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Polystoma vernoni n. sp. (Monogenea: Polystomatidae) from the sharp nosed grass frog *Ptychadena oxyrhynchus* (Smith, 1849) in South Africa

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Abstract

Polystoma vernoni (Polystomatidae) is a new species in the urinary bladder of *Ptychadena oxyrhynchus* in KwaZulu-Natal province, South Africa. *Ptychadena* is a true African species and the 49 currently known species in Africa harbour 11 polystome parasites. *Ptychadena oxyrhynchus* has an extensive distribution through Africa and harbours *Polystoma prudhoei* in West Africa. This new parasite differs significantly from *P. prudhoei*. The new species is distinguished by its long body relative to its haptor length, its unique shape and its ratio of marginal hooklets. In a sample of 13 frogs, 53.8% were infected with up to 18 parasites per host (mean intensity 4.86).

Introduction

Ptychadena is a true African frog and belongs to the anuran family Ptychadenidae, with 49 species currently recognized (Frost, 2010). These species are widespread in savanna bushveld where they are especially common in low-lying areas, ranging south from Egypt through subSaharan Africa, excluding the south-western parts of Africa. They are also known from the Seychelles, the Mascarene Islands and Madagascar (Du Preez & Carruthers, 2009). Du Preez & Kok (1992a) stated that *Ptychadena* species fulfil the requirements for the perfect host for a polystome in that they breed in stagnant or slow-flowing water and are opportunistic breeder with extended breeding seasons.

Polystomatids of anurans are represented in Africa by the genera *Eupolystoma* Kaw, 1950, *Metapolystoma* Yamaguti, 1963, *Polystoma* Zeder, 1800 and *Protopolystoma* Bychowsky, 1957. The genus *Polystoma* has a widespread occurrence in all zoogeographical realms except the Australian realm and 31 of the 64 currently known species are known from Africa. Of these, 13 are known from the grass frog genus *Ptychadena*. Polystomes known from *Ptychadena* include *Polystoma aeschlimanni* Bourgat & Murith 1980 from *Pt. pumilio*, *P. assoulinei* Bourgat 1975 from *Pt. tellinii*, *P. baeri* Maeder *et al.* 1970 from *Pt. bibroni*, *P. dawiekoki* Du Preez *et al.* 2002 from *Pt. anchietae*, *P. ebriensis* Maeder, 1973 from *Pt. aequiplicata*, *P. lamottei* Bourgat & Murith 1980 from *Pt. pumilio*, *P. mangenoti* Gallien 1956 from *Pt. superciliaris*, *P. pricei* Vercammen-Grandjean, 1960 from *Ptychadena* sp., *P. prudhoei* Saoud 1967 from *Pt. oxyrhynchus*, *P. sodwanensis* Du Preez & Kok, 1992b from *Pt. porosissima* and *P. togoensis* Bourgat, 1977 from *Pt. hylaea*. Furthermore *Ptychadena* also harbours *Metapolystoma* in that *M. brygoonis* (Euzet & Combes, 1964) is known from *Pt. mascareniensis* in Madagascar and *M. cachani* (Gallien, 1956) is known from *Pt. longirostris*. During further studies of African polystomes, *Pt. oxyrhynchus* has been found infected with an undescribed species of *Polystoma* in the KwaZulu Natal province of South Africa. This paper reports the second polystome species from *Pt. oxyrhynchus,* but the fifteenth record of a polystome from the anuran genus *Ptychadena*.

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Materials and methods

Collection and examination of frogs

Adult frogs were collected in KwaZulu-Natal (South Africa) during December 1990, November 1991, May 1992, October 1992, March 1993 and October 2007. Prior to dissection, frogs were anaesthetized with benzocaine. They were dissected and the complete urinary bladder of infected frogs was removed and transferred to 0.3% saline solution. Following fixation for 2 h in 10% neutral buffered formalin (NBF) while under coverslip pressure, parasites were removed and preserved in 10% NBF. Prior to staining, parasites were rinsed in tap water for 1 h, agitating the Petri dish every 10 min and replacing the water after 30 min, stained overnight in a weak solution of acetocarmine, dehydrated, cleared in xylene and mounted using Canada balsam. Two of the specimens were fixed in 96% ethanol (EtOH) for future molecular studies. Parasite eggs were harvested from infected hosts prior to dissection. Eggs were collected by sieving water through plankton netting with a mesh size of 112 μm and incubated in 10 ml of aged tap water in glass Petri dishes. Oncomiracidia were mounted in ammonium picrate glycerine. Marginal hooklet pairs were numbered one to eight, with pair one being the posteriormost pair closest to the median (see Murith, 1981a). All measurements are in micrometres and are given with the mean followed by the range in parentheses.

