of time holding single egg (but up to three eggs

observed); egg capsule length 185 (174 –197), width142 (132 – 147). No indication of intrauterine development, eggs operculated. Vitellaria throughout most of body. Genito-intestinal canal prominent, on same side as ovary, joining intestinal caecum posterior to ovary (Figure 2).

Oncomiracidium: Ciliated larva 313 (275-358) with narrow cylindrical body and circular cup-shaped opisthaptor bearing 16 marginal hooklets. Marginal hooklet C1 36.6 (33.3–38.6), marginal hooklet C2–7 18.6 (17.0–21.0), marginal hooklet C8 34.9 (31.8–38.0); hamulus primordia 9.8 (9.4 – 10.2) (Figure 4).

FIGURE 1. Partial map of Africa with Nigeria enlarged and Edo State where specimens were collected in grey. Type locality of *Polystoma africanum* marked as a black dot and other known localities as grey dots.

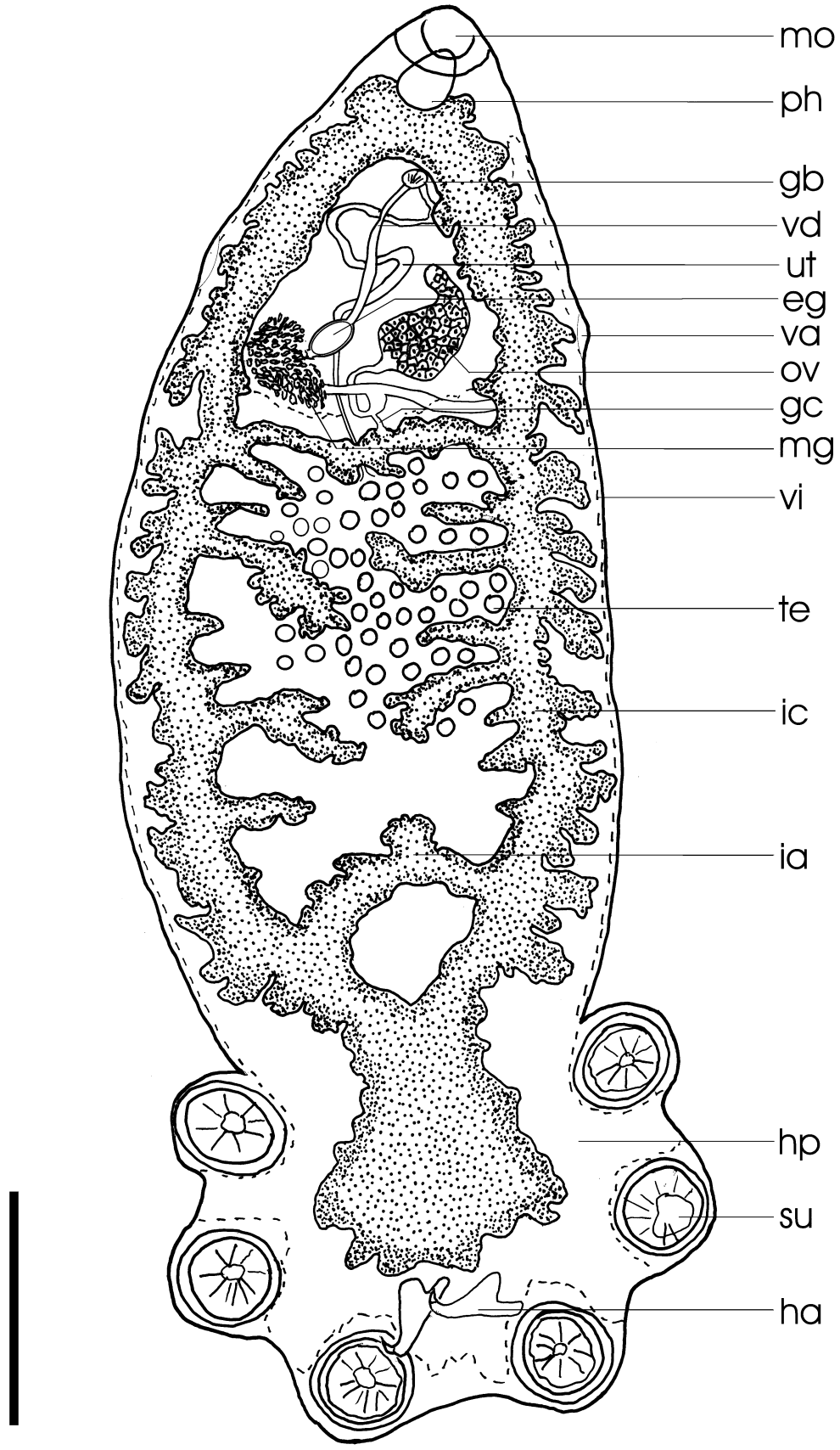

FIGURE 2. *Polystoma africanum*. Ventral view; dotted line indicates the outline of the vitelline system. Abbreviations: eg, egg; gb, genital bulb; gc, genitor-intestinal canal; ha, hamulus; hp, haptor; ia, intercaecal anastomosis; ic, intestinal caecum; mg, Mehlis' gland; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; ut, uterus; va, vagina; vd, vas deferens; vi, vitellaria. Scale-bar: 1 mm.

