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

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Combes & Channing (1978 – 79) expressed the opinion that the discontinuity between the range of *Polystoma natalensis*, described by them from Natal in South Africa, and those of other African species of the genus, was probably due to a lack of documentation. An investigation into the parasite loads of several anuran species in the central and eastern parts of the Orange Free State led to the discovery of another new South African species of *Polystoma*, harboured in running frogs of the genus *Kassina* which has not previously been known as a host of adult polystomes. Detailed information is given on the morphological characteristics of the larval, juvenile and mature parasites, while information on parasite and host ecology and distribution is also included.
S. Afr. J. Zool. 1986, 21: 189 – 196

Combes & Channing (1978 – 79) het die mening uitgespreek dat die onderbrokenheid tussen die verspreidingsgebied van *Polystoma natalensis*, wat deur hulle beskryf is uit Natal in Suid-Afrika, en dié van ander Afrikaspesies van die genus, waarskynlik te wyte is aan gebrekkige opnames. 'n Studie van die parasietbeladings van verskeie Anura-soorte uit die sentrale en oostelike gedeeltes van die Oranje-Vrystaat, het gelei tot die ontdekking van nog 'n nuwe Suid-Afrikaanse spesie van *Polystoma*, gehuisves in vleipaddas van die genus *Kassina*, 'n genus waarin nog nie voorheen volwasse *Polystoma*-parasiete gevind is nie. Omvattende inligting word gegee ten opsigte van die morfologiese kenmerke van die larwale, onvolwasse en volwasse parasiete terwyl inligting ook gegee word ten opsigte van die ekologie en verspreiding van beide die gasheer en die parasiet.
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The anuran Polystomatidae are represented in Africa by four genera viz. *Eupolystoma* Kaw, 1950, *Metapolystoma* Combes, 1976, *Polystoma* Zeder, 1800 and *Protopolystoma* Bychowsky, 1957. The genera, with the exception of *Polystoma* which includes more than 20 species, are small. Most of the anuran polystomatids have a mainly tropical distribution, the most obvious exception being *Protopolystoma xenopodis* (Price, 1943), the only species of the genus, which is endemic, abundant and widely distributed throughout the continent with *Xenopus* spp. as hosts.

Only three polystomatid species have been recorded from South Africa: *Eupolystoma anterorchis* has been described from *Bufo pardalis*, a host with a restricted southern distribution (Tinsley 1978); *Polystoma natalensis* has been recorded from Natal specimens of *Strongylopus grayi*, a host with a fairly wide distribution in southern Africa (Combes & Channing 1978 – 79), and *Protopolystoma xenopodis* which occurs throughout South Africa.

In the African species of *Polystoma*, the problem of distinguishing between species on the ground of adult morphological characteristics alone, is still a formidable one. The establishment of new species has often been based largely on the presupposition of rigid host specificity, an approach which led Prudhoe & Bray (1982) to conclude that '. . . a species based primarily on so-called host-specificity must be arbitrary, unless its identity is recognized without knowing the name of its host'.

The problems with species identification were naturally a primary concern of many authors. Prudhoe & Bray (1982) clearly outlined the main trends of the earlier approaches towards a clearer definition of species identity, up to Tinsley's (1974) proposal of a *Polystoma africanum* 'species complex'. Combes & Channing (1978 – 79) again emphasized the remarkable host specificity of *Polystoma* spp., as confirmed experimentally by several researchers in Africa, although they admitted that possible exceptions to habitual specificity might be expected. Combes & Channing (1978 – 79) still referred to an '*africanum* complex' which they divided into three subgroups, based on the relative development of the medial intestinal diverticula.

Mainly as a result of the comprehensive contributions to the knowledge on African anuran polystomatids by French researchers over the past decade or more, the rigid host specificity of these parasites seems to be well established and positive progress has been made towards a more definite species differentiation. The most positive approach is that by Murith (1981) who recognized the importance and stability of the morphology of the larval hooklets as a specific

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taxonomic character. Murith (1981) still recognized an *'africanum* complex', but redefined it and allocated some species to a newly-established *'togoensis* complex'. In the present description of a new species of *Polystoma* Murith's approach is followed closely.

The new *Polystoma* species from *Kassina* Girard, 1853 is the second *Polystoma* species recorded from South Africa. The only previous record of *Polystoma* from *Kassina* is that by Murith (1981) of larval parasites from the gills of *K. cochranae* tadpoles collected in the Ivory Coast. Maeder, Euzet & Combes (1970) found no polystomes in adult *K. maculata* and *K. senegalensis* examined in the Ivory Coast.

Materials and Methods

Live adult specimens of *Kassina wealii* Boulenger, 1882 and *K. senegalensis* (Dumeril & Bibron, 1841) were collected from June 1984 to January 1985. Data pertaining to these hosts is given in Table 1. Preserved specimens of both host species in the collections of the National Museum, Bloemfontein, were also examined for the presence of parasites. Figure 1 indicates the distribution of all infected hosts reported on during the present study.