Results

Levels of infection

The single host specimen collected during December 1990 was infected with a single parasite and the specimen collected during November 1991 with two parasites. The two immature specimens collected during May 1992 were infected with one and two immature parasites, respectively. The four specimens collected during October 1992 were not infected. One of the two immature specimens collected during March 1993 was infected with 18 immature parasites. Of the three frogs collected in October 2007 two were infected with respectively six and four mature parasites, which were identified as *Polystoma vernoni* n. sp., with a prevalence and mean intensity of 53.8% and 4.9, respectively.

Polystoma vernoni n. sp.

Thirteen sexually mature worms, 21 immature worms. Holotype (NMB P312) and five paratypes (NMB P313–317) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, 9300 Bloemfontein, South Africa; two paratypes (2010.8.27.1-2) in the Parasitic Worms Collection, Natural History Museum, Cromwell Road, London; remaining specimens in the collection of the author.

Type host. *Ptychadena oxyrhynchus*, sexually mature male (AACRG 695) deposited in the Amphibian Collection, African Amphibian Conservation Research Group, North West University, Potchefstroom, South Africa.

Site. Urinary bladder.

Type locality. Vernon Crookes Game Reserve, KwaZulu-Natal, South Africa (30°16′39″S, 30°36′33″E).

Etymology. The specific name refers to the Vernon Crookes Nature Reserve which was named after naturalist Vernon Crookes.

Morphological characteristics. General characteristics of mature, egg-producing parasite (fig. 1) typical of *Polystoma*. Body elongate; total body length 7244

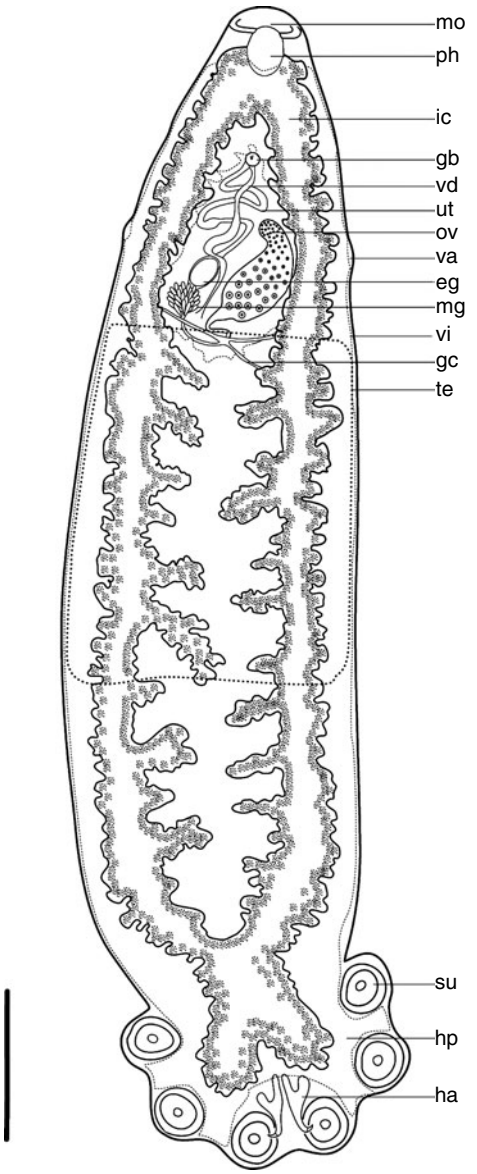

Fig. 1. *Polystoma vernoni* n. sp. Ventral view of holotype; the dotted line indicates the outline of the testis and the dashed line indicates the outline of the vitelline system. Abbreviations: eg, egg; gc, genito-intestinal canal; gb, genital bulb; ha, hamulus; hp, haptor; ic, intestinal caecum; mg,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

