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(Smith)**

Louis H. du Preez

Richard C. Tinsley

Rafael O. de Sá

University of Richmond, rdesa@richmond.edu

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Polystomatidae (Monogenea) of Southern African Anura: *Eupolystoma vanasi* n. sp. parasitic in *Schismaderma carens* (Smith)

Louis H. du Preez¹, Richard C. Tinsley² & Rafael de Sá³

¹*School of Environmental Sciences and Development, Potchefstroom University for CHE, Private Bag X6001, Potchefstroom 2520, South Africa*

²*School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK*

³*Department of Biology, University of Richmond, Richmond, VA23173, USA*

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Abstract

Eupolystoma vanasi is described as a new species of the Polystomatidae parasitic in the urinary bladder of *Schismaderma carens* in Northern Province and KwaZulu-Natal Province, South Africa. This is the third *Eupolystoma* species described from Africa and the first polystomatid from *Schismaderma*, an anuran genus that is primitive with respect to the other African bufonids in which *Eupolystoma* has been recorded. The species is distinguished by body size (this is the largest *Eupolystoma* known; mean length of adults 6 mm), by genital spine number (4 in comparison with 6–9 in other species), marginal hooklet length (greater than in other African species), and by the small size of the ovary and testis. In a sample of 27 toads, 37% were infected with up to 130 parasites per host (mean intensity 37). Worm burdens of this magnitude are exceptional amongst polystomatids in general but are characteristic of *Eupolystoma*, where there may be repeated re-infection of adult hosts and, uniquely, a direct, internal cycle of auto-infection.

Introduction

Polystomatids of anurans are represented in Africa and in South Africa by the genera *Eupolystoma* Kaw, 1950, *Metapolystoma* Combes, 1976, *Polystoma* Zeder, 1800 and *Protopolystoma* Bychowsky, 1957. *Eupolystoma* is known only from Africa and India and is presently represented by four species: *E. alluaudi* (de Beauchamp, 1913) from Central, East and West Africa, *E. anterorchis* Tinsley, 1978 from the Cape, South Africa, and *E. chauhani* Pandey, 1969 and *E. rajai* Kaw, 1950, both from India.

The first member of the genus was originally named *Polystomum alluaudi* by de Beauchamp (1913), but taxonomic assessment of both the parasite and its hosts has been subject to repeated revision. Ozaki (1935) placed *P. alluaudi* in *Parapolystoma*, and Yamaguti (1963) then erected *Beauchampia* for this species, but it changed genus a fourth time to become *Eupolystoma alluaudi* (see Euzet & Combes, 1967). The original description of *E. alluaudi* was based on a single specimen recovered from either

Bufo regularis Reuss or *B. taitanus* Peters in Kenya (see Bourgat, Morere & Kulo, 1983). These *Bufo* species were later re-identified by Grandison (1972), Tandy (1972) and Hulselmans (1977) and assigned to *B. kerinyagae* Keith and *B. loennbergi* Andersson, respectively. Despite these taxonomic re-assignments, the type-host of *E. alluaudi* remains uncertain. Subsequent studies have produced widely-distributed parasite records from a series of host species. Beverley-Burton (1962) described representatives under the name *Parapolystoma alluaudi* from Zimbabwe based on two specimens found in *Rana adspersa* (Tschudi) and one specimen in *B. regularis*. These hosts were subsequently transferred to *Pyxicephalus adspersus* Tschudi and *B. gutturalis* Power (see Frost, 1985). Euzet & Combes (1967) recorded *E. alluaudi* from a *Bufo* species in northern Tchad. Various authors, including Bourgat & Salami-Cadoux (1974), Combes, Bourgat & Salami-Cadoux (1973a,b, 1976) and Salami-Cadoux (1975, 1979) carried out studies on *E. alluaudi* in *B. regularis* in Togo. Bwathondi (1977) reported

E. alluaudi in *B. regularis* in Tanzania, and Tinsley (1978a) recorded material tentatively assigned to *E. alluaudi* in *B. regularis* in the Cameroon and Togo and in *Nectophrynoides malcolmi* Grandison in Ethiopia. Bourgat, Morere & Kulo (1983) found *E. alluaudi* in *B. pentoni* Andersson in Togo and in *B. xeros* Tandy, Tandy, Keith & Duff-Mackay in Senegal. *E. alluaudi* is thus reported from at least seven different host species occupying a very wide geographical range in Africa, from Senegal in the west to Ethiopia in the east, and from Tchad in the north to Zimbabwe in the south.