Live frogs collected out of their breeding season (June to August) were dissected within 24 h of collection. Frogs collected after the first spring rains in October (no frogs were found in September) were kept individually for 5 to 7 days in 600-ml jars containing 50 ml of tap water. The water was replaced every 24 h and parasite eggs harvested by sieving through plankton netting with mesh size of 112 µm. All frogs were eventually dissected and the parasites they harboured removed.

Prior to dissection frogs were narcotized with MS 222 (Sandoz) and parasites found were transferred live from the urinary bladder to 0,7% saline. Individual parasites were confined between two microscope slides which were separated from each other by small amounts of petroleum jelly between their ends. Parasites could then be studied live under low power transmitted light for a short period before transferring the slides to Gilson's fixative for 12 – 24 h. Parasites were then separated from the slides, washed in 50% ethanol, treated with 70% iodine ethanol for 5 – 7 h, washed in 70% ethanol for 24 h, stained in Borax carmine for 2 – 3 h, destained in 70% acid ethanol, dehydrated, cleared in xylene and mounted in Entellan (Merck) (*cf.* Humason 1979). All the important

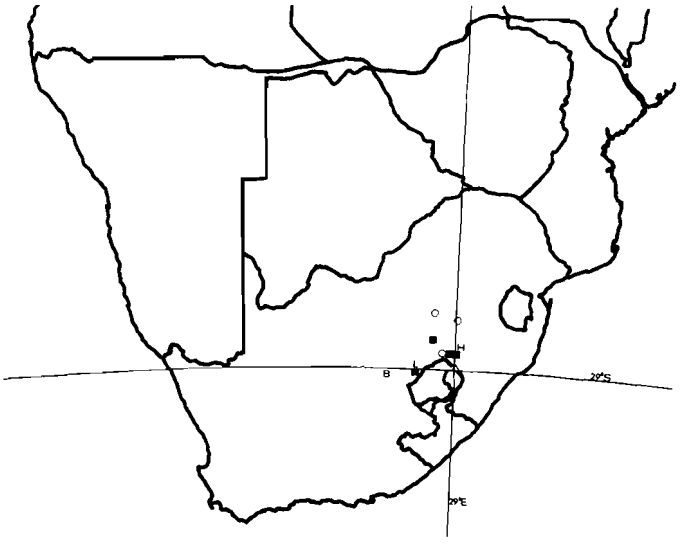

Figure 1 The locality records of *Polystoma* species recorded from South Africa. (■ *P. australis* sp. nov. in *Kassina wealii*; ○ infected *Kassina senegalensis*; △ *P. natalensis* (Combes & Channing 1978 – 1979); B = Bloemfontein; L = Ladybrand; H = Harrismith).

morphological features could be discerned in these whole mounts but regarding the shape of the hamuli and larval hooklets more satisfying results were obtained by digestion of the opisthaptor with concentrated HCl followed by mounting in lactophenol. The limited number of parasites available precluded the extensive application of this procedure.

Parasite eggs harvested were pipetted into small petri dishes containing chlorine-free tap water and kept at a constant temperature of 23°C (± 1,5°C) while exposed to 12 h light and dark periods with simulated dawn and dusk.

Hatched oncomiracidia were mounted in lactophenol for the detailed study of haptoral hooklet morphology.

The genus *Kassina* in South Africa

Taxonomy

Poynton (1964) recognized two species of *Kassina* Girard, 1853 in South Africa: *K. senegalensis* (Dumeril & Bibron, 1841) and *K. wealii* Boulenger, 1882. China & Melville (1968), however, included *Hylambates maculata* in the genus *Kassina*.

Drewes (1985) suggested the elevation of *Kassina wealii* to

Table 1 Numbers and distribution of infected and uninfected host frogs collected from June 1984 to January 1985 at different localities in the Orange Free State

Host species	Number & sex	Month	Locality	Hosts infected	Number of parasites	Parasite development
K. wealii	6♂2♀	June	Harrismith[a]	1	1	Very small juvenile
K. wealii	3♂3♀	July	Harrismith	1	6	Immature
K. wealii	7♀	August	Harrismith	2	1 + 4	Immature
K. wealii	13♂	October	Harrismith	3	1,1 + 2	Mature but only 3 producing eggs
K. wealii	4♂	November	Harrismith	0	–	–
K. wealii	1♂	November	Ladybrand[b]	0	–	–
K. wealii	11♂	January	Ladybrand	2	?	Mature and producing eggs
K. senegalensis	9♂	October	Bloemfontein[c]	0	–	–
K. senegalensis	1♀	November	Harrismith	1	1	Mature and producing eggs
K. senegalensis	11♂4♀	January	Bloemfontein	0	–	–
K. senegalensis	3♂	January	Ladybrand	0	–	–

Localities: [a]28°17'S/29°00'E; [b]29°15'S/27°30'E; [c]29°10'S/26°10'E (See Figure 1).