(5067–9628); greatest width 1816 (956–2578); haptor length 1407 (1008–2291); haptor width 1796 (1119–2404); haptor length to body length ratio 0.19; six haptoral suckers, mean diameter 356 (255–444); hamulus length to tip of handle 395 (332–459); hamulus length to tip of guard 338 (291–381), handle longer than guard and mean X/Y ratio 1.16 (fig. 2); hamulus hook length 73 (70–77). Mouth subterminal, ventral. False oral sucker 422 (303–531) wide; pharynx length 279 (207–312); pharynx width 223 (182–249). Intestine bifurcate, caeca confluent posteriorly extending into haptor; caeca with 16 (13–23) medial diverticula, 42 (30–54) small lateral diverticula; no pre-haptoral anastomoses.

Testis single and follicular, well developed and extensive, situated post-ovarian, ventral, extending full width of body and halfway towards the haptor (fig. 1). Seminal vesicle prominent. Genital atrium median, ventral; genital bulb 84 (71–90) posterior to intestinal bifurcation, 8–9 genital spines 29 (28–29) long. Ovary sinistral, submedian; anterior in body, ovary length 719 (487–959); ovary width 254 (146–360). Ootype well developed. Genito-intestinal canal present on same side as ovary, joining intestinal caecum posterior to ovary. While observing a live parasite under coverslip pressure it was observed how waste products were rhythmically pumped via the genito-intestinal canal to the intestine. Uterus tubiform and, judging from the length, it may hold about ten eggs; specimens studied had a single egg *in utero* and one specimen had two. Eggs operculate. Egg length 201 (180–219), egg width 127 (116–140). No intrauterine development of eggs observed. Vitellarium distributed throughout the body excluding oral region, region around ovary and uterus, and haptoral suckers (fig. 1).

