Polystomatidae (Monogenea) parasitic in the anuran genus *Natalobatrachus* in South Africa

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In southern Natal adult polystomes have been found in 10,6% of a sample (n = 47) of *Natalobatrachus bonebergi*, a host species with a restricted eastern coastal distribution. The parasite is described as *Polystoma umthakathi* sp. nov., and relevant information is also given on the ecology and geographical distribution of both parasite and host. The taxonomic problems within the African polystomes and parasite adaptations to the ecology of the host are discussed.

In Natal is volwasse polystome gevind in 10,6% van 'n monster (n = 47) van *Natalobatrachus bonebergi*, 'n gasheerspesie met 'n beperkte oostelike kusstreek-verspreiding. Die parasiet word beskryf as *Polystoma umthakathi* sp. nov., terwyl toepaslike inligting ook gegee word met betrekking tot die ekologie en geografiese verspreiding van die parasiet sowel as van die gasheer. Die taksonomiese probleme rakende die Afrika-polystome en die aanpassings van die parasiet by die ekologie van die gasheer word bespreek.

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Very few anuran Polystomatidae are known from South Africa. Until recently only one species of each of the three genera occurring in South Africa, namely *Eupolystoma*, *Polystoma* and *Protopolystoma*, was known. Whereas the latter is a monospecific and *Eupolystoma* a small genus, *Polystoma* is represented by more than twenty species, of which most are known only from central Africa.

When *Polystoma natalensis* was described by Combes & Channing (1979), the only other southern species known was *P. mashoni*, described by Beverley-Burton (1962) from Zimbabwe. Combes & Channing (1979) emphasized the existence of a discontinuity between the geographical distribution of the southern *P. natalensis* and the distribution of other species within the '*africanum* group' of African anuran polystomes. They regarded this as mainly due to a lack of investigation of the parasitic fauna of amphibians occurring in the southern half of the continent.

Investigations in the eastern Orange Free State led to the discovery of *P. australis* Kok & van Wyk, 1986 from *Notokassina wealii* and *Kassina senegalensis*. Recent investigations in southern Natal, led to the recovery of another polystomatid parasite from *Natalobatrachus bonebergi*, a little-known anuran with a restricted eastern coastal distribution. The parasite is described here as a new species of *Polystoma*.

Materials and Methods

Live adult specimens of *Natalobatrachus bonebergi* Hewitt & Methuen, 1913, were collected during March 1986 in the Vernon Crookes Nature Reserve in southern Natal.

Parasite egg production was used to determine the presence of parasites. Live frogs were kept in 600-ml jars containing 50 ml of tap water. Parasite eggs were harvested by sieving the water through plankton netting with a mesh size of 112 µm. All frogs from which parasite eggs were collected were eventually necropsied and the parasites they harboured were removed. Most of the remaining frogs were necropsied in order to determine the possible presence of non-egg-producing parasites.

Prior to necropsy, frogs were anaesthetized with M.S. 222 (Sandoz) and parasites found were transferred alive from the urinary bladder to 0,7% saline. Individual parasites were fixed in 70% ethanol while under light coverslip pressure. Parasites were stained in borax carmine or safranin after 24-h fixation, and then dehydrated, cleared and mounted.

Parasite eggs were kept in small petri dishes at a constant temperature of 23°C (± 1,5°C). Hatched oncomiracidia were mounted directly in lactophenol for examination of haptoral sclerite morphology.

Natalobatrachus bonebergi in South Africa

The monotypic genus *Natalobatrachus* and its species *bonebergi* were described by Hewitt & Methuen (1913) from Marianhill Monastery near Durban (Figure 1). Although there have been differences of opinion as to its generic status relative to the genus *Phrynobatrachus*, its ecological peculiarities alone have been considered sufficient to keep it separate (Poynton 1964).

N.bonebergi is restricted to the coastal forests of southern Natal and Pondoland below about 900 m (Poynton 1964; Passmore & Carruthers 1979) — Figure 1. The geographical area is relatively small, over and above which the availability of forest within it is greatly restricted owing to large-scale agricultural and urban development.