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a separate genus: *Notokassina*. However, the authors need to evaluate this suggestion before accepting separate generic recognition for *K. wealii*. We therefore, followed the above taxonomic grouping in this paper.

Distribution

Distributions have been mapped using published records (Poynton 1964; Van Dijk 1977; Bourquin & Channing 1980; Pienaar, Passmore & Carruthers 1976) and the collections in the Transvaal Museum, Pretoria; Durban Museum, Durban; Natal Museum, Pietermaritzburg; South African Museum, Cape Town; McGregor Museum, Kimberley and the National Museum, Bloemfontein.

K. wealii is restricted to the eastern coastal margins of South Africa, the eastern Orange Free State and southern Transvaal (Figure 2). Poynton (1964) recognized *K. wealii* as part of the Cape fauna.

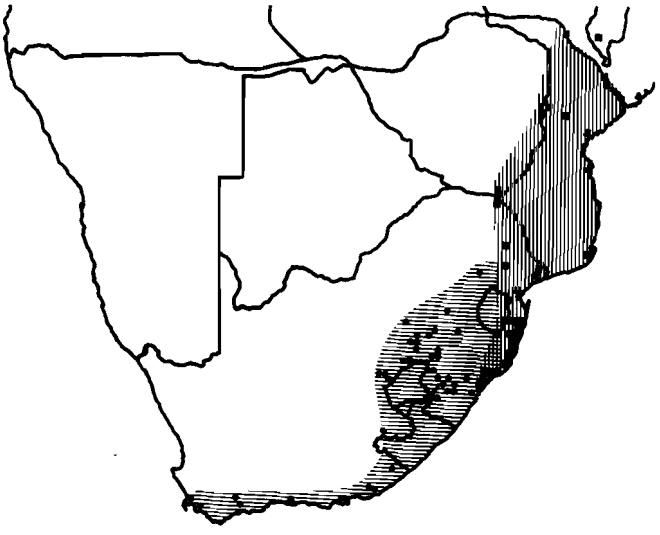

Figure 2 The distribution of *Kassina wealii* (horizontal shading) and *Kassina maculata* (vertical shading) in southern Africa. Locality records are indicated.

K. senegalensis is found throughout southern Africa, excluding the western and northern Cape and southern Namibia (Figure 3). Its range extends northwards into all the savanna regions south of the Sahara and these frogs are thus part of the tropical fauna (Poynton 1964).

K. maculata is recorded from the eastern coastal plains of Africa, extending as far south as Stanger near Durban (29°30′S/31°00′E) (Figure 2) and northwards into Kenya. This species is also included into the tropical fauna (Poynton 1964).

K. senegalensis is sympatric with *K. wealii* on the eastern borders of its distribution and with both *K. wealii* and *K. maculata* in the Richards Bay area (Figures 2 & 3) (Channing 1976).

Ecological notes

Out of the breeding season, members of the genus *Kassina* are known to be completely terrestrial (Balinsky 1969; Jacobsen 1982). *K. wealii* were collected (*cf.* Table 1) from the burrows of the lizard, *Cordylus giganteus* during the period June – August, confirming their preference for this microhabitat (*cf.* Branch & Paterson 1975; De Waal 1978; Newbury, pers. comm.). In the present study area, Harrismith (28°17′S/29°00′E), the highest number of frogs (n = 85) were collected from lizard burrows in May, whereas at the Amersfoort (27°00′S/29°50′E) study area frogs (n = 82) were collected from burrows during September (Newbury, pers. comm.). *K. senegalensis*, although occurring sympatrically with *K. wealii* in both study areas, was never found in the burrows of *C. giganteus*. Eloff (1952), however, reported *K. senegalensis* from mole rat burrows. The absence of *K. wealii* from the lizard burrows during the breeding season (from September onwards) may suggest movement towards water bodies. This is in accordance with the suggestion by Jacobsen (1982) that *K. senegalensis* move long distances in short periods towards water bodies.

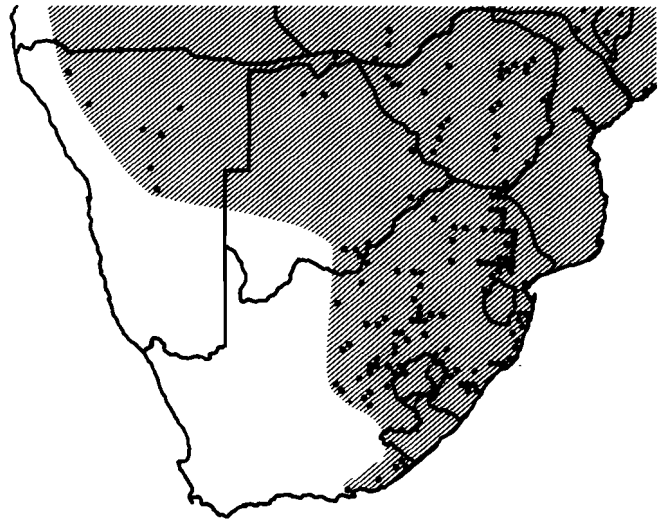

Figure 3 The distribution of *Kassina senegalensis* in southern Africa. Locality records are indicated.