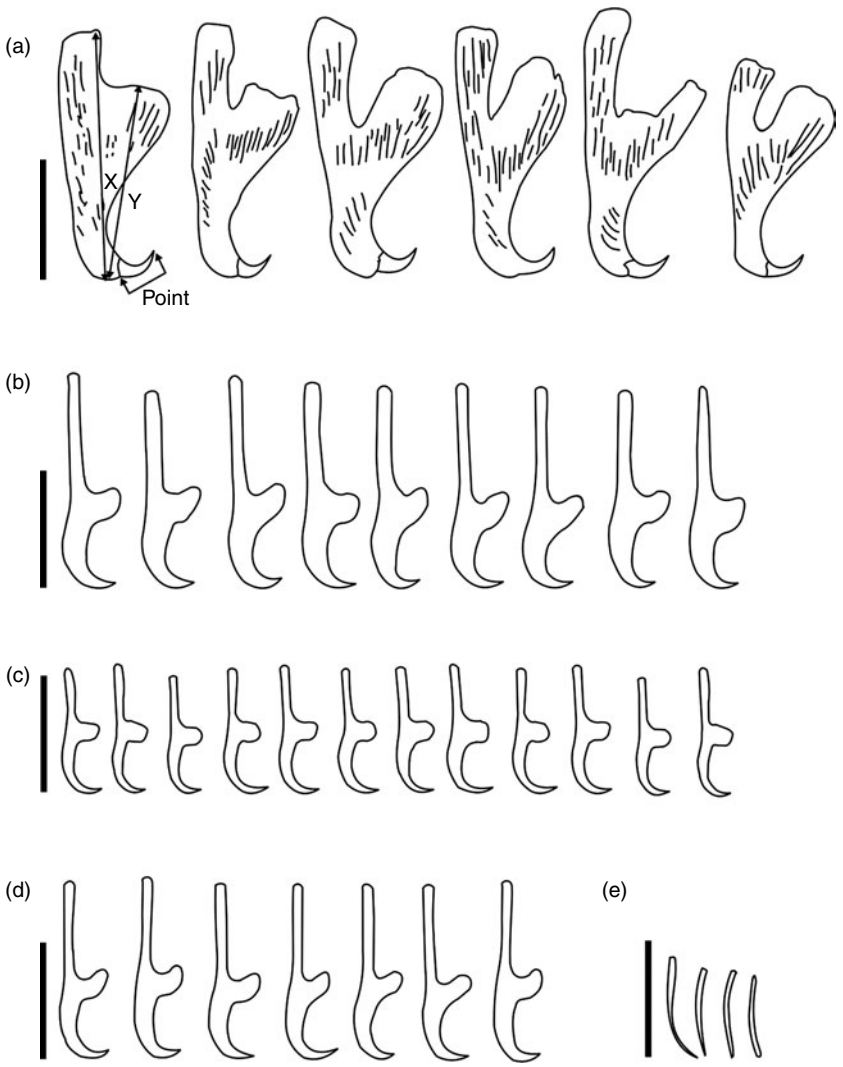

Fig. 2. *Polystoma vernoni* n. sp. (a) Hamuli from holotype and paratypes; (b) marginal hooklets 1 from oncomiracidia hatched from eggs laid by holotype and paratypes; (c) marginal hooklet 2–7; (d) marginal hooklet 8; (e) genital spines. Abbreviations: X, distance from hook to tip of handle; Y, distance from hook to tip of guard. Scale bars: 200 μm (a), 20 μm (b)–(e).

Oncomiracidium. Ciliated oncomiracidium has narrow cylindrical body with circular cup-shaped opisthaptor and resembles a typical polystomatid oncomiracidium. Opisthaptor bears 16 marginal hooklets, which are retained in adult parasites and do not increase in size. Marginal hooklet 1 (postero-medial), 36 (34–38) in length; hooklets 2–7, 21 (20–23) in length and hooklet 8, 31 (30–31) in length (fig. 2). Hamulus primordia 13 (13–14) in length.

Diagnosis

Polystoma vernoni n. sp. differs from all 11 other members of the genus *Polystoma* that infect *Ptychadena* by a combination of characteristics. Whereas most other polystomes have a spindle-shaped body with a haptor length–body length ratio of between 0.2 and 0.3, *P. vernoni* n. sp. has a long cylindrical shape with a mean haptor length–body length ratio of 0.19 (fig. 3). The only other African polystome for which this ratio is below 0.2 is *P. chiromantis* Dupouy & Knoepffler, 1978 from the tree frog *Chiromantis rufescens* (Günther, 1868) with a ratio of 0.18. Although the body measurements for *P. chiromantis* do overlap with that of *P. vernoni* n. sp., their hosts belong to two totally different anuran families, namely Rhacophoridae and Pyxicephalidae and, based on the high degree of host specificity that has been documented for African polystomes (Combes, 1966, 1968; Tinsley, 1973, 1974a; Euzet *et al.*, 1974a, b; Bourgat & Salami-Cadoux, 1976; Combes & Channing, 1979; Murith, 1981a, b, 1982; Kok & Van Wyk, 1986; Kok & Du Preez, 1987; Du Preez & Kok, 1992b, 1993, 1997), it is highly unlikely that the two would be related.

A plot of the products of the total length (a in fig. 4) and the width at the level if the guard (c in fig. 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 fig. 4) of marginal hooklet 1 (Du Preez & Maritz, 2006) reveals that *P. vernoni* n. sp. does not overlap with any other *Polystoma* species. It borders the plots for two other polystomes known from South Africa, namely *P. sodwanensis* and *P. dawiekoki*. *Polystoma vernoni* n. sp. differs from both of these regarding the shape and number of intestinal diverticulae as well as the length of marginal hooklet 1.

Discussion

Ptychadena is one of the most successful anuran genera in Africa and, according to Perret (1979), it is currently undergoing an explosive radiation. Du Preez & Kok (1992a) referred to *Ptychadena* as an extremely suitable host for polystomes as it fulfils ecological and physiological requirements for the completion of the polystome life cycle. With the high degree of host specificity within the genus, *Polystoma* host–parasite co-evolution is a reality, implying that diversification of hosts might be mirrored by diversification of polystome diversity. A study of the origin and evolution of African *Polystoma* conducted by Bentz *et al.* (2001) included five specimens retrieved from *Ptychadena*. Within the robust African clade, two subgroups were identified, among which polystomes of *Ptychadena* fall in a tight group supported with high bootstrap values in both neighbour-joining and maximum-parsimony analyses. Based on experimental infection and cross-infection studies Du Preez & Kok (1997) suggested that not all polystomes exhibit the same degree of host specificity. Bentz *et al.* (2001) proposed an alternative hypothesis, namely that some polystomes may be generalists rather than specialists and that differences in host ecology and physiology might result