The habitat of this species is stream beds and adjacent litter in densely forested kloofs (Passmore & Carruthers 1979). Eggs are laid in adhesive masses on the underside of any of a large variety of objects (e.g. branches, fern fronds, rocks) overhanging small streams or pools. Each egg capsule adheres strongly to the rest and contains an

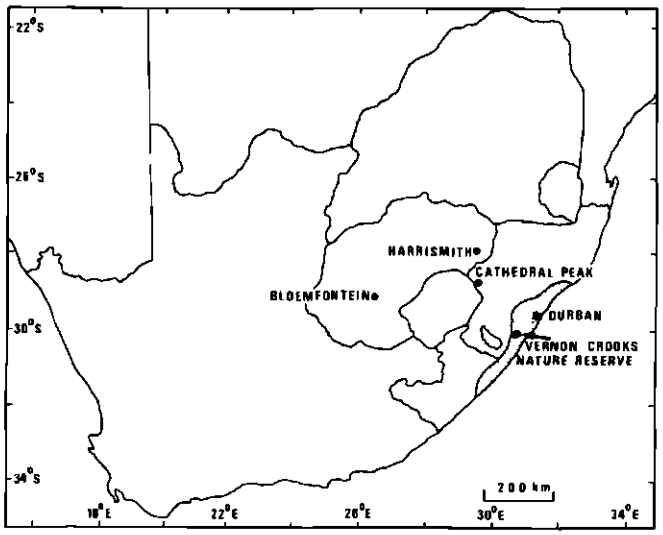

Figure 1 Geographical distribution of *Natalobatrachus bonebergi* (shaded) and localities relevant to the present study.

aqueous fluid in which the tadpole develops and is able to wriggle around. At about seven days (Wager 1931, 1952) hatching begins and tadpoles drop from the egg capsules into the stream below where they follow a benthonic existence. Post-metamorphic frogs are presumably able to reproduce during the following breeding season.

Description of *Polystoma umthakathi* sp. nov.

Type specimens. Seven specimens, all collected in March 1986 at the type locality.

Holotype. National Museum, Bloemfontein (NMB-P17). Mature egg-producing parasite from the urinary bladder of the type host.

Paratypes. Six mature egg-producing parasites from male and female hosts (NMB-P18 to P23).

Type host and locality. *Natalobatrachus bonebergi* Hewitt & Methuen, 1913, sexually mature male from the umThakathi forest in the Vernon Crookes Nature Reserve (30°16'S/30°37'E), Natal, Republic of South Africa. Relevant data on the total host sample and parasite occurrence in this sample are given in Table 1.

Etymology. The specific name *umthakathi* relates to the umThakathi forest type locality. uMthakathi is the Zulu word for sorcerer.

Table 1 Occurrence of *Polystoma umthakathi* sp. nov. in the total sample of mature adult *Natalobatrachus bonebergi* hosts, collected in the Vernon Crookes Nature Reserve during March 1986

Number of hosts	47
Ratio ♂ : ♀	34 : 13
Hosts parasitized	5 (4♂♂, 1♀)
Parasite prevalence (%)	10,6%
Parasites/infected host (mean intensity)	1,4
Parasites/host (relative density)	0,15

Species description

The species description and measurements are based on all seven egg-producing parasites collected in March 1986. Data on larval sclerites are based on these structures as seen in oncomiracidia hatched from eggs laid by the holotype or paratype specimens before host necropsy.

General characteristics of the mature, egg-producing parasite (Figure 2) are typical of *Polystoma* spp. of the '*africanum* complex'. The more important body and organ measurements are given in Table 2.

Diagnostic characters: Medial intestinal diverticula extend beyond the midline but anastomoses are infrequent (frequency 0,29) and very narrow when present; mean body length is 7,29 mm with a haptor length to body length ratio of 0,28; the length of each hamulus is somewhat smaller than, and the diameter of each haptoral sucker at least as large as, those of most other species with a comparable body length; freshly laid