A second species of the genus in Africa was described by Tinsley (1978a), *E. anterorchis* from *Bufo pardalis* Hewitt. This host species was formerly known from two disjunct populations in South Africa, in the Western Cape and Eastern Cape respectively (Passmore & Carruthers, 1995). Poynton & Lambiris (1998) recognised the taxonomic separation of these populations and retained the name *B. pardalis* for the Eastern Cape form, while that from the Western Cape, the host for *E. anterorchis*, was assigned to *B. pantherinus* Smith.

During present studies of South African polystomatids, *Schismaderma carens* (Smith) has been found infected with a species of *Eupolystoma*. Parasite material was obtained during field investigations and from preserved museum collections of the host. This paper provides a formal description of these representatives.

Materials and methods

Parasites were removed from the urinary bladders of adult *Schismaderma carens* collected at Warmbaths, South Africa during January 1992, February 1994, November 1995, January 1997 and January 2000. Toads were anaesthetised with Benzocaine and, at dissection, the urinary bladder, urinary ducts and kidneys were removed and examined in 0.3% saline solution with a stereo binocular microscope. To trigger egg-laying by gravid adult parasites, the saline was replaced with dechlorinated tapwater.

Following fixation for 24 h in 10% neutral buffered formalin 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 h, embedded in paraffin wax, sectioned at 6 μ m and stained with Harris' haematoxylin and eosin.

Further parasites were obtained from the urinary bladder of a preserved specimen of *S. carens* (KU 195745) in the collection of the Museum of Natural History, University of Kansas, Lawrence, USA (one of a series of 6 toads collected by W.E. Duellman, L. Trueb and D.T. Duellman in KwaZulu-Natal Province, South Africa, 10 January 1984).

Actively swimming oncomiracidia that hatched from eggs laid by parasites collected during January 1997 were used in infection experiments. Four *S. carens* and one each of *Bufo garipeensis*, *B. gutturalis* and *Breviceps mossambicus*, maintained in the laboratory for more than 30 days after capture, were placed individually in 600 ml plastic bottles with water 15 mm deep. These species show no systematic relationship to *S. carens* but were representative of anurans occupying the same habitat and therefore potentially exposed to the same infection. The 30 day pre-exposure maintenance period ensured that experimental invasion could be distinguished from pre-existing burdens. Ten oncomiracidia were transferred into each bottle. Toads were dissected after 14 days and the urinary bladder and renal system examined for the presence of parasites (the kidneys were finely teased apart with dissecting needles).

To demonstrate the distribution of tegumental ciliated cells, active oncomiracidia were pipetted into 1% ice-cold silver nitrate and exposed to sunlight for 1-5 minutes before rinsing in distilled water and mounting under a coverslip in glycerine.

The ratio of ovary to body size was calculated from published measurements for *Eupolystoma anterorchis* (see Tinsley, 1978a) and *E. rajai* (see Kaw, 1950) and from published diagrams of *E. alluaudi* (see Salami-Cadoux, 1979) and *E. chauhani* (see Pandey, 1969).

Observations

The host

Schismaderma is a monotypic genus of the Bufonidae. *S. carens* has a maximum body length of 86 mm and a more slender build than members of *Bufo*; it is more lively and mobile than most other toads and seldom mixes with other species. Adults favour deep pools or dams for breeding, which is explosive, with a succession of mating assemblies during early spring and mid-summer. Tadpoles congregate in compact schools in deep water (Du Preez, 1996). *S. carens* is widespread in savannas and is distributed from the

northern Cape Province, northern Free State Province and southern Kwazulu-Natal Province in South Africa northwards to southern Democratic Republic of the Congo and Tanzania (Poynton & Broadley, 1988).

Levels of infection

Two of 6 *S. carens* collected during January 1992 were infected with respectively 1 and 5 immature parasites. In February 1994, 3 toads were examined but none was infected. Of 5 specimens collected in November 1995, 3 were infected with, respectively, 5 (all immature), 99 (89 mature) and 130 (86 mature) parasites. In January 1997, 4 of 8 toads examined were infected with, respectively, 4 (1 mature), 5 (3 mature), 30 (9 mature) and 33 (10 mature) parasites; one had a single parasite in the rectum. One of 5 specimens collected in January 2000 was infected with 58 parasites: only one was mature while the remainder could be divided into 5 size classes probably reflecting different infection events. For the total sample (n=27), prevalence was 37% and mean intensity 37 worms/ host. None of the hosts examined had parasites in the kidneys.