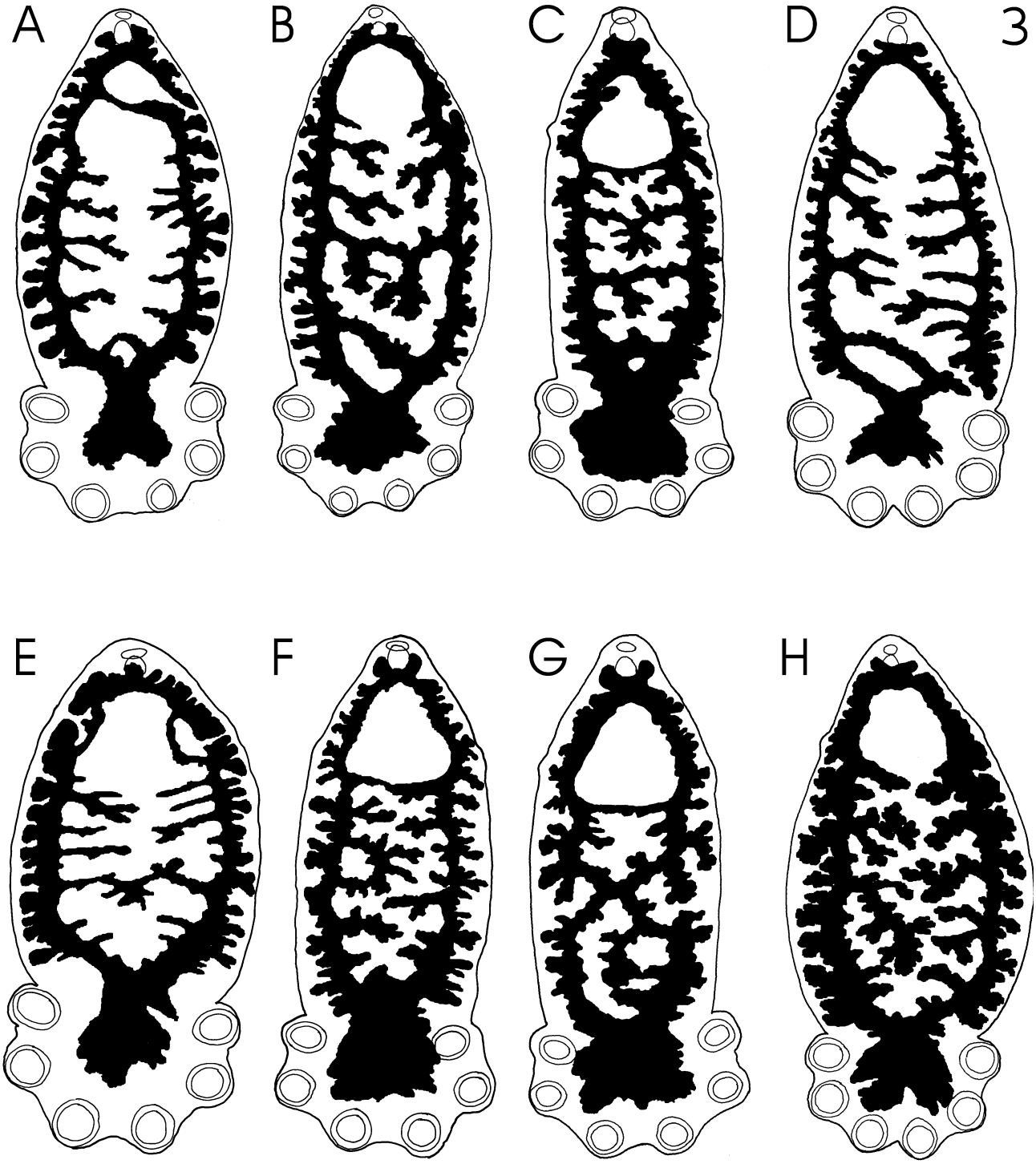

FIGURE 3. *Polystoma africanum*. Variation in intestinal arrangement.

Discussion

Although the arrangement of the intestine may show significant variation within a polystome species, it remains an important and informative character to identify species. Tinsley (1974a) refers to the disposition of the intestine as one of the principal systematic characteristics for polystomes. A key intestinal character is the presence or absence of caecal anastomoses. Provided that one works with a sufficient sample size, the presence and nature of intercaecal anastomoses is of great taxonomic importance.