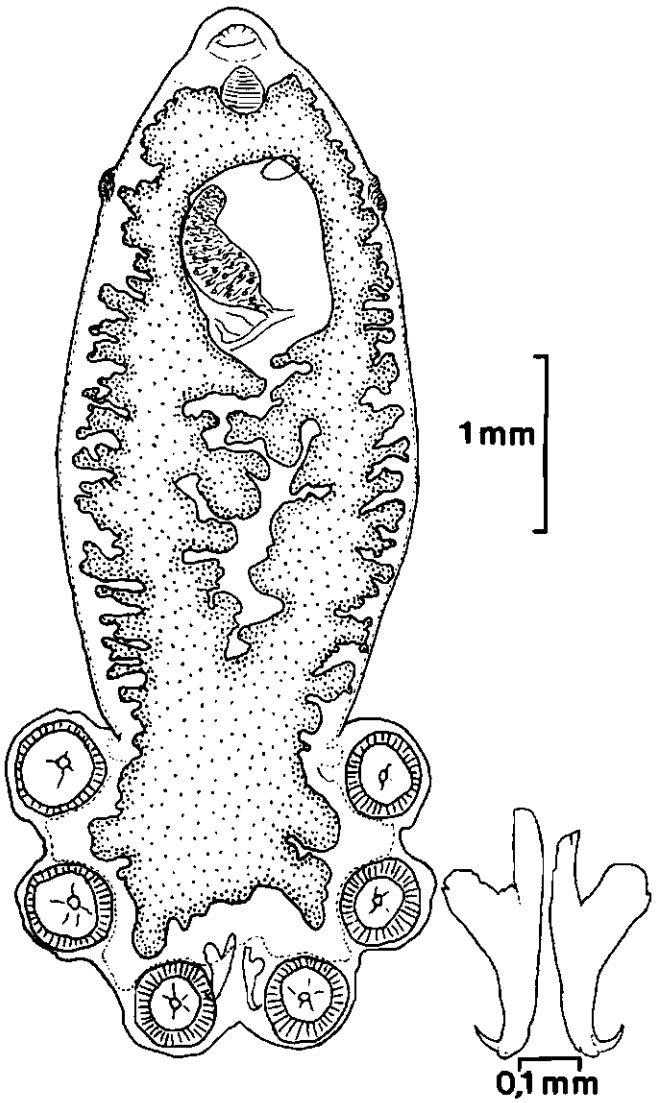

Figure 2 *Polystoma umthakathi* sp. nov. Ventral view of type specimen, opisthaptoral hamuli enlarged. Only the ovary, vaginal protrusions and position of the single intra-uterine egg are shown in the reproductive system.

Table 2 Measurements of sexually mature, egg-producing specimens of *Polystoma umthakathi* sp. nov., compared to the relevant measurements for *P. australis* and *P. natalensis* (*cf.* Kok & van Wyk 1986; Combes & Channing 1979)

	P. umthakathi sp. nov.				*P. australis*	*P. natalensis*
	Mean	*S.D.*	*CV* (%)*	*n*	Mean	Mean
Body length (L) (mm)	7,29	1,41	19,3	5	6,73	4,05
Greatest width (mm)	2,45	0,44	18,0	5	2,53	1,42
Width at vaginae (mm)	1,77	0,25	14,3	5	1,58	–
Haptor length (H.L.) (mm)	2,06	0,17	8,1	5	1,74	1,25
Haptor width (mm)	2,76	0,29	10,5	5	2,49	1,72
H.L./L ratio	0,28	–	–	–	0,26	ca. 0,33
Pharynx width (μm)	300	45,5	15,1	5	238	–
Pharynx length (μm)	295	42,2	14,3	5	251	–
Ovary length (μm)	862	167,0	19,4	5	784	481
Sucker diameter (μm)	474	78,4	16,5	21	376	329
Hamulus length (μm)	408	64,6	15,9	14	326	345
Egg length (μm)	219	17,9	8,2	25	237	207
Egg diameter (μm)	155	4,1	2,6	25	180	123
Intestinal anastomoses	0,29	–	–	7	0	>1

*CV (%) = Coefficient of variation expressed as %.

eggs are of intermediate length but of larger diameter than those of most other species; first larval hooklets are 33 μm long with an a/b ratio (*cf.* Figure 5) of 1,7.

Intestine (Figures 2 and 3): Lateral diverticula from 25 to 30 and of varying but small size; from 4 to 5 large and often branched, medial diverticula are interspersed with from 4 to 6 smaller and unbranched diverticula; large medial diverticula extend beyond the midline and may form narrow anastomoses (Figure 3b and c) with those on the opposite side (frequency 0,29); from 4 to 8 often unbranched diverticula of varying size extend into the opisthaptor.