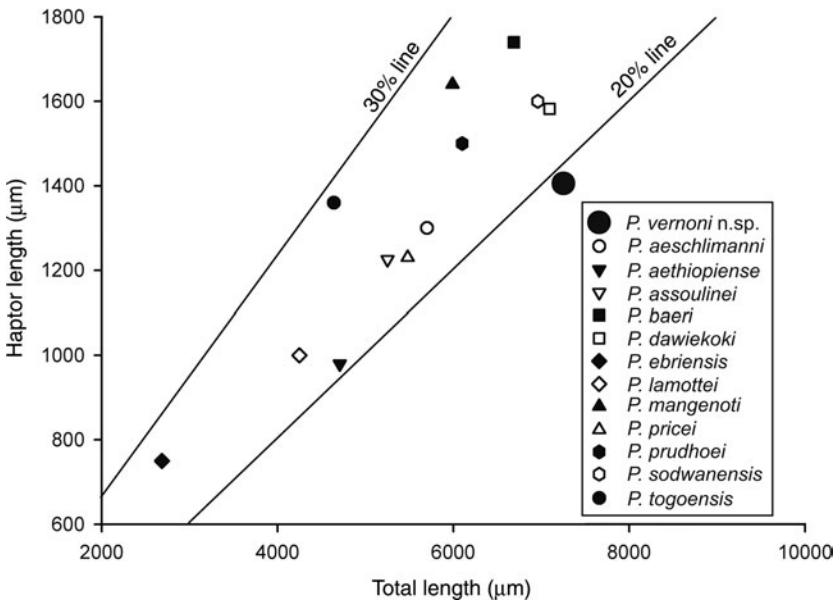

Fig. 3. Scatter diagram of haptor length relative to the total length for *Polystoma vernoni* n. sp. and other *Polystoma* species known from *Ptychadena*. The 20% and 30% lines are indicated to show the relative position for the various species.

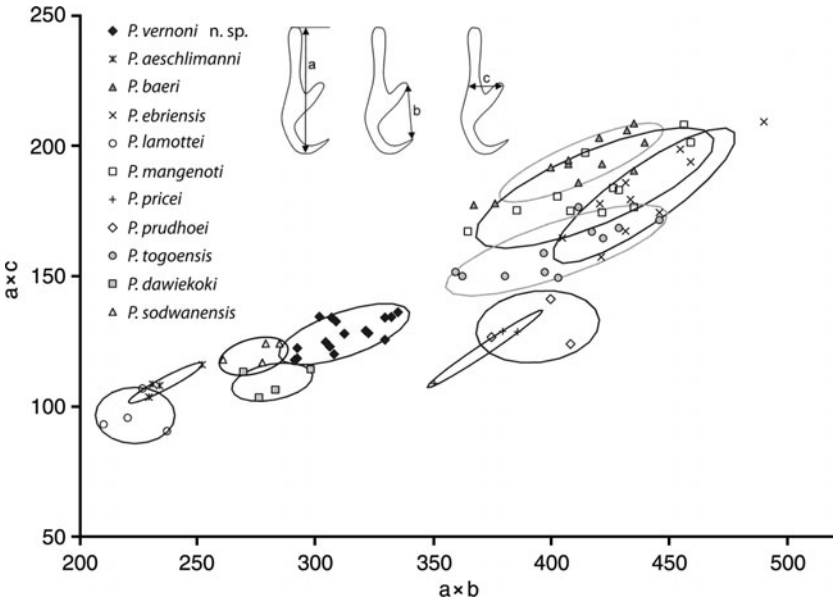

Fig. 4. Scatter diagram of the product of the total length (a) and the width at the level if the guard (c) versus the product of the total length and the length of a tangent between the tip of the blade to the guard (b) of marginal hooklet 1 for *Polystoma vernoni* n. sp. and other *Polystoma* species known from *Ptychadena*.

in either temporal or behavioural isolation precluding polystome speciation, and that the isolation within non-closely related hosts would generate speciation.