The breeding season of *K. wealii* extends from September to December in both summer and winter rainfall areas (Carruthers & Carruthers 1979; Visser 1979). Balinsky (1969) suggested a longer breeding season, October to May, for *K. senegalensis*. The *Kassina* males are known to call throughout the breeding season (Passmore, pers. comm.). *K. senegalensis* mostly call from concealed positions at the base of a clump of grass and *K. wealii* from above ground level while clinging to plants (Channing 1976). According to Passmore (pers. comm.) the females only respond to the calls of the males when they are ready to spawn. The suggested number of eggs by *Kassina* spp. females ranges from 40 (Wager 1965) to 150 (Balinsky 1969). Egg hatching may take place within six days. The young tadpole shows large external gills, two suckers, no mouth and a deep tail (nectonic tadpole). The external gills and suckers disappear after three days (Wager 1965). Development from egg laying to the end of metamorphosis ranges from 50 to 65 days (Balinsky 1969). Compared to other frog species, *Kassina* spp. show a relatively slow development (Balinsky 1969). Jacobsen (1982) reported a high frequency of *K. senegalensis* sub-adults entering the grassland after the breeding season. During the present study, two fully metamorphosed *K. senegalensis* of 23 and 25 mm respectively, reached 42 and 43 mm within 65 days of metamorphosis. This is close to the 45,6 mm (SD = 1,69; n = 9) of sexually mature males collected near Bloemfontein and it seems reasonable to suggest that these frogs grow to an adult size and sexual maturity before the next breeding season.

Description of *Polystoma australis* sp. nov.

Polystoma australis sp. nov.

Type specimens. Sixteen specimens, all collected at the type locality.

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Holotype. National Museum, Bloemfontein (NMB-P1). Mature egg-producing parasite from the urinary bladder of a male host collected 17 October 1985 by D.J. Kok.
Paratypes. Fifteen immature and mature parasites from male and female hosts collected at the type locality (NMB-P2-16).
Type host and locality. Kassina wealii Boulenger, 1882 from the farm Greenlands (1245), Harrismith (28°17'S/29°00'E) Orange Free State, Republic of South Africa collected by J.H. van Wyk.
Etymology. The specific name *australis* is the Latin adjective, meaning southern.

Species description

The type specimen is represented in Figure 4 but the species description and measurements are based on all three egg-producing parasites collected in October 1984 (*cf.* Table 1).

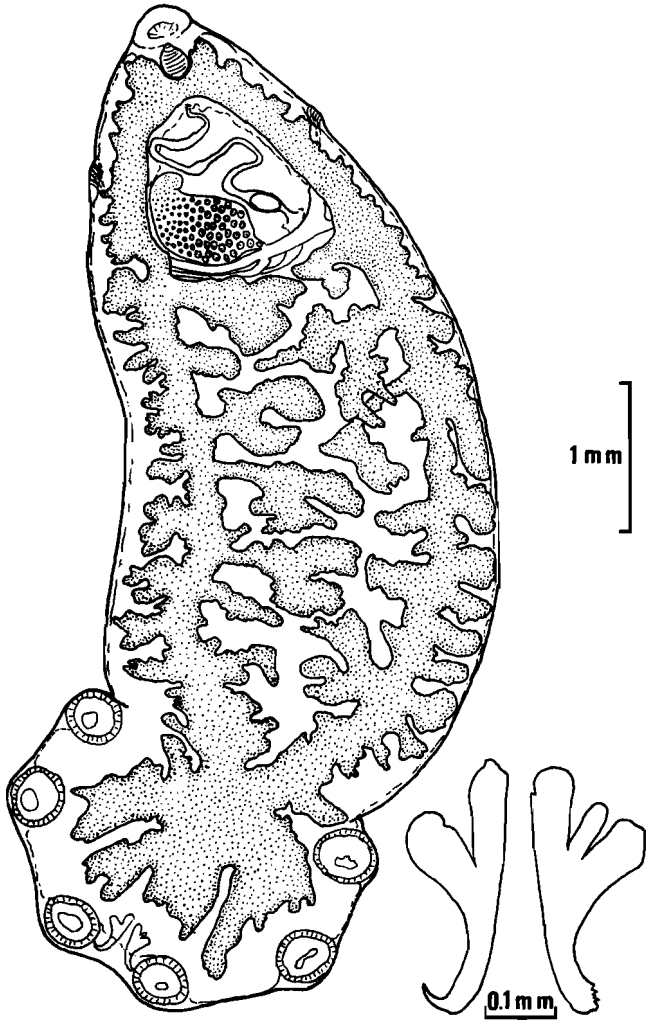

Figure 4 *Polystoma australis* sp. nov. Ventral view of type specimen; testis follicles and vitellaria not shown.

Data on immature stages are based on paratypes and hatched oncomiracidia. Parasites found in preserved frogs could not be used for morphological study and served only to supplement data on geographical distribution.