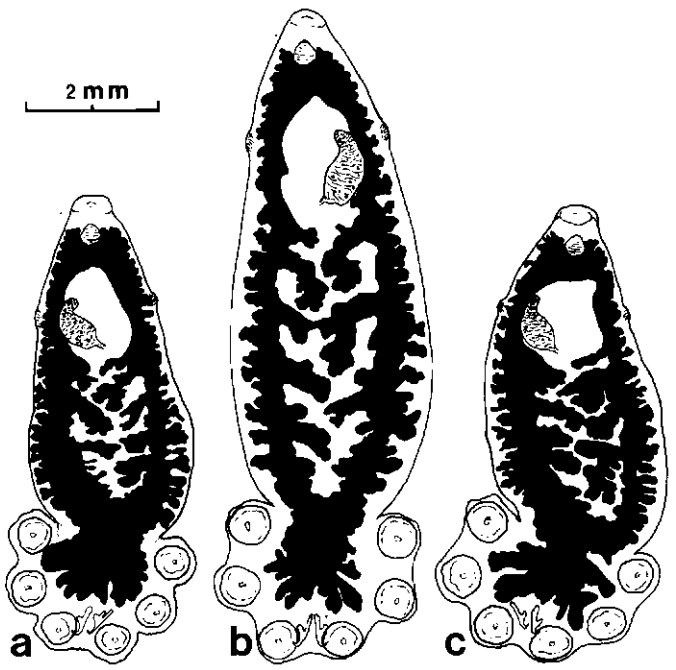

Figure 3 *Polystoma umthakathi* sp. nov. Intestinal silhouette of three paratypes, a: without pre-haptoral intestinal anastomoses; b and c: each with one pre-haptoral intestinal anastomosis.

Genital system: The placement of organs is typical of the genus *Polystoma (cf.* Williams 1960); the uterus is coiled but short and contained at least one and a maximum of three intra-uterine eggs in the actively egg-producing type specimens (see egg production below); the 7 to 9 hooklets of the penial crown are 33 to 40 μm long.

Opisthaptor (Figures 2 and 4, Table 2): Mean sucker diameter does not differ significantly between posterior, middle and anterior suckers and is at least as large as and often larger than in other African species of comparable size; the hamuli are somewhat smaller than in most species of comparable size and though variable in shape (Figure 4) the relatively great length of the handle is in most cases immediately evident; a ratio x/y (*cf.* Figure 4a) was used to quantify relative handle length for the three South African species: the mean ratio was 1,43 for *P. umthakathi* sp. nov. against the 1,24 and 1,14 for *P. natalensis* and *P. australis* respectively (based on published illustrations of adult parasite hamuli).

Larval sclerites (Figure 5): Hooklet no. 1 is 33 μm long and a plot of its total length (a) against handle length (b) occupies a distinct position in a scatter diagram; hooklets 2 to 7 are 22 to 24 μm, no. 8 is 31 μm and the hamulus primordium 17μm.

Parasite reproductive activity

Parasite egg production was used to determine the presence of reproductively active parasites before host necropsy. In four of the hosts, eggs were harvested on