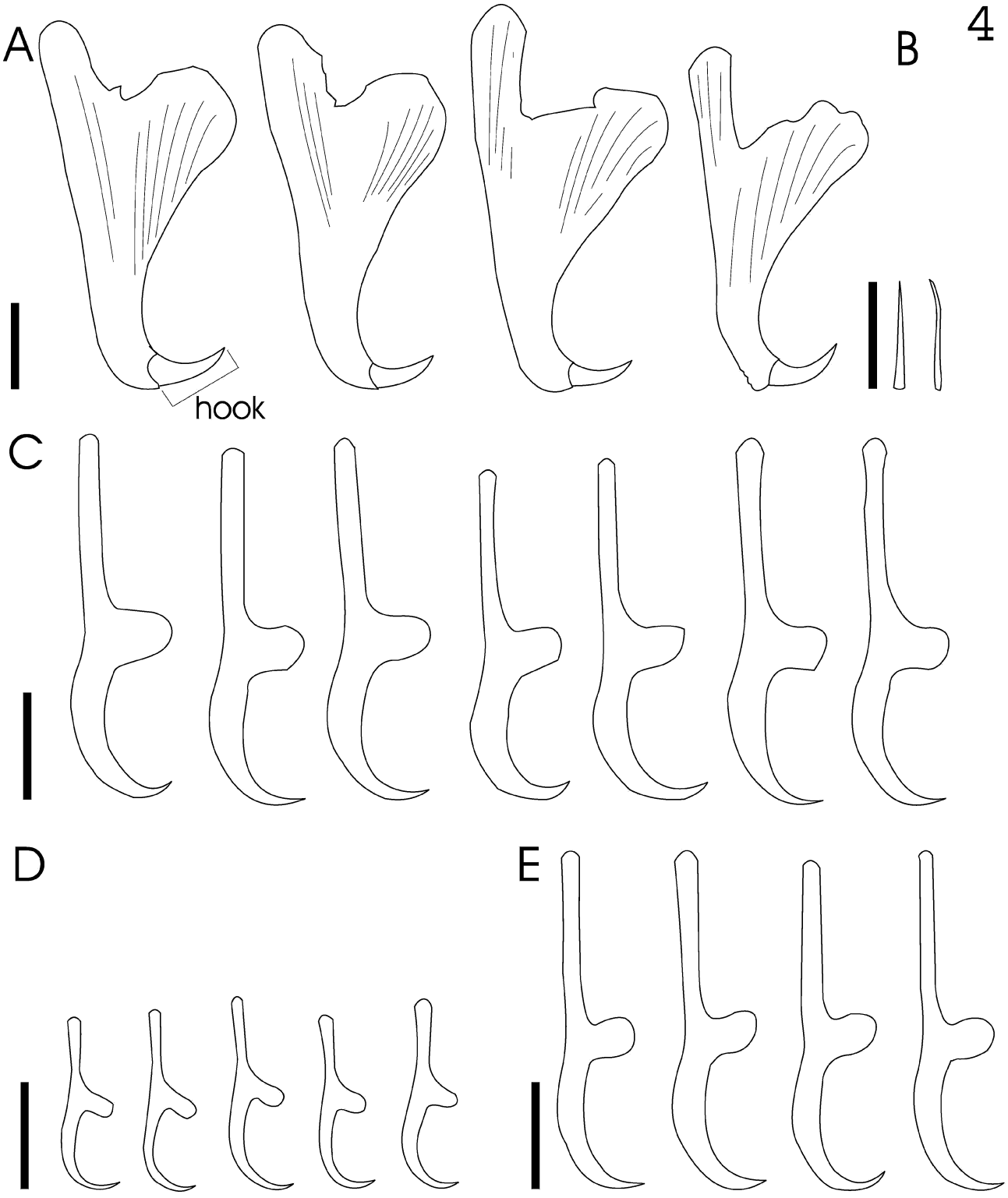

FIGURE 4. *Polystoma africanum*. A, hamuli (drawn from adult specimens); B, hamulus primordia; C, marginal hooklets C1; D, marginal hooklets C2-7; E, marginal hooklets C8, (drawn from oncomiracidia). *Scale-bars*: A 100 µm, B–E 10 µm.

The single type specimen, on which the species description of *P. africanum* was based, lacked intercaecal anastomosis. Euzet *et al*. (1966) re-examined the type material and confirmed the absence of the anastomoses. Tinsley (1974a) reported that specimens isolated from *A. regularis* in Uganda were adult parasites and that the precise disposition of the intestine was impossible to determine, but that the caeca were apparently heavily

branched. Several authors have commented on the absence of intercaecal anastomoses in *P. africanum* (see Szidat 1932, Tinsley 1974a,b, Euzet *et al*.1966). Tinsley (1974a) assigned a number of specimens he examined as *P. africanum* and concluded that *P. pricei* could not be separated from *P. africanum* and that neither species had caecal anastomoses. The lack of caecal anastomosis in Szidat's type specimen is the one aspect that led to confusion and misidentification.

Although the arrangement of the intestine of *P. africanum* as presented here shows significant variation, there are recurring common features, several of which are shared with the type specimen of Szidat. *Polystoma africanum* is characterized by highly branched medial diverticula, the presence of loops formed by the joining of two neighboring medial diverticula, intercaecal anastomoses and a posterior prehaptoral anastomosis spanning the position where the two intestinal caeca join.

In contrast, the specimens from *Pty. mascareniensis* in Uganda identified as *P. africanum* had small lateral diverticula and finger-like medial diverticula, rarely reaching the mid-line of the parasite with no overlapping and no intercaecal anastomoses. In the light of new information provided herein, it becomes evident that the Ugandan material from *Pty. mascareniensis* is not *P. africanum*, but most likely is *P. pricei*.