General characteristics of the mature, egg-producing parasite (Figure 4) are typical of the *Polystoma* spp. in the '*africanum* complex'. The more important body and organ measurements are given in Table 2.

This species is characterized by the following: Medial intestinal diverticula extending beyond the midline but never forming any anastomoses; mean body length is 6,73 mm with a haptor length to body length ratio of 0,26; diameter of the posterior suckers and the length of the hamuli are smaller than in most other species with a comparable body length; eggs are large (based on measurements not of intra-uterine but of freshly laid eggs) and the diameter is conspicuously larger than in most other species; first larval hooklets of the oncomiracidium are 42 µm long with an a/b ratio (*cf.* Murith 1981) of 1,8.

Table 2 Measurements of sexually mature, egg producing and immature specimens of *Polystoma australis* sp. nov.

	Mature parasites (n = 3) October			Immature parasites (n = 4) August		
	$\bar{x}$	*SD*	*CV* (%)	$\bar{x}$	*SD*	*CV* (%)
Body length (mm)	6,73	0,46	6,8	3,57	0,10	2,8
Greatest width (mm)	2,53	0,07	2,8	1,05	0,04	4,5
Width at vaginae (mm)	1,58	0,09	6,0	0,84	0,03	3,8
Haptor length (mm)	1,74	0,02	1,2	1,05	0,09	8,4
Haptor width (mm)	2,49	0,06	2,7	1,46	0,22	14,9
Haptor/body length	0,26	–	–	0,29	–	–
Pharynx width (µm)	238	9,8	4,1	189	12,8	6,8
Pharynx length (µm)	251	6,4	2,6	185	10,7	5,8
Ovary length (µm)	784	54,1	6,9	342	28,2	8,2
Sucker diam. (µm)	376	13,0	3,5	291	9,2	3,2
Hamulus length (µm)	326	31,0	9,5	238	26,2	11,0
Egg length (µm) (n=25)	237,4	12,3	5,2	–	–	–
Egg diam. (µm) (n=25)	179,7	3,3	1,8	–	–	–

The intestine bifurcates immediately posterior to the pharynx, the two longitudinal caeca again fusing in the anterior region of the opisthaptor. From 28 to 34 lateral diverticula of varying but small size are present along each caecum. Posterior to the genital complex each caecum has 3 – 6 large and often branched medial diverticula which extend beyond the midline but never form anastomoses with those on the same or opposite sides. The large medial diverticula of each caecum are interspersed with from 2 to 5 smaller unbranched diverticula with a size comparable to that of the lateral diverticula. Three to five branched diverticula extend from the posterior caecal union into the opisthaptor (Figure 4).

The placement of organs in the genital system is typical of the genus *Polystoma*. The genito-intestinal canal was clearly visible in live specimens. The uterus is short and will probably contain no more than 5 to 7 eggs under conditions of active egg production. The parasites stopped producing eggs under laboratory conditions (see egg production below) and only the type specimen contained one intra-uterine egg. Yolk glands filled the whole body with the exception of the area anterior to the pharynx and the anterior intercaecal area. Actively moving sperm were observed in the vas deferens and uterus. The penial crown bears 7 or 8 (usually 8) hooklets 25 to 30 µm long.

The opisthaptor bears six suckers and the diameters of anterior, middle and posterior suckers do not differ significantly. The relative distances between suckers may be taxonomically important. With the distance between the anterior suckers taken as one unit, the other mean distances can be given as follows: 1,17 between middle suckers, 0,26

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between posterior suckers, 0,19 between anterior and middle suckers and 0,39 between middle and posterior suckers. Larval hooklets with the exception of pairs 3 to 5 are present but the anterior pairs are usually very difficult to distinguish.

The shape of the hamuli of the adult parasites is as given in Figure 4 and Figure 5 a – d. It is evident that, even in the same specimen, variation occurs in the relative length and width of both guard and handle and in the depth of the incision between the two. The general shape and the relatively small size of the hamuli does, however, aid in differentiating this species.