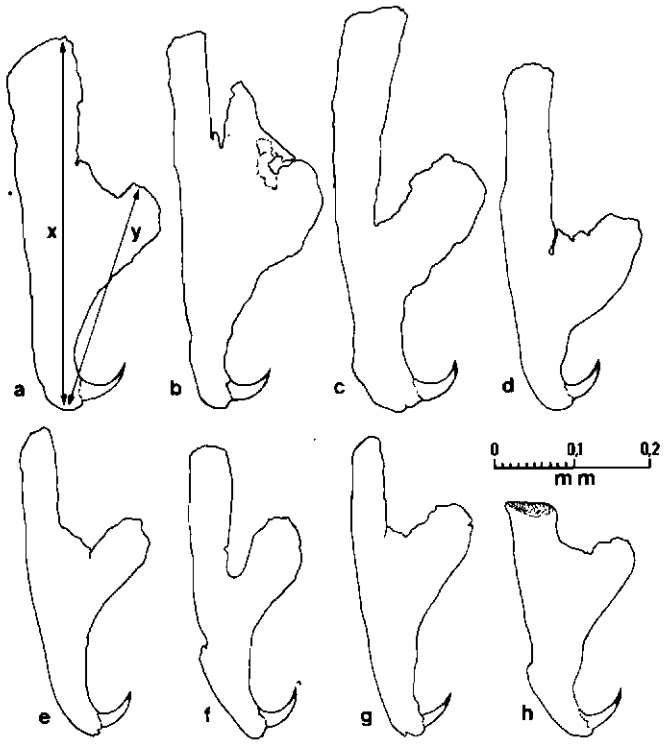

Figure 4 *Polystoma umthakathi* sp. nov. Opisthaptoral hamuli, indicating the extent of variation in size and shape, a and b: from single specimen; c and d: from different specimens; e and f: from single specimen; g and h: from different specimens.

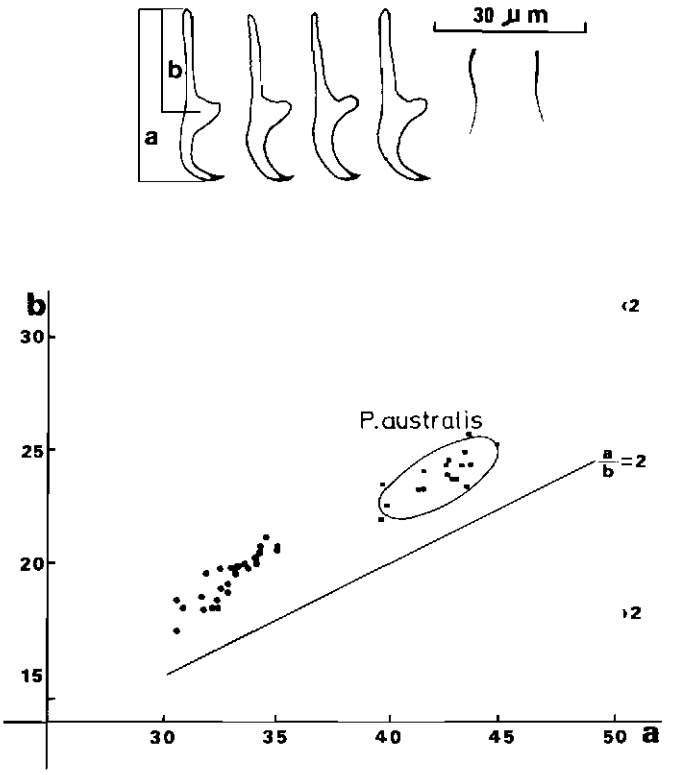

Figure 5 First larval hooklets and hamulus primordia of *Polystoma umthakathi* sp. nov., and scatter diagram of hooklet length (a) against handle length (b) for both *Polystoma umthakathi* sp. nov. (●) and *P. australis* (■).

only three or four consecutive days and the following numbers of eggs were harvested:

47	16	27	–	(♂ host, 1 parasite)
35	24	42	–	(♂ host, 2 parasites)
53	4	21	–	(♂ host, 1 parasite)
1	15	16	0	(♀ host, 1 parasite)

One male host, kept under laboratory conditions, harboured two mature parasites which produced 1433 eggs over a period of 21 days (mean = 68,2 per day; *S.D.* = 39,9; *CV* % = 58,5). Over the last five days before host necropsy 68, 70, 56, 21 and 82 eggs were harvested, indicating that egg production was not declining. At necropsy 13 eggs were found in the host's bladder and the two parasites contained one and three intra-uterine eggs. In both parasites massive amounts of actively moving sperm were observed in and around the vaginal openings. Many vitelline cells were also extruded from the vaginal openings. All these aspects are indicative of persistent reproductive activity, unusual for adult anuran polystomes, especially under laboratory conditions.

