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Eupolystoma namibiensis n. sp. (Monogenea: Polystomatidae) parasitic in *Poyntonophrynus hoeschi* (Ahl, 1934) of Namibia

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Described species of the polystomatid flatworm genus *Eupolystoma* are only known from Africa and India. A survey conducted in January 1994 in Namibia revealed a previously unknown species from the urinary bladder of Hoesch's pygmy toad *Poyntonophrynus hoeschi*. Toads were screened from eight different localities within their known distribution. The toads found to be infected were from three localities, namely Mariental, Outjo and Kamanjab in Namibia. Morphologically, this polystome is distinguised from the other known species by a combination of characteristics including the size of the parasite, number of genital spines, and the size and shape of the marginal hooklets. *Eupolystoma namibiensis* n. sp. is the first polystome to be described from Namibia.

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Keywords: *Eupolystoma*, Hoesch's pygmy toad, monogenean, Namibia, polystome

Introduction

Polystomatid flatworms (Monogenea: Polystomatidae) infect mainly amphibian hosts and freshwater terrapins but are also known from the Australian lungfish and the African hippopotamus. Of the 24 currently known polystome genera, only *Eupolystoma, Metapolystoma, Polystoma* and *Protopolystoma* are known from African amphibians (Raharivololoniaina et al. 2011). While *Polystoma* has a cosmopolitan distrubution, *Metapolystoma* and *Protopolystoma* are restricted to the Ethiopian Realm and *Eupolystoma* is known only from Africa and India. Within *Eupolystoma*, five species are currently recognised. In Africa, *Eupolystoma anterorchis* is known from *Amietophryus pantherinus, E. alluaudi* from *A. regularis* and *E. vanasi* from *Schismaderma carens*. In India, *E. chauhani* is known from an unidentified *Bufo* sp. and *E. rajai* from an unidentified *Rana* sp. (du Preez et al. 2003).

Eupolystoma is a fairly poorly known polystome genus with taxonomic contributions by de Beauchamp (1913), Kaw (1950), Pandey (1969), Tinsley (1978), Beverly-Burton (1962), Yamaguti (1963), Euzet and Combes (1967) and du Preez et al. (2003). The first member of the genus *Eupolystoma* was originally named *Polystomum alluaudi* (de Beauchamp, 1913) but after taxonomic assessment both the parasite and hosts have been revised. Ozaki (1935) placed *Polystomum alluaudi* in the genus *Parapolystoma*, which Yamaguti (1963) revised and erected the genus *Beauchampia*. The genus was subsequently changed again to *Eupolystoma* (see Euzet and Combes 1967). Tinsley (1978) made a significant contribution to the current knowledge of *Eupolystoma* with his detailed study on *E. anterorchis* in which he discussed various aspects of its taxonomy and biology. In a major revision of the amphibians of the world, Frost et al. (2006) split the genus *Bufo* in southern Africa into *Amietophrynus, Poyntonophrynus* and *Vandijkophrynus*.

Eupolystoma is characterised by its extended uterus, which takes up most of the central body, the posterior displacement of the gonads, lack of hamuli, and marginal hooklets of the same length. The capacity of this group to accumulate eggs provides an opportunity for the development of eggs *in utero*, allowing for an internal reproductive cycle where the oncomiracidia may establish in the urinary bladder of the same host individual as the parent. Unlike most other known polystomes, *Eupolystoma* has the capacity to build up large infrapopulations. Tinsley (1978) reported that *A. pantherinus* may harbour as many as 2 000 *E. anterorchis* in a single host. This reproductive strategy enables *Eupolystoma* to exploit arid-adapted hosts with brief and infrequent contact with water. In Africa, there are several arid-adapted anurans and an extensive search will probably reveal as yet undiscovered eupolystomes. This paper reports on the discovery of a new *Eupolystoma* sp., the first to be found in Namibia.

Materials and methods

Infected specimens of Hoesch's pygmy toads *Poyntonophrynus hoeschi* (Ahl, 1934) were collected along rocky pools, below the Hardopdam near the town of Mariental, Namibia (Figure 1). Toads were collected by hand at night with the aid of strong flashlights, euthanised using MS222 (etyl-4-aminobenzoate, Sigma) and dissected for parasites and host tissue collection using a Nikon SMZ 645 microscope. The urinary bladder and all reproductive

and excretory ducts were thoroughly screened for polystomes. Live parasites were immediately placed in a drop of water on a slide and briefly heated from below with a butane lighter until they relaxed and stopped moving. Most specimens were fixed in 10% neutral buffered formalin under very gentle coverslip pressure, whereas remaining specimens, earmarked for molecular studies, were fixed in 96% ethanol. Semi-permanent lactophenol or ammonium picrate preparations were made from some of the smaller parasites to reveal sclerites. Specimens destined for permanent mounting were stained overnight in a weak acetocarmine solution, dehydrated and mounted in Canada balsam. Preparations were studied using a Nikon E800 compound microscope fitted with a high-end digital camera connected to a computer. Measurements were taken using the NIS-Elements D software (Nikon).