The hamuli as observed in the present study are characterized by a distinct division between the handle and the guard (Fig. 4A). Although Szidat (1932) did not describe the hamuli, he indicated a distinct division between the handle and guard in his drawing of the type specimen. The mean length of the hamuli from the Nigerian specimens (388µm) is in the range of the 370µm reported for the type specimen. Furthermore, irrespective of the oblique orientation of the hamuli of the three specimens of *P. africanum* recovered from *A. regularis* (syn. *B. regularis*) in Uganda, it was possible according to Tinsley (1974a) to conclude that the straight handles were well separated from the wing-like guard. This overwhelming agreement in the shape of the hamuli of *P. africanum* recovered from *A. regularis* from three different countries shows that this morphological character is a reliable diagnostic feature for this parasite. Although the hamuli from the polystome specimens recovered from *Pty. mascareniensis* in Uganda (Tinsley 1974a) also have the handles separated from the guard, they are not only differentiated from the Nigerian specimens by their larger size range (446-471µm), but ultimately by the absence of intercaecal anastomoses in the Ugandan polystome specimens.

Murith (1981) studied the marginal hooklets of several species of Central and West African polystomes. Based on these studies, especially on the total length to handle length ratio measured for marginal hooklets C1, material from Uganda, identified by Tinsley (1974a) as *P. africanum*, compared well with *P. pricei*, *P. aethiopiense* and *Polystoma togoensis* Bourgat, 1977. She therefore suggested that *P. africanum* be treated as a parasite specific to *A. regularis*.

A high level of host-specificity has been well documented for the genus *Polystoma* (see Combes 1966, 1968; Euzet *et al*. 1974a, b; Tinsley 1973, 1974a; Bourgat & Salami-Cadoux 1976; Murith 1981, 1982; Combes & Channing 1979; Kok & Van Wyk 1986; Kok & Du Preez 1987; Du Preez & Kok 1992, 1993, 1997). Assuming strict host-specificity coupled with hamulus shape and intestinal disposition, Maeder *et al*. (1970) described three new polystomes, namely *Polystoma baeri* Maeder, Euzet & Combes, 1970 from *Ptychadena maccarthyensis* Dubois (now *P. bibroni*) (Ivory Coast), *Polystoma dorsalis* Maeder, Euzet & Combes, 1970 from *Afrixalus dorsalis Peters* (Liberia), and *Polystoma ragnari* Maeder, Euzet & Combes, 1970 from *Phrynobatrachus alleni* Parker (Ivory Coast). In view of the new information presented in this study on the intestinal disposition (especially, the presence of intercaecal anastomosis) and hamulus shape of *P. africanum*, we conclude that this parasite is specific to *A. regularis*. Therefore other polystomes from host species other than *A. regularis* hitherto referred to as *P. africanum* should be re-examined and appropriately assigned.

When Meskal (1970) described a new polystome from Ethiopia he compared his material with the then available descriptions of *P. africanum sensu stricto*, *P. africanum ivindoi* Euzet, Combes & Knoepffler, 1966 and *P. africanum gabonensis* Euzet, Combes & Knoepffler, 1966 and named this parasite *P. africanum aethiopiense*. In a study of polystomes from Ivory Coast and Liberia, Maeder *et al.* (1970) proposed a systematic ranking elevating these subspecies of *P. africanum* to species level, namely *P. ivindoi*, *P.*

gabonensis and *P. aethiopiense*. Examination of *P. aethiopiense* from *Pty. mascareniensis* from Ethiopia led Tinsley (1974a) to conclude that these parasites were conspecific with *P. pricei*. He further stated that it was practically impossible to separate *P. africanum* and *P. pricei* based on morphology.

In the light of limited interspecific morphological variation among polystomes the value of molecular studies cannot be underestimated. Therefore, a thorough study of African polystomes at the molecular level would be of great importance to verify the validity of species.

Acknowledgments

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