Discussion

(i) *Polystoma umthakathi* sp. nov. belongs to the '*africanum* complex' of species, a taxonomic grouping originally proposed by Tinsley (1974) and later subdivided and redefined by other authors (Combes & Channing 1979; Murith 1981a). The limited morphological diversity within the '*africanum complex*' has led to a dependence on host-specificity, the extent of which is still unknown and open to controversy (Prudhoe & Bray 1982), as an important factor in determining species identity. Murith's (1981a) quantification of haptoral sclerite parameters was an important step towards a more reliable system of species determination.

Among the South African polystomes the relationship between total length and handle length of the first larval hooklets distinctly separates *P. umthakathi* sp. nov. from *P. australis*, for which details of larval hooklet morphology are also known. The distinct position which the new species occupies in the scatter diagram of hooklet length against handle length (Figure 5) also separates *P. umthakathi* sp. nov. from most other African species for which these parameters are known (*cf.* Figure 27 in Murith 1981a). *P. natalensis*, for which larval hooklet morphology is unknown, may be distinguished from the new species by the greater number of prehaptoral intestinal anastomoses, the large haptor length to body length ratio, the relatively smaller hamular handle length and the smaller dimensions of all other body parts measured. However, since the geographical ranges of the hosts of *P. umthakathi* sp. nov. and *P. natalensis* overlap, it is necessary to obtain further information on the extent of morphological variation and on the ecology of both species. *P. umthakathi* sp. nov. does not show a particularly close relationship to any of the other African species of the genus and can, apart from the first larval hooklet morphology already mentioned, be distinguished by the

diagnostic characters given in the first part of the species description.

The unique ecology of the host *Natalobatrachus bonebergi*, separates it phylogenetically and ecologically from most other African anurans. The two *Polystoma* species described from species of the related genus *Phrynobatrachus,* namely *Polystoma makereri* Tinsley, 1973 and *Polystoma ragnari* Maeder, Euzet & Combes, 1970, are the only two African species with the alimentary canal reticulated or at least extensively branched. This feature is thus completely different from that of *Polystoma umthakathi* sp. nov.

(ii) Within the anuran polystomatid parasites the intimate adaptation of the parasite life cycle to the ecology of the host is well known (Tinsley 1978; Murith 1981b & 1982; Tinsley 1983). In general, parasite reproduction is continuous in aquatic hosts (*cf. Protopolystoma* in *Xenopus*) but becomes progressively more punctuated in parasites of more terrestrial hosts (*cf. Polystoma australis* and many others), ending with the occurrence of ovoviviparity in some polystomatids (*cf.* Tinsley 1983).

Natalobatrachus, the host of *Polystoma umthakathi* sp. nov., is not well known but field studies have shown its intimate association with water although it is seldom found in the water. Other significant aspects of its ecology include the occurrence in a specialized habitat, egg laying out of the water and the scarcity of tadpoles despite the abundance of developing and hatching eggs. In view of these aspects of the host's ecology, it is thought that the present rather scanty evidence indicating persistent reproductive activity in adult *P. umthakathi* sp. nov. will probably be substantiated by extended field studies. The punctuated egg production reported for many *Polystoma* species is typically found in *P. australis* and is related to the host's punctuated visits to water bodies, following heavy downpours (*cf.* Kok & van Wyk 1986). Directly related to this is a massive build up of neotenic parasites, of up to more than 90% prevalence, in *Notokassina wealii* and *Kassina senegalensis* (unpublished results), making the neotenic stage and not the adult of *P. australis* by far the more important source of recruitable infective larvae. In *P. umthakathi* sp. nov., on the other hand, the neotenic stage seems to be of less importance than the adult stage as a source of recruitable material. The extended release of host tadpoles from egg masses will also have favoured an adaptation towards continuous egg production by adult parasites. Further studies on the host's reproductive ecology during the whole breeding season is fundamental to a proper understanding of the ecological and phylogenetic adaptations of the parasite.

(iii) The *Natalobatrachus bonebergi* — *Polystoma umthakathi* host-parasite system offers unique possibilities, among the South African anuran polystomatids, for the study of branchial parasite population dynamics under laboratory and under natural conditions. The almost total absence of pigment ventral to the branchial chambers facilitates the exact determination of parasite numbers and locations by microscopical examination of anaesthetized tadpoles. Parasite population parameters can thus be determined without killing the hosts. Under natural conditions such procedures would eliminate the necessity of destructive sampling.

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