Following the discovery of the parasites, archived museum specimens were obtained from the Bayworld Museum, Port Elizabeth, and the private collection of Dr Mike Cherry at the University of Stellenbosch. Archived specimens were carefully dissected and, if infected, parasites were removed and processed.

Specimens in alcohol were examined using molecular tools, but we were unable to extract useable DNA. This was most likely a result of specimens being stored for a number of years before DNA extraction was attempted. The formal description of the species was postponed, and thus far additional live parasites have not been found.

Results

***Eupolystoma namibiensis* n. sp.** (Figures 2–4)

Frogs screened and levels of infection

In total, four live and 22 museum archived specimens of Hoesch's pygmy toads were screened for polystomes (Table 1, Figure 1). Infected toads were found from three localities, namely Hardapdam near Mariental, Outjo and Kamanjab (Figure 1). Of the four specimens from Hardapdam, two were infected with two mature and 23 immature parasites (prevalence 50%, mean intensity 12.5); three of the five specimens from Outjo were infected with three mature and 15 immature specimens (prevalence 60%, mean intensity 6.0); and one of the five specimens from Kamanjab was infected with four immature parasites (prevalence 20%). Specimens from Leonardville, Noabis, Rehoboth, Trekkopje and Windhoek were not infected (Table 1).

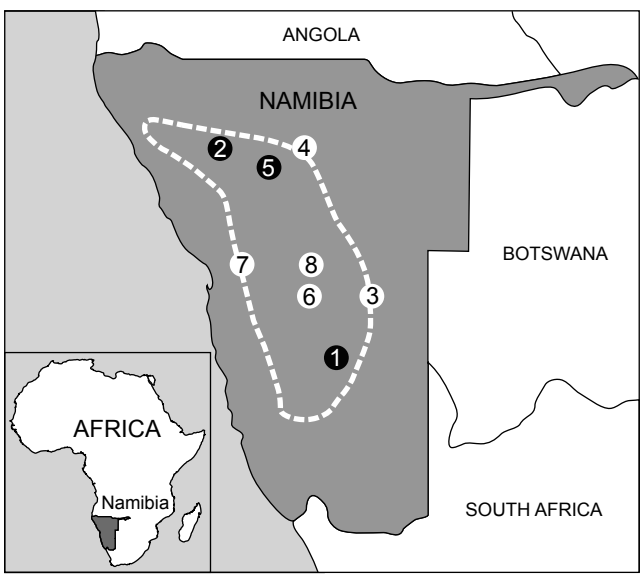

Figure 1: Map of Namibia with the distribution of Hoesch's Pygmy Toad indicated by a dashed line. Localities where infected toads were found are indicated by solid dots and non-infected ones by open dots. Localities: 1, Hardapdam; 2, Kamanjab; 3, Leonardville; 4, Noabis; 5, Outjo; 6, Rehoboth; 7, Trekkopje; 8, Windhoek

Species description

Description of the new polystome species is based on six sexually mature and eight immature parasites. Measurements are given in micrometres. The mean measurement is given followed by the range in parentheses. Marginal hooklet measurements are based on eight immature specimens.

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

General characteristics as for other *Eupolystoma* spp. (Figure 2). Body elongated and pyriform, total length 4 401 (3 477–6 522), greatest width 1 438 (826–2 496), haptor length 876 (575–1 267), width 1 091 (873–1 572); haptor length approximately 20% of body length. Six haptorial suckers, mean diameter 299 (223–436); lacking sclerotised supports. No hamuli present. Mouth subterminal with false oral sucker 315 (206–401) and pharynx length 177 (147–228); pharynx width 143 (109–214). Intestine bifurcate without prominent lateral diverticula. No prehaptoral anastomoses.