An in-depth study of the polystomes infecting *Pt. mascareniensis* might shed further light on host specificity and speciation among polystomes infecting *Ptychadena*. This frog is known to be widespread in Africa and also occurs on Madagascar, in the Seychelles and the Mascarene Islands, including the type locality Reunion. *Metapolystoma brygoonis* has originally been described as *Polystoma brygoonis* parasitizing *Pt. mascareniensis* from Madagascar and was later placed in a separate genus based on internal arrangement of the reproductive organs and the extent of the uterus. *Ptychadena mascareniensis* furthermore has been reported as host for *Polystoma africanum* from Zaire (Vercammen-Grandjean, 1960), Uganda and Kenya (Tinsley, 1974a) and *Polystoma aethiopiense* from Ethiopia (Meskal, 1970). On the basis of this, Bentz *et al.* (2001) postulated that specificity in this case was operating below the species level of the host, which might reveal that the host itself was undergoing speciation. Aisien & Du Preez (2009) redescribed *P. africanum* and concluded that the Ugandan material from *Pt. mascareniensis* was not *P. africanum* but most likely *P. pricei*. After re-examining the types from *P. pricei* Vercammen-Grandjean and *P. aethiopiense* Meskal, Tinsley (1974b) concluded that these two species were conspecific but that the name *P. pricei* takes precedence. This implies that polystomes known from *Pt. mascareniensis* from Africa and the Mascarene Islands include *M. brygoonis* and *P. pricei*. Genetic studies, however, revealed that mitochondrial haplotypes from Madagascar are similar to those from the Seychelles and Mascarene Islands but very different to those in Africa. In addition, those from Africa were very divergent from each other, indicating that up to six different species might be involved (Vences *et al.*, 2004). This could imply that the high degree of host specificity that has been reported for *Polystoma* is still being upheld. However, only an in-depth study of *Pt. mascareniensis* and its polystomes conducted throughout its known range of extension will shed light on this question.

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References

Aisien, M. & Du Preez, L.H. (2009) Redescription of *Polystoma africanum* (Polystomatidae onogenea). *Zootaxa* **2095**, 37–46.

Bentz, S., Leroy, S., Du Preez, L.H., Mariaux, J., Vaucher, C. & Verneau, O. (2001) Origin and evolution of African *Polystoma* (Monogenea: Polystomatidae) assessed by molecular methods. *International Journal for Parasitology* **31**, 697–705.

Bourgat, R. & Salami-Cadoux, M.L. (1976) Recherches expérimentales sur la spécificité parasitaire des polystomes de *Bufo regularis*, *Rana galamensis* et *Hylarana albolabris* au Togo. *Revue des Sciences Médicales et Biologiques der Togo* **1**, 41–42.

Combes, C. (1966) Recherches expérimentales sur la spécificité parasitaire des polystomes de *Rana temporaria* et de *Pelobates cultripes* (Cuv.). *Bulletin de la Société Zoologique de France* **91**, 439–444.

Combes, C. (1968) Biologie, écologie des cycles et biogéographie de digènes et monogènes d'amphibiens dans l'est des Pyrénées. *Mémoire du Muséum National d'Histoire Naturelle Série A Zoologie* **51**, 1–195.

Combes, C. & Channing, A. (1979) Polystomatidae (Monogenea) d'amphibiens d'Afrique du Sud: *Polystoma natalensis* n. sp., Parasite de *Strongylopus grayii* (Smith 1849). *Vie Milieu, Série C* **28–29**, 61–68.

Du Preez, L.H. & Carruthers, V.C. (2009) *A complete guide to the frogs of southern Africa*. 488 pp. Cape Town, Struik Nature.

Du Preez, L.H. & Kok, D.J. (1992a) The frog genus *Ptychadena* as host for polystomatid (Monogenea) parasites in Africa. *African Journal of Herpetology* **40**, 47–50.

Du Preez, L.H. & Kok, D.J. (1992b) Syntopic occurrence of new species of *Polystoma* and *Metapolystoma* (Monogenea: Polystomatidae) in *Ptychadena porosissima* in South Africa. *Systematic Parasitology* **22**, 141–150.