A single infected host specimen from the Kansas Museum of Natural History collection carried 69 (56 mature) worms in the bladder.

Eupolystoma vanasi n. sp.

Specimens studied

Thirty-eight sexually mature worms and 25 oncomiracidia. Holotype (NMB P262) and 8 paratypes (NMB P263-270) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein 9300, South Africa; 4 paratypes (2002.5.7.3-6) in the Parasitic Worms Collection, The Natural History Museum, London; remaining specimens in the collections of the authors.

Type-host: *Schismaderma carens* (Smith) sexually mature female (NMB A 6315) deposited in the Amphibian Collection, National Museum, Bloemfontein 9300, South Africa.

Type-locality: Smallholding on the outskirts of Warmbaths in the Northern Province, South Africa (24°53' S, 28°16' E).

Other records: Infected specimen of *S. carens* (KU 195745) in the Kansas Museum of Natural History collected 17 km north of Mtubatuba, KwaZulu-Natal Province, South Africa.

Site: Urinary bladder.

Etymology: The specific name *vanasi* acknowledges Professor J.G. van As, Department of Zoology & Entomology, University of the Free State, who collected the initial infected host specimens from the type-locality.

Description (Figures 1–3)

Based on egg-producing adults; measurements (in micrometres) are means from 38 specimens. Larval sclerite characters based on oncomiracidia hatched from eggs released from uterus of holotype, paratypes and other specimens.

Adult

General characteristics of mature, egg-producing parasite (Figure 1) typical of *Eupolystoma*. Body elongate, total length 5,977 (3,775-7,733), greatest width 2,356 (1,505-3,275), haptor length 836 (564-1,128), width 1,447 (1,076-1,924); haptor length to body length ratio 0.14 (0.12-0.18); haptoral suckers 6, mean diameter 226 (160-285); hamuli absent. Mouth subterminal, ventral. Oral sucker 222 (160-290) wide; pharynx length 206 (165-270), width 166 (131-230). Intestine bifurcate with small lateral diverticula but no median branches; caeca confluent posteriorly, extending into haptor, without pre-haptoral anastomoses.

Testis diffuse consisting of several follicles, ventrally, medially and posteriorly, restricted to small area just anterior to haptoral anastomosis (Figure 1). Seminal vesicle present. Genital atrium median, ventral, posterior to intestinal bifurcation; genital bulb with 4 spines 31.8 (29.5-32.5) in length. Small ovary sinistral, in posterior 20% of intercaecal field, length 250 (184-470), width 121 (100-150). Genito-intestinal canal prominent, on same side as ovary, joining intestinal caecum posterior to ovary. Uterus extends to hindbody, and then anteriorly to genital atrium, occupying most of intercaecal space lateral and anterior to ovary, contains up to 81 eggs; egg capsule length 129 (136-150), width 83 (79-89); many eggs fully embryonated. Eggs not operculate. Vitellarium forming 2 discrete lateral fields of follicles, lateral to intestine, in posterior half of body (Figure 1).

Oncomiracidium

Ciliated larva with narrow cylindrical body and circular cup-shaped opisthaptor bearing 16 marginal hooklets of equal length, 34.9 (33.5-36.0); ratio of marginal