Testis follicular, diffuse and restricted posteriorly at the level of the ovary anterior to the intestinal haptorial anastomosis. Seminal vesicle and genital bulbus situated median, ventrally and posterior to the intestinal bifurcation posterior to the pharynx. Genital bulbus 68 (53–81) armed with 5 genital spines, 35 (32–38) in length. Vitellaria confined to the posterior half of the body proper and in two lateral fields. Ovary small 244 (202–278) × 95 (66–126), lateral and posteriorly positioned in the body on the same side as the genito-intestinal canal. In specimens where the uterus did not contain any eggs the gonads were positioned

Table 1: *Poyntonophrynus hoeschi* specimens examined, collected during this study and from museum collections

Locality	No. of frogs	No. infected	Mature parasites	Immature parasites
Hardapdam	4	2	2	23
Kamanjab	5	1	4	0
Leonardville	2	0	0	0
Noabis	3	0	0	0
Outjo	5	3	4	14
Rehoboth	1	0	0	0
Trekkopje	1	0	0	0
Windhoek	6	0	0	0

about two-thirds from the anterior end of the body proper. A pair of vaginae situated laterally and one-third from anterior with the vaginal canal decending laterally to join the vitelline canal to form the vitello-vaginal canal that join from both sides to form a vitello-vaginal chamber. At this position the oviduct enters and the oovitelline canal branches off to lead to the ootype. The genito-intestinal canal also branches off and connects to the one intestinal caecum. Uterus tubiform occupying most of the intercaecal space of the body proper holding as many as 118 eggs. *Intra uterine* eggs 156 (128–183) × 96 (70–113). Eggs develop *in utero* and were organised from unembryonated posteriorly to fully embryonated anteriorly. Oncomiracidia were observed moving inside the *intra uterine* egg capsules. Eggs are light yellow in colour and embryonated eggs are semi-transparent. Oncomiracidia in fully embryonated eggs with 16 marginal hooklets of equal length 32 (31–34).

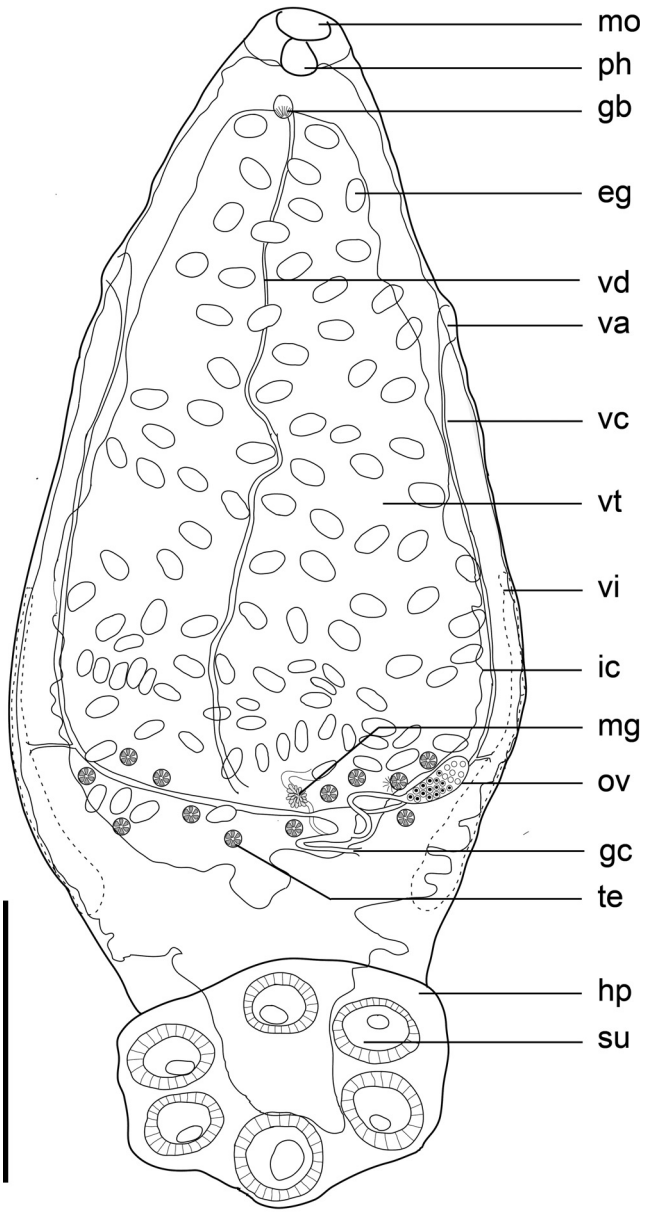

Figure 2: *Eupolystoma namibiensis* n. sp. Ventral view of the holotype. The dotted line indicates the outline of the vitelline system. eg, egg; gb, genital bulb; gc, genito-intestinal canal; hp, haptor; ic, intestinal caecum; mg, Mehlis' gland; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; va, vagina; vc, vaginal canal; vd, vas deferens; vi, vitellaria; vt, vitelline duct. Scale bar = 1 mm

Taxonomic summary

Type specimens

Holotype (NMB P 372) and seven paratypes (NMB P 373–379) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa. Two paratypes (BMNH no. NHMUK 2015.4.16.1-2) deposited in the Parasitic Worms Collection at the Natural History Museum, London.