Du Preez, L.H. & Kok, D.J. (1993) Polystomatidae (Monogenea) of Anura in southern Africa: *Polystoma testimagna* n. sp. parasitic in *Strongylopus f. fasciatus* (Smith, 1849). *Systematic Parasitology* **25**, 213–219.

Du Preez, L.H. & Kok, D.J. (1997) Supporting evidence of host-specificity among southern African polystomes (Polystomatidae: Monogenea). *Parasitology Research* **83**, 558–562.

Du Preez, L.H. & Maritz, M.F. (2006) Demonstrating morphometric protocols using polystome marginal hooklet measurements. *Systematic Parasitology* **63**, 1–15.

Euzet, L., Bourgat, R. & Salami-Cadoux, M.-L. (1974a) *Polystoma galamensis* (Monogenea) parasite de *Rana galamensis* Duméril et Bibron, 1841, au Togo. *Annales de Parasitologie Humaine et Comparée* **49**, 63–68.

Euzet, L., Combes, C. & Knoepffler, L.Ph. (1974b) Parasites d'amphibiens de la République Centrafricaine. Polystomatidae (Monogenea). *Vie Milieu, Série C* **24**, 141–150.

Frost, D.R. (2010) Amphibian species of the world: an online reference. Version 5.4 (8 April 2010). American Museum of Natural History, New York, USA. Electronic Database available at http://research.amnh.org/vz/herpetology/amphibia/ (accessed 1 September 2010).

Kok, D.J. & Du Preez, L.H. (1987) *Polystoma australis* (Monogenea): life cycle studies in experimental and natural infections of normal and substitute hosts. *Journal of Zoology (London)* **212**, 235–243.

Kok, D.J. & Van Wyk, J.H. (1986) Polystomatidae (Monogenea) parasitic in the anuran genus *Kassina* in South Africa. *South African Journal of Zoology* **21**, 189–196.

Meskal, F.S. (1970) Trematodes of anurans from Ethiopia. *Årbok for Universitetet i Bergen, Matematisk-Naturvitenskapelig Serie* **1**, 1–73.

Murith, D. (1981a) Contribution à l'étude de la biologie du développement des polystomes (Monogenea) parasites d'amphibiens anoures de basse Côte-d'Ivoire. *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **104**, 5–33.

Murith, D. (1981b) Contribution à l'étude de la systématique des polystomes (Monogènes, Polystomatidae) parasites d'amphibiens anoures de basse Côte-d'Ivoire. *Revue Suisse de Zoologie* **88**, 475–503.

Murith, D. (1982) Etude *in vivo* de la nature des relations hôte-parasite dans le complexe amphibien-polystome (Monogenea). *Revue Suisse de Zoologie* **89**, 957–965.

Perret, J.L. (1979) Remarques et mises au point sur quelques espéces de *Ptychadena* (Amphibia Ranidae). *Bulletin de la Société Neuchatêloise des Sciences Naturelles* **102**, 5–21.

Tinsley, R.C. (1973) Observation on Polystomatidae (Monogenoidea) from East Africa with a description of *Polystoma makereri* n. sp. *Zeitschrift für Parasitenkunde* **42**, 251–263.

Tinsley, R.C. (1974a) Observations on *Polystoma africanum* Szidat with a review of the inter-relationships of *Polystoma* species in Africa. *Journal of Natural History* **8**, 355–367.

Tinsley, R.C. (1974b) A redescription of *Polystoma pricei* Vercammen-Grandjean, 1960 (Monogenoidea: Polystomatidae). *Revue de Zoologie Africaine* **88**, 65–174.

Vences, M., Kossuch, J., Rödel, M.-O., Lötters, S., Channing, A., Glaw, F. & Böhme, W. (2004) Phylogeography of *Ptychadena mascareniensis* suggests transoceanic dispersal in a widespread African-Malagasy frog lineage. *Journal of Biogeography* **31**, 593–601.

Vercammen-Grandjean, P.H. (1960) Les trématodes du Lac Kivu Sud (Vernes). *Sciences Zoologiques* **5**, 1–171.