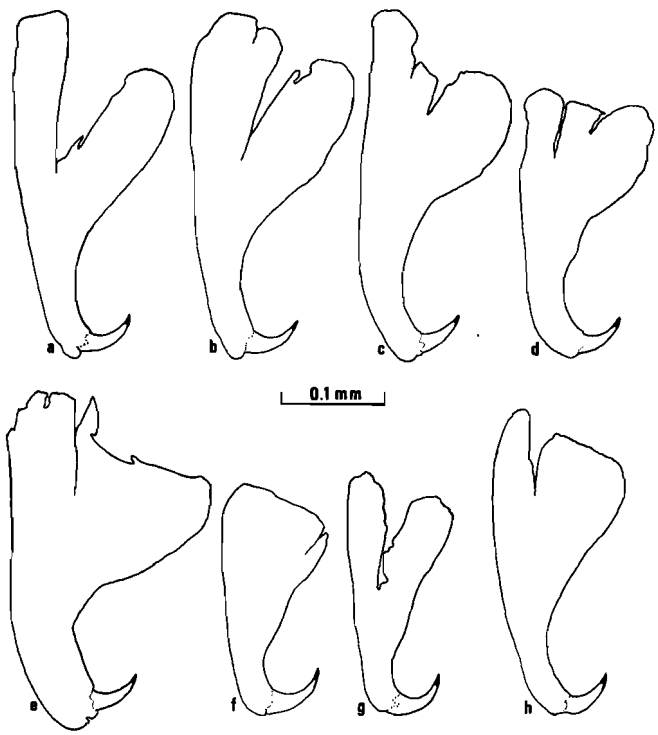

Figure 5 Opisthaptoral hamuli of polystomes from *Kassina* spp: a – d: *Polystoma australis* sp. nov., mature specimens from *K. wealii*; e: *P. autralis* sp. nov., immature specimen from *K. wealii*; f – g: *P. australis* sp. nov., near mature specimen from *K. wealii*; h: mature parasite from *K. senegalensis*.

The oncomiracidium has the full complement of 16 hooklets and the primordia of the hamuli. The shape of the first hooklets and primordia of the hamuli is as indicated in Figure 6. Measurements of the larval sclerites are: No. 1 — 42 µm, No's 2 to 7 — 23 to 26 µm, No. 8 — 38 µm and the primordium — 13 µm. In Figure 6, a and b represent Murith's (1981) measurements of hooklet No. 1. When these measurements are plotted in a scatter diagram the plot occupies a distinct position (Figure 6) separating this species from many others with which it has other characters in common. The ratio a/b is still considered to be approximately two, a value typical of the members of the '*africanum* complex', although, in this case, the plot position lies close to the < 2 region typical of the '*togoensis* complex' (*cf.* Murith 1981).

Kassina wealii collected during the winter months of July and August harboured only immature parasites which could be grouped into a size class distinctly different from that of the egg-producing parasites. The more important body and organ measurements, based on four representative specimens collected in August, are given in Table 2 together with the corresponding measurements of the mature parasites. The

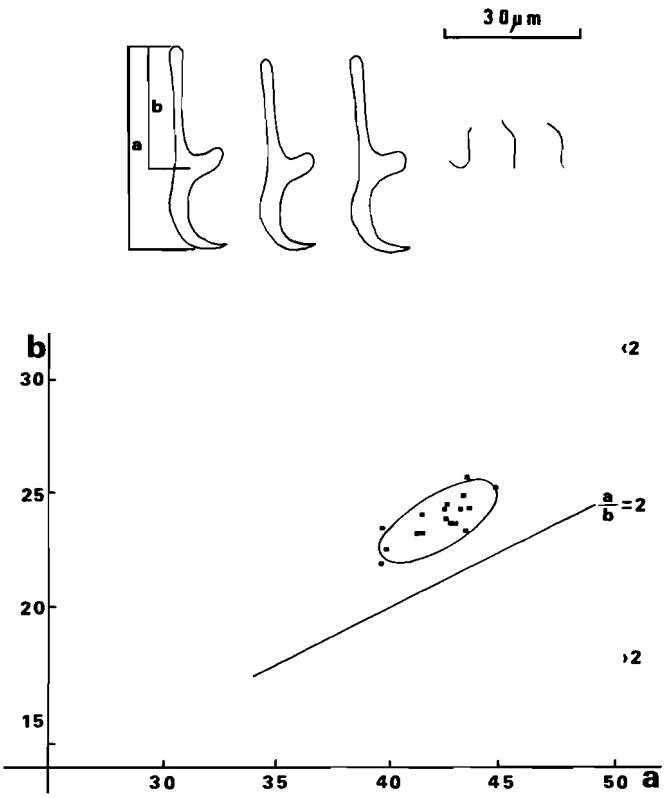

Figure 6 First larval hooklets and hamulus primordia of *Polystoma australis* sp. nov. and scatter diagram of hooklet length (a) against handle length (b).

hamuli of the immature parasites (Figure 5e), apart from being shorter than those of the mature parasites, also do not have the distinct incision between guard and handle. One parasite collected in October fits into the same size class as the above as far as body length is concerned, but it has an obviously greater volume owing to a well developed genital system, especially the vitelline glands. An incision between guard and handle of the hamulus is present in only one of the hamuli of this parasite (Figure 5f & g). It is evident that the parasite represents a developmental stage intermediate between the immature 'winter' and the egg-producing 'spring' parasites. The general size, shape and intestinal silhouette of the distinct size classes and the intermediate form are comparatively illustrated in Figure 7.

The single parasite collected in June was very small (1,26 mm) with the anterior suckers of the opisthaptor still much smaller (93 µm) than the posterior suckers (137 µm). The hamuli (118 µm) are also relatively undeveloped and without a guard, while all the larval hooklets can still be distinguished.

Egg production and egg hatching

Two host specimens collected shortly after the first spring rains in October harboured egg-producing parasites. One and two parasites respectively were eventually recovered from these hosts. In the latter case it is not known whether both parasites or only one were actively producing eggs and none of them contained any intra-uterine eggs at the time of removal from the host. Both parasites showed a similar maturity of the genital system and in both animals gametes and vitelline cells filled the respective genital canals. During five consecutive 24-h periods the following numbers of eggs were harvested from the parasites:

Host with one parasite: 79, 31, 33, 4, 1.
Host with two parasites: 57, 17, 17, 44, 8.

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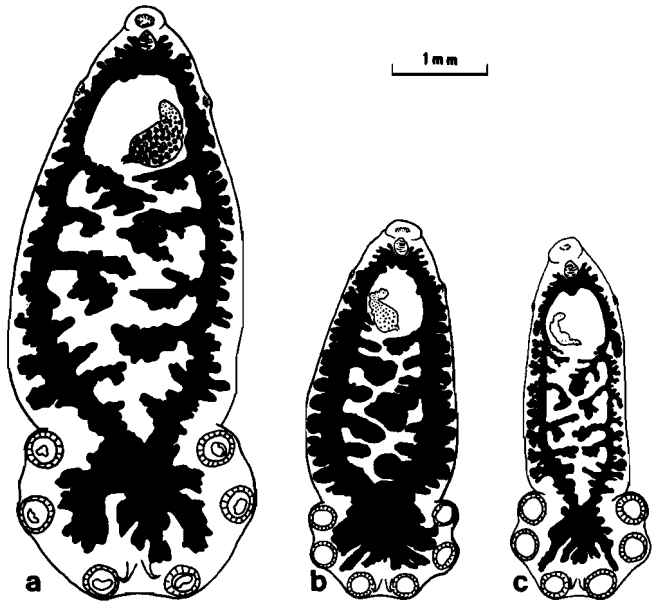

Figure 7 *Polystoma australis* sp. nov. Intestinal silhouette of mature, egg-producing (a), near mature, non-egg-producing (b) and immature (c) specimens.

Since it was evident that egg production was declining, the hosts were killed on the fifth day and the parasites recovered. The urinary bladders contained no eggs and only the single parasite had one intra-uterine egg. Eggs hatched after 10 days at 23°C ± 1,5°C.

Kassina senegalensis as host

During the examination of preserved museum specimens of *K. senegalensis* nine small immature polystomes were recovered from three hosts collected in February and April 1974 near Vrede and Heilbron, about 1° north and north-west of Harrismith (*cf.* Figure 1). All parasites were stained and mounted but owing to improper *in situ* fixation they were of little use for morphological study. Twenty-seven *K. senegalensis* collected at Bloemfontein and Ladybrand (*cf.* Table 1) were all free of parasites. Parasite eggs were recovered from one female host specimen collected in November at Harrismith and it was later established that only one parasite was present. On five consecutive days, 104, 13, 45, 3 and 1 eggs were harvested, a pattern very similar to that found in parasites harboured by *K. wealii*. On the fifth day the female host was injected into the dorsal lymph sac with 30 I.U. of human chorionic gonadotrophin (Pregnyl-Organon) well known to stimulate reproductive activity in *Xenopus laevis* (Nieuwkoop & Faber 1967). After 24 h 33 eggs were harvested. The host was again injected and 5 eggs recovered during the next 24 h, after which period the host was dissected and the parasite removed. No eggs were present in the urinary bladder and the parasite contained only one intra-uterine egg. The parasite seemed to be still in a state of 'reproductive readiness' with an abundance of all materials necessary for egg production. Morphologically it closely resembled those parasites found in *K. wealii* and most measurements of adult and oncomiracidial structures fall within the range reported for *Polystoma australis* sp. nov. However, in view of differences such as *inter alia* a smaller haptor/body length ratio (0,21) and smaller suckers and hamuli (*cf.* Figure 5h) we prefer to withhold a final opinion on the specific identity of the *K. senegalensis* parasites until after the completion of cross-infestation experiments.

Discussion

(i) Considering the traditional approach to species description within the Polystomatidae of African Anura, the newly recorded host species and the geographical distribution of *Polystoma australis* sp. nov. is sufficient to merit the establishment of the new species. On the other hand Prudhoe & Bray (1982) are certainly entitled to doubt the validity of species descriptions 'based primarily on so-called host specificity'.

Regarding the question of the 'so-called' host specificity, the comprehensive studies and unequivocal verdicts of mainly French researchers can leave little doubt that a remarkable degree of host-specificity is shown by the genus *Polystoma* in Africa, despite some definite or apparent exceptions. *Ptychadena mascareniensis*, for example, harbours three different but closely related (Murith 1981) *Polystoma* spp., while *Bufo regularis* is host to *Eupolystoma alluaudi* and three different species of *Polystoma* which do not seem to be closely related. Also, *P. aethiopiense* allegedly occurs in three anuran genera. Murith (1981) has already shown that *P. africanum* occurs in *Bufo regularis*, and was wrongly described by Tinsley (1974) as a parasite of *Ptychadena mascareniensis*. Further studies may similarly reveal other inconsistencies which will presumably strengthen the case for rigid host specificity.

Furthermore, although the question of host-specificity may have been decisive in the establishment of some new species, it has always been found that such species could also be distinguished by morphological characters. Although the different species can be recognized without knowing the names of their hosts, this may not always be an easy task on account of intraspecific variation of those characters traditionally regarded as specifically important. Care should be taken not to overemphasize the taxonomic importance of single characters such as intestinal silhouette, the shape of the hamuli, the ratio of haptor length to body length, etc. During the present study it was possible to distinguish between practically all existing species of *Polystoma*, using the following combination of characters of mature, egg-producing parasites: Total body length (L), opisthaptor length (LH), ratio LH/L, mean number of prehaptoral intestinal anastomoses, mean diameter of haptoral suckers, mean hamulus length, egg length and diameter, length of first larval hooklets and the ratio a/b of the first larval hooklets (although these hooklets are present in all developmental stages they can be studied best in the oncomiracidium).

Apart from the obvious general relationships with other species within the '*africanum* complex', *Polystoma australis* sp. nov. cannot be said to show a particularly close relationship to any one species within the complex. *P. australis* sp. nov. can briefly be characterized as a medium-sized polystomatid without any prehaptoral intestinal anastomoses and with relatively small haptoral suckers and hamuli and very large eggs.

(ii) It has already been pointed out that a final conclusion cannot be reached on the identity of the parasite found in *Kassina senegalensis*. Different species of the same anuran genus may harbour different polystomatid species e.g. *Polystoma assoulinei* and *P. baeri* in *Ptychadena huguettae* and *Ptychadena maccarthyensis* respectively (Bourgat 1975; Maeder, Euzet & Combes 1970). On the other hand *Polystoma gabonensis* has been recorded from three different species of *Hylarana* (*cf.* Murith 1981). The information presently available indicates that *Kassina senegalensis* is only infected in those regions where its geographical distribution overlaps with that of *K. wealii*, the host of *Polystoma australis* sp. nov. (*cf.* Figures 1 – 3). It thus seems likely that only one polystome species occurs in this region and while *Kassina*

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wealii is its main host, *K. senegalensis* may also be infected. Further field investigations and cross-infestation experiments are intended and will lead to a better understanding of parasite prevalence and abundance and the factors determining possible differences in this regard between the two host species. The life cycle of the *K. senegalensis* parasite has already been successfully completed experimentally in larval and post-metamorphosis *K. senegalensis* hosts. Neither *Polystoma australis* sp. nov. eggs nor *K. wealii* larvae were available at that time for cross-infestation experiments.

(iii) Any problems relating to the specific identity of the recently found South African polystomes are typical of the identification problems within the '*africanum* group' which shows restricted morphological diversity of the adult parasites, in contrast to the '*togoensis* group' within which a more marked morphological diversity is seen. Preliminary indications are that, even where adult parasites may show few morphological differences, detailed studies on the life cycle may reveal more conspicuous differences. Aspects which need much more detailed investigation are, for example, egg hatching and factors influencing larval development within the egg, maturation of neotenic forms, egg production by adults and neotenics, intensity of infestation by neotenics, parasite mortality, invasion strategies as related to host ecology, etc. In this regard an interesting aspect is the degree of larval development at the time of egg deposition. An advanced development of the oncomiracidium at the time of deposition is normally associated with an enlarged uterus permitting an accumulation of eggs which may develop *in situ* and hatch at or shortly after deposition (*cf.* Tinsley 1983). Members of the genus *Metapolystoma* typically show the lengthened uterus with great egg storage capacity. This is not found within the '*africanum* group' and therefore it is surprising that advanced development within the eggs (or some eggs) has been reported for *Polystoma mashoni* and *P. natalensis* (Beverley-Burton 1962 and Combes & Channing 1978–79). Although Llewellyn (1981) referred to the accumulation of large numbers of eggs in the uterus of *P. integerrimum*, it is actually a question of the stockpiling of reserves permitting the production of thousands of eggs in a very short space of time (Combes 1981). Very few eggs can be retained in the short uterus of *Polystoma* spp. and eggs are not retained during the short period of active egg production and deposition. During the present investigation it was found that egg production decreased rapidly, probably owing to hormonal changes in the host, following mating, or, under laboratory conditions, associated with the removal of the reproductively active host from its natural habitat. At the end of a period of active egg production polystomes may retain a few eggs in the uterus and larval development may continue in such eggs. This is known for *P. integerrimum* and *P. pelobatis* where one egg is often retained (Tinsley 1983). In *P. australis* sp. nov., only one egg was found in one of the mature parasites after active egg production had stopped. The problem of intra-uterine development in *P. mashoni* and *P. natalensis* further serves to emphasize the necessity of more detailed studies on the factors influencing egg production and subsequent development in different species of anuran polystomes. Such studies are currently being undertaken on the parasites occurring in *Kassina wealii* and *K. senegalensis*.

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