Type host

Poyntonophrynus hoeschi (Ahl, 1934)

Type locality

Rocky pools below damwall of the Hardapdam near the town of Mariental, Namibia (24°29′49″ S, 17°52′19″ E).

Site of predilection

Urinary bladder.

Etymology

Named after Namibia as this is the first polystome to be described from this country.

Differential diagnosis

Eupolystoma namibiensis n. sp. differs from other members of *Eupolystoma* by a combination of characteristics. The maximum length recorded for previously described species

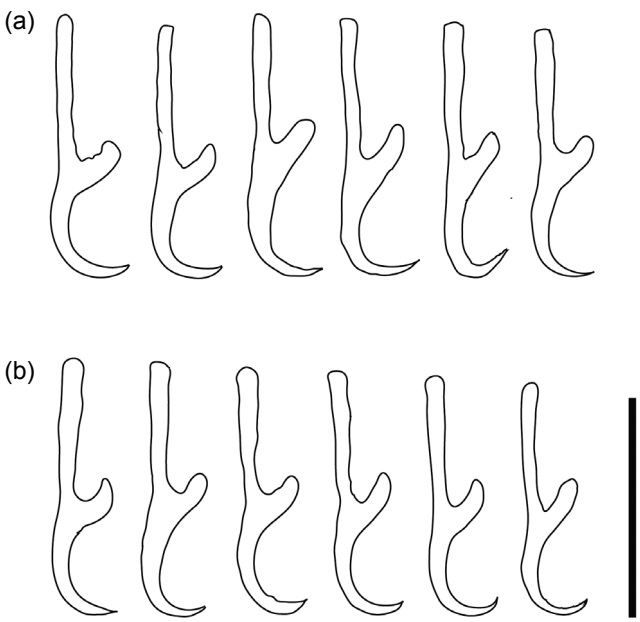

Figure 3: *Eupolystoma namibiensis* n. sp. (a) Marginal hooklets 1 from the holotype and paratypes. (b) Marginal hooklets 2–8 from the holotype and paratypes. Scale bar = 30 µm

of *Eupolystoma* is around 4 700 for both *E. anterorchis* and *E. alluaudi* and 7 733 for *E. vanasi*. *Eupolystoma namibiensis* n. sp. with a maximum length of 6 522 thus differs from all known species except *E. vanasi*. It differs from *E. vanasi* in that it has five genital spines whereas *E. vanasi* has four. A plot of the products of the total length (a in Figure 4) and the width at the level of the guard (c in Figure 4) versus the product of the total length and the length of a tangent between the tip of the blade to the guard (b in Figure 4) of marginal hooklet 1 (du Preez and Maritz 2006) reveals that *E. namibiensis* n. sp. occupies a distinct position and only marginally overlaps with *E. vanasi* (Figure 4).

Concluding remarks

Hoesch's pygmy toad is endemic to central and western Namibia where it occurs in very dry areas and is associated with rock outcrops, breeding in sandy-bottomed temporary pools (du Preez and Carruthers 2009). This arid environment poses a challenge not only to the toad, but also to its parasites. Polystomes display a remarkable adaptation and co-evolution with their hosts, this being especially evident, as demonstrated by field and laboratory investigations, among the anuran hosts to which they are very host specific (du Preez and Kok 1997; Tinsley and Jackson 1998). While some infect primarily aquatic hosts such as *Protopolystoma* in aquatic clawed frogs (*Xenopus*), others infect arid-adapted frogs. *Pseudodiplorchis* from the Arizona desert infects the arid-adapted spade foot toad *Scaphiopus couchi* that breeds in a single night or two per year, restricting the opportunity for parasite transmission to one or two days per year (Tinsley and Earle 1983). *Raharivololoniaina* et al. (2011) described *Kankana* from Madagascar as a sister group to *Eupolystoma*. In Africa and India *Eupolystoma* is mostly known from toads, some of which are arid-adapted. *Eupolystoma*, *Kankana* and *Pseudodiplorchis* share characteristics that fit with the reproductive strategy of their explosive breeding hosts. All three have extended uteri providing the opportunity for accumulation of eggs, intrauterine development and the option of an internal cycle whereby parasites can infect the parent host, boosting the number of parasites in a single host. Unlike most polystomes, where the infective stage of the parasite can only infect the host in the tadpole stage, the free-swimming oncomiracidium of *Eupolystoma* infects adult frogs directly (Tinsley 1983).