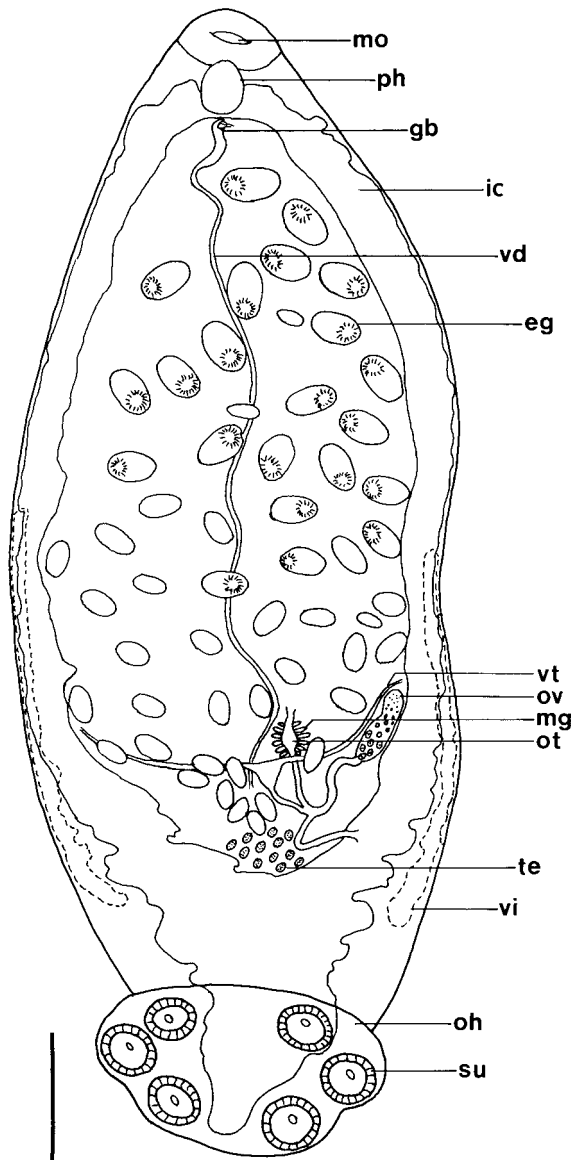


Figure 1. *Eupolystoma vanasi* n. sp. Ventral view of holotype; dotted line indicates the outline of the vitelline system. Abbreviations: eg, egg; gb, genital bulb; ic, intestinal caecum; mg, Mehlis' gland; mo, mouth; oh, opisthaptor; ot, oötype; ov, ovary; ph, pharynx; su, sucker; te, testis; vd, vas deferens; vi, vitelline follicles; vt, vitelline duct. Scale-bar: 0.5 mm.

hook total length to handle length 1.62 (1.55-1.73) (Figure 2). Hamulus primordia absent.

Tegumental ciliated cells distributed in consistent pattern, conforming to 5 cell groups recognised for polystomatid oncomiracidia by Tinsley (1981). Total cell number 55, arranged as: apical group, 1 cell; cephalic group, 2×12 cells, dorsal, lateral and ventral; medioanterior group, 2×3 cells, ventral; mediopos-

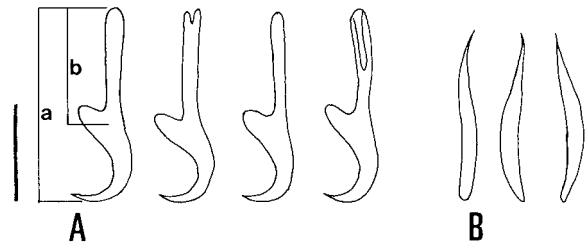


Figure 2. *Eupolystoma vanasi* n. sp. A, marginal hooklets I from holotype and paratypes; B, genital spines drawn from paratypes. Abbreviations: a, total length of marginal hooklet I; b, handle length of marginal hooklet measured from tip to centre of guard base (see Muriith, 1981). Scale-bars: 20 μ m.

terior group, 2×6 cells, dorsal, lateral and ventral; haptor group, 2×6 cells, dorsal, lateral and ventral.

Differential diagnosis

Eupolystoma vanasi n. sp. differs from other members of the genus by a combination of characters (Table 1). It is the largest of the known species: the maximum length recorded for previously-described species of *Eupolystoma* is around 4,700 for both *E. anterorchis* and *E. alluaudi*, while *E. vanasi* n. sp. reaches a length of 7,733. It has only four genital spines; this separates it from the other species: *E. alluaudi* has eight to nine genital spines, *E. anterorchis* six to eight, *E. chauhani* six and *E. rajai* eight. Marginal hooklets are larger in *E. vanasi* n. sp. (34-36) than in *E. alluaudi* (17-23) and *E. anterorchis* (26); this distinction is demonstrated by a plot of hooklet total length against handle length in the African species (Figure 3).

The reproductive organs of egg-producing *E. vanasi* n. sp. are relatively small: ovary length as a percentage of body length is 3.2% for *E. vanasi* n. sp. compared with 10.4% for *E. alluaudi*, 10.9% for *E. anterorchis*, 9.3% for *E. rajai* and c. 8% for *E. chauhani*. The testis follicles are small, post-ovarian and median, not reaching the outer perimeter of the gut as in the other known African species. The distribution of the vitