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Polystomatidae (Monogenea) of African Anura: *Polystoma dawiekoki* n. sp. parasitic in *Ptychadena anchietae* (Bocage)

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Abstract

Polystoma dawiekoki n. sp. is described as a new species of the Polystomatidae parasitic in the urinary bladder of the plain grass frog *Ptychadena anchietae*. This parasite was collected at Mkuze town and Mkuze Game Reserve in northern Kwazulu-Natal Province, in the Kruger National Park in Mpumalanga Province, South Africa, and at Bulwa in Tanga Province, East Usambara Mountains, Tanzania. It is distinguished from other African *Polystoma* species by a combination of characters, including the body size, size and shape of marginal hooklets and the haptor length to body length ratio. The presence of adult, as well as subadult, parasites in the same individuals, as is known for *Eupolystoma*, represents a significant evolutionary departure from the pattern of transmission typical of *Polystoma* in most of the other anuran hosts.

Introduction

Polystomatids of anurans are represented in Africa by the genera *Eupolystoma* Kaw, 1950, *Metapolystoma* Yamaguti, 1963, *Polystoma* Zeder, 1800 and *Protopolystoma* Bychowsky, 1957. *Polystoma* has a widespread occurrence in all zoogeographical regions except the Australian region. To date more than 55 species are known of which more than half are known from Africa.

During the period 1966-1979 several African polystomes were described mainly from the former French colonies. Between 1979 and 1985 very few species were added to the list, but since then, a series of *Polystoma* has been described from South Africa: *P. australis* Kok & Van Wyk, 1986 from *Semnodactylus wealii* (Boulenger); *P. umthakathi* Kok & Seaman, 1987 from *Natalobatrachus bonebergi* Hewitt & Methuen; *P. sodwanensis* Du Preez & Kok, 1992 from *Ptychadena porosissima* (Steindachner); *P. marmorati* Van Niekerk, Kok & Seaman, 1992 from *Hyperolius marmoratus* Rapp; *P. testimagna* Du Preez & Kok, 1993 from *Strongylopus fasciatus* (Smith); and *P. claudecombesi* Du Preez & Kok, 1995 from *Afrana angolensis* (Bocage). During further studies of African polystomes, *Ptychadena anchietae* (Bocage) has been found infected with an undescribed species of *Polystoma* in the Kwazulu-Natal and Mpumalanga Provinces of South Africa and in Tanga Province of Tanzania. This paper reports the first species from *P. anchietae*, but the sixteenth record of a polystomatid from the anuran genus *Ptychadena* Boulenger.

Materials and methods

Adult *Ptychadena anchietae* from South Africa were obtained (L.d.P. coll.) from the following localities: two specimens from Hoedspruit Air Force Base (24°20′S, 30°59′E) during March 1988; one specimen from the town of Mkuze (27°37′S, 32°02′E) during July, 1990; six specimens from Skukuza camp in the Kruger National Park (24°59′S, 31°35′E) during August 1991; two specimens from Berg & Dal camp in the Kruger National Park (27°37′S, 32°02′E) during August, 1995; 62 specimens from Mkuze Game Reserve (28°45′S, 28°52′E) during October, 1995; two specimens from Ndumu Reserve (26°54′S,

32°18′E) during June, 1996; two specimens from Bayala (27°51′S, 32°06′E) during April, 2000; and one specimen from Ndumu Reserve during April, 2000. A single *P. anchietae* was examined from a tea plantation in Bulwa, near Tanga, East Usambara Mountains (5°3′S, 38°38′E), 29th November, 1999 (J.M. coll.).

Prior to dissection, frogs were anaesthetised with Benzocaine. In the case of South African material, the complete urinary bladder of infected frogs was removed and transferred to 0.3% saline solution. Following fixation for 24 hours in 10% neutral buffered formalin while under coverslip pressure, parasites were rinsed in water, stained in alum carmine, dehydrated, cleared and mounted in Canada balsam. Material for histological sectioning was fixed in Bouin's solution for 24 hours and transferred to 70% ethanol. For histology, material was embedded in paraffin wax, sectioned at 6 μm and stained routinely in Harris' haematoxylin and eosin. Some specimens were fixed in 96% ethanol for future molecular studies. As part of another project parasite material collected during the present study was analysed and compared at the DNA level at the University of Perpignan in France. The terminal 18S rDNA plus the complete ITS1 region were PCR-amplified and compared.

Specimens from Tanzania were fixed without coverslip pressure in 4% neutral buffered formalin and some in 80% ethanol. Specimens were stained in hydrochloric carmine and mounted in Canada balsam.

The host, Ptychadena anchietae (Ranidae)

The distribution of *P. anchietae* extends from Ethiopia and Zaire to Angola and Kwazulu-Natal Province in South Africa (Frost, 1985). The body is streamlined with powerful hindlimbs and a pointed snout, features which according to Passmore & Carruthers (1995) facilitate equally efficient movement on land, through dense vegetation and in water. *P. anchietae* is a typical savanna amphibian and is rarely found far from water. Adults prefer to breed in shallow pools (Lambiris,1989a) and spawning takes place during the middle of summer (November to February) (Lambiris, 1989b).

Levels of infection

The single host specimen from the town of Mkuze collected during July, 1990 was infected with 23 parasites of which only 7 were mature and egg-producing. One of the 6 specimens collected in the Kruger Park during August 1991 was infected with a single parasite (prevalence 17%, mean intensity 1). Five of the 62 specimens collected in the Mkuze Game Reserve during October, 1995 were infected with respectively 1, 2, 6, 10 and 16 parasites (prevalence 8%, mean intensity 7). The host specimen infected with 6 parasites had one immature parasite. The single host specimen examined in Tanzania harboured 9 mature specimens. For the total sample the prevalence was 11% and the mean intensity 8.5.

Polystoma dawiekoki n. sp.

Specimens studied

Twenty-six sexually mature worms and 30 oncomiracidia. Holotype (NMB P256) and 7 paratypes (NMB P257-263) deposited in the Parasitic Worm Collection, National Museum, Aliwal street, Bloemfontein 9300, South Africa; 2 paratypes (BMNH 2001.7.26.1-2) in the Parasitic Worms Collection, Natural History Museum, London; remaining South African specimens in the collection of the senior author. Three stained and mounted specimens (MHNG 29656 INVE) and 6 remaining specimens stored in formalin or ethanol deposited in the Geneva Museum Collection.

Type-host: *Ptychadena anchietae* (Bocage), sexually mature female (NMB A 5934) deposited in the Amphibian Collection, National Museum, Aliwal Street, Bloemfontein 9300, South Africa.

Site: Urinary bladder.

Type-locality: Kumahlala pan, Mkuze Game Reserve, Kwazulu-Natal Province, South Africa (27°35′50"S, 32°13′12"E).

Etymology: The specific name *dawiekoki* refers to Professor Dawie Kok who initiated the study of southern African Polystomatids.

Description (Figures 1-3)

Measurements and description based on egg-producing parasites and measurements of larval sclerites on oncomiracidia hatched from eggs released from uterus of holotype and paratype specimens, as well as from adult parasites from South Africa. General characteristics of mature, egg-producing parasite (Figure 1) typical of *Polystoma*. Body and organ measurements are given in micrometres based on means of 26 specimens.