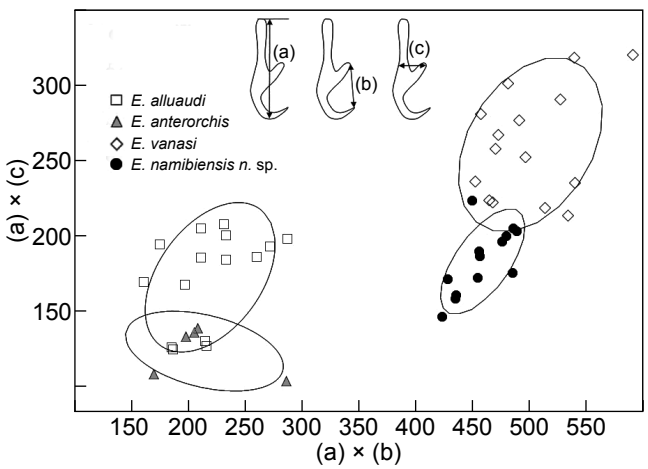

Figure 4: Scatter diagram with confidence ellipses of the products of the total length (a) and the width at the level if the guard (c) versus the product of the total length and the length of a tangent between the tip of the blade to the guard (b) of marginal hooklet 1 for known African *Eupolystoma* species

Combes et al. (1973) and Fournier and Combes (1979) have demonstrated a population regulation mechanism, whereby *Eupolystoma* produce two different types of oncomiracidia. Ciliated oncomiracidia are active swimmers and are released into the external environment for transmission to new hosts, whereas unciliated oncomiracidia are destined to remain within the original host to boost existing infrapopulations. Fournier and Combes (1979) reported that these unciliated oncomiracidia never developed cilia during embryo development. Du Preez et al. (2003) reported that for *E. vanasi* fully embryonated eggs were released *en masse* and while some oncomiracidia remained actively swimming, others lost their ability to swim shortly after hatching. They also observed that some oncomiracidia already hatched *in utero*. This phenomenon has been observed and reported for *E. anterorchis* by Tinsley (1990). In the present study oncomiracidia were not expelled in spite of the fact that they were observed moving inside embryonated eggs. Mature egg-producing and immature *E. namibiensis* n. sp. were observed together in the same host. The same phenomenon was reported for *E. vanasi* by du Preez et al. (2003), who reported as many as five size classes in the same host individual.

Field and experimental studies suggest a high level of anuran polystome host specificity (du Preez and Kok 1997; Tinsley and Jackson 1998). However, not all genera and species dislplay the same degree or level of host specificity. *Eupolystoma alluaudi*, for example, has been reported from at least seven anuran species representing four genera and two families. Bourgat et al. (1983) referred to this apparent lack of host-specificity in the case of *E. alluaudi*. This low level of host-specificity was also noted by Salami-Cadoux (1975), who experimentally infected *Bufo maculatus* with *E. alluaudi*, a parasite usually infecting *B. regularis* in Togo. Indications are that *Eupolystoma* are not as host-specific as *Polystoma* and that the mechanism of host-specificity is different. Du Preez and Kok (1997) postulated that host specificity for *Polystoma* is determined by the oncomiracidium's ability to recognise only the correct host tadpole. In the case of *Eupolystoma*, the tadpole is not involved at all. It could be that the degree of host-specificity is reduced. Preliminary results indicated the latter, and that *Eupolystoma* oncomiracidia can enter substitute hosts, but that these parasites do not survive longer than a few days (LHdP and Aisien unpublished data). However, species of *Protopolystoma* and *Pseudodiplorchis* also infect only post-metamorphic stages of their respective hosts and the tadpoles are not involved in the life cycle at all but they still display a high degree of host-specificity. Rejection of parasites in foreign hosts occurs in the sites of juvenile development, in the kidneys and lungs (Jackson and Tinsley 1998).

The observation in the present study that a uterus packed with eggs may push back the reproductive organs in the body proper serves as a caution, highlighting that the the placement of the reproductive organs may be plastic. This may have far-reaching implications for the taxonomy of polystomes, as considerable weight has been placed on such morphological characteristics, for example, the difference between *Polystoma* and *Metapolystoma*. This possible degree of plasticity needs to be investigated.

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