Body elongate; total body length 7,096 (4,050-8,200); greatest width 2,031 (1,250-2,425); hap-

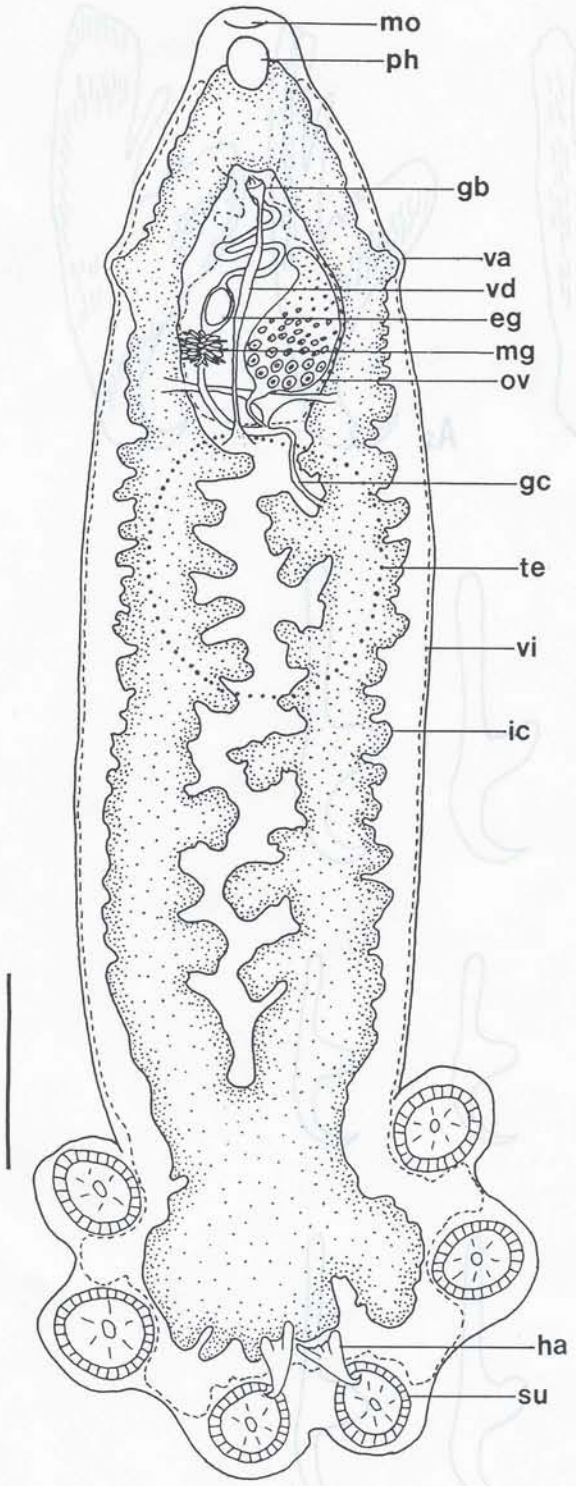

Figure 1. Polystoma dawiekoki n. sp. Ventral view of holotype; the dotted line indicates the outline of the testis and the dashed line indicates the outline of the vitelline system. *Abbreviations*: eg, egg; gc, genito-intestinal canal; gb, genital bulb; ha, hamulus; ic, intestinal caecum; mg, Mehlis gland; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis distribution; va, vagina; vd, vas deferens; vi, vitelline distribution. *Scale-bar*: 1 mm.

tor length 1,582 (1,000-1,825); haptor width 2,432 (1,325-2,750); haptor length to body length ratio 0.34; 6 haptoral suckers, with mean diameter 455 (252-543); hamulus length 440 (281-485); handle longer than guard and mean x/y ratio 1.36 (Figure 2); hamulus hook length 66 (62-71). Mouth subterminal, ventral. Oral sucker 286 (160-329) wide; pharynx length 270 (170-314); pharynx width 245 (177-280). Intestine bifurcate, caeca confluent posteriorly extending into haptor; caeca with 12-15 medial diverticula, 31-49 small lateral diverticula; 2 pre-haptoral anastomoses observed in only 1 specimen and 2 other specimens with 1 each.

Testis well developed, post-ovarian, ventral, median (Figure 1). Seminal vesicle prominent. Genital atrium median, ventral; genital bulb posterior to intestinal bifurcation, with 7-8 genital spines 32 (29-34) long. Ovary sinistral, submedian, 760 (534-911) × 410 (281-475). Oötype well developed. Genito-intestinal canal present on same side as ovary, joining intestinal caecum posterior to ovary. Uterus holding up to 9 eggs but majority of egg producing specimens with single egg in the uterus. Eggs operculate. Egg 201 (180-219), × 127 (116-140). No *intra uterine* development of eggs observed. Vitelline follicles distributed throughout body excluding oral region, region around ovary and uterus and haptoral suckers (Figure 1).

The Tanzanian specimens (n=6) fixed without coverslip pressure have the following main measurements: total body length 2,980-4,140; greatest width 1,630-1,690; haptor length 1,330-1,370; haptor width 1,570-1,730; haptoral sucker diameter 344-507; hamulus length 384-507; oral sucker 270-335 wide; pharynx length 188-209; egg 172-205; × 110-131.

Worms (n=23) initially found in one host specimen were not of same age class. Total lengths varied from 517 to 5,392, average 2,498. Two smallest parasites had only 4 suckers, 12 had ovary present and 7 of these had single egg *in utero*. Hamulus length varied from 101 to 405.

Oncomiracidium

Ciliated oncomiracidium has narrow cylindrical body with circular cup-shaped opisthaptor and resembles typical polystomatid oncomiracidium. Opisthaptor bears 16 marginal hooklets, which are retained in adult parasites and do not increase in size. Marginal hooklet I (postero-medial), 32 (31-33) in length; hooklets 2-7 20 (19-20) in length; hooklet 8 28 (28-29) in length

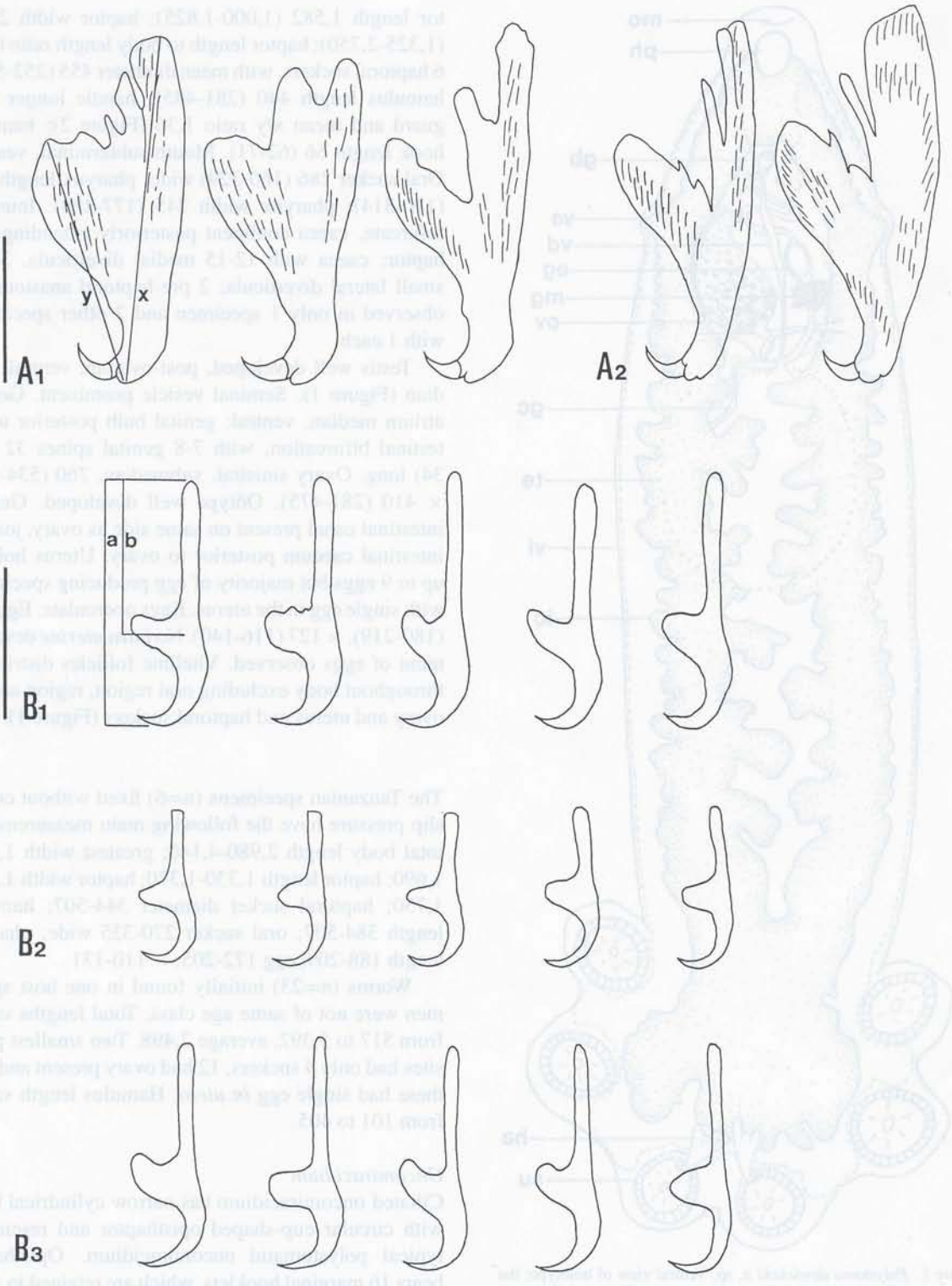

Figure 2. Polystoma dawiekoki n. sp. A1, hamuli from holotype and paratypes collected in South Africa; A2, hamuli from Tanzanian material; B, marginal hooklets from oncomiracidia hatched from eggs laid by holotype and paratypes; B1, marginal hooklet 1; B2, marginal hooklets 2–7;. B3, marginal hooklet 8. *Abbreviations*: a, total length of marginal hooklet I; b, handle length of marginal hooklet measured tip to centre of guard base (see Murith, 1981); ×, distance from hook to tip of handle; *y*, distance from hook to tip of guard. *Scale bars*: A, 200 μm; B, 20 μm.

(Figure 2). Ratio of total length (a in Figure 2) to handle length (b in Figure 2) for marginal hooklet I was 1.56 (1.53-1.62). Hamulus primordia 11 (11-12) in length.

Diagnosis

In a study on the origin and evolution of African *Polystoma*, assessed from molecular phylogeny, material of *P. dawiekoki* n. sp. from South Africa and Tanzania were compared and found to differ by less than 1%, indicating that they belong to the same species (Bentz et al., 2001).

P. dawiekoki n. sp. differs from other members of the genus by a combination of characters. *P. grassei* Euzet et al., 1966 is the only other African Polystomatid known where parasites of different size classes were found in the same host (Dupouy & Combes, 1977; Murith et al., 1978). With a body length of 7,096 (4,050–8,200) *P. dawiekoki* n. sp. is significantly larger than *P. grassei* (2,100–3,600).

P. dawiekoki n. sp. differs from all 12 other members of the genus *Polystoma* that infect the host genus *Ptychadena*. The length of marginal hooklet 1 of 32 for *P. dawiekoki* n. sp. separates it from *P. africanum* Szidat, 1932 (38), *P. aethiopiense* Meskal, 1970 (36), *P. baeri* Maeder *et al.*, 1970 (41), *P. mangenoti* Gallien, 1956 (42), *P. pricei* Vercammen-Grandjean, 1960 (38), *P. prudhoei* Saoud, 1967 (43), *P. sodwanensis* Du Preez & Kok, 1992 (38) and *P. togoensis* Bourgat, 1977 (40). The a/b ratio for marginal hooklet 1 (see Figure 2) of 1.56 for *P. dawiekoki* n. sp. separates it from *P. aeschlimanni* Bourgat & Murith, 1980 (1.89), *P. baeri* (1.86), *P. ebriensis* Maeder, 1973 (1.65), *P. lamottei* Bourgat & Murith, 1980 (1.94), *P. mangenoti* (1.83), and *P. sodwanensis* (1.86). Furthermore, the structure of the intestine separates *P. dawiekoki* n. sp. from *P. assoulini* Bourgat, 1975, which has 2–4 anastomoses, *P. mangenoti* which has up to five and *P. lamottei* which has a network of anastomoses.

The plot of the a/b ratio for marginal hooklet 1 for southern African polystomes (Figure 3) separates *P. dawiekoki* n. sp. from all other known southern African polystomes, except *P. umthakathi*. *P. umthakathi*, however, has a haptor length to body length ratio of 0.28, while the ratio for *P. dawiekoki* n. sp. is 0.22.

Discussion

In the present study as many as 23 parasites per host were found, which is higher than the average for *Polystoma*. The high infection levels recorded for *P. dawiekoki* n. sp. are however not exceptional. Murith et al. (1978) reported 116 individuals of *Polystoma grassei* from the bladder of its host *Leptopelis calcaratus* (Boulenger). Secondary re-infection has been recorded for *P. grassei* by Dupouy & Combes (1977).

It is well documented that, in the case of *Polystoma*, one or more eggs could remain *in utero* at the end of the breeding season and that these eggs could develop and hatch, resulting in auto-reinfection of the host (Tinsley, 1983). Intra-uterine development of oncomiracidia was recorded in 1872 by Zeller for the European polystome *Polystoma integerrimum* Frölich, 1791 and by Paul (1935) for the North American *P. nearcticum* (Paul, 1935). Intra-uterine development of oncomiracidia has been reported for a number of other African polystomes. These include *P. mashoni* (*cf.* Beverley-Burton, 1962), *P. galamensis* (see Euzet et al., 1974; Tinsley, 1978a), *P. togoensis* (see Bourgat, 1977; Murith, 1981), *P. natalensis* (see Combes & Channing, 1979) and *P. africanum* (see Salami-Cadoux, 1979). In most of these cases it is, however, not known whether the oncomiracidia hatch *in situ* and augment the established parasite burden, or whether they hatch after deposition in water, enabling rapid re-infection of the same or another host (Tinsley, 1983). No evidence of intra-uterine development has been observed in the present study. Auto-reinfection would imply that the newly established parasites would be more or less of equal size. The recording of different size clusters of *P. dawiekoki* n. sp in *Ptychadena anchietae* varying in size from 517 to 5,392 thus excludes auto-reinfection and implies secondary re-infection of the adult host. This occurrence of adult and subadult parasites in the same individuals is well documented for *Eupolystoma* (see Combes et al., 1973; Salami-Cadoux, 1975; Tinsley, 1975, 1978b). Tinsley (1978b) pointed out that heavy worm burdens pose a fundamental advantage in Polystomatid evolution. The occurrence of this phenomenon in *Polystoma* represents a significant evolutionary departure from the pattern of transmission typical of *Polystoma* in most of the other anuran hosts.

No less than 52% of all *Polystoma* and *Metapolystoma* in Africa infect species of *Ptychadena*. One could ask the question why *Ptychadena* is such a susceptible host for polystomatid parasites. *Ptychadena*

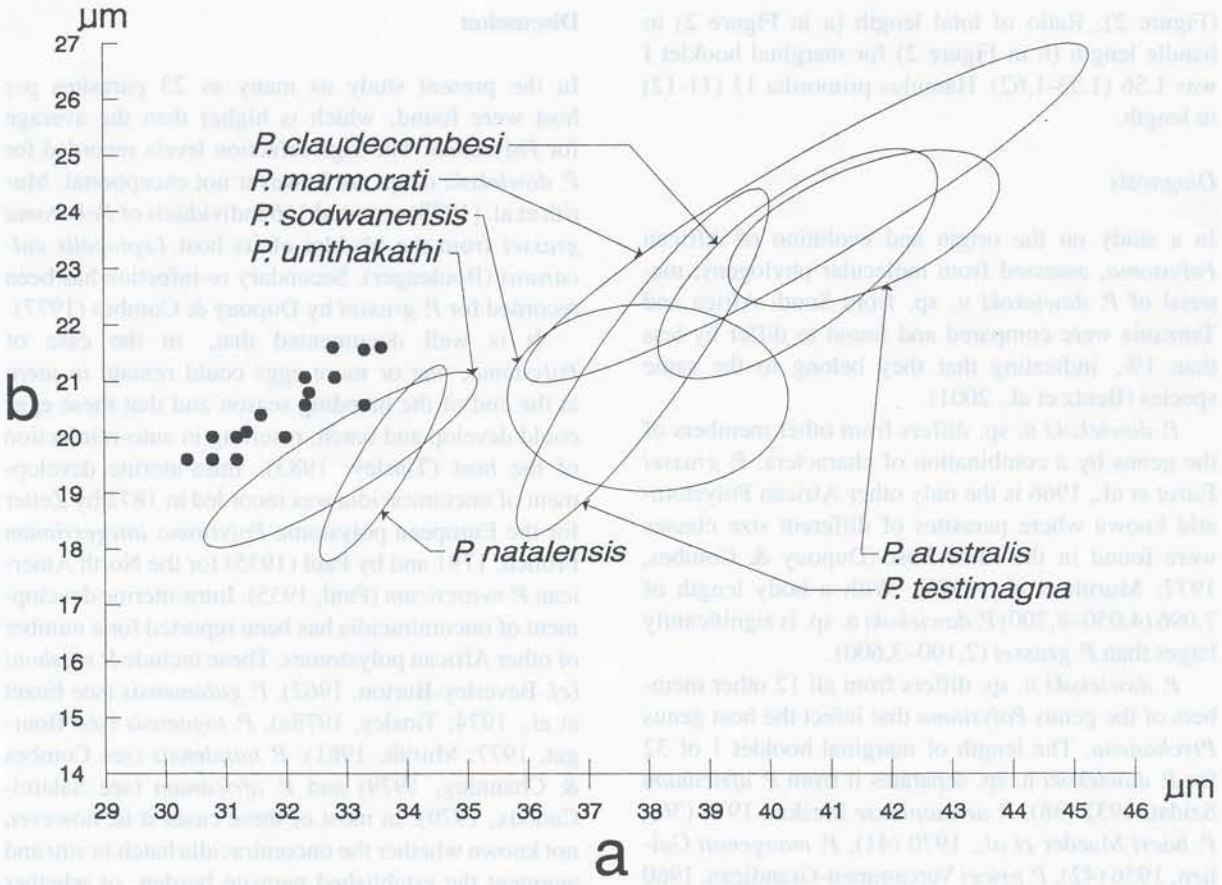

Figure 3. Scatter diagram of total length (a) against handle length (b) of marginal hooklets I for *Polystoma dawiekoki* n. sp. (black dots) and other southern African *Polystoma* spp. indicated on the figure.

is a very large and successful genus in Africa and is in an active phase of evolution (Stewart, 1967). Furthermore, *Ptychadena's* habitat and breeding behaviour favour polystomatid transmission (Du Preez & Kok, 1992).

Polystomatids, in general, display a degree of intraspecific morphological variation and very little interspecific variation (Du Preez & Kok, 1995). The present study confirms the value of measuring the marginal hooklets which display little intraspecific variation. Murith (1981) showed how measurements of marginal hooklets assist species separation. The characterisation of the marginal hooklet morphology, however, needs to be studied in more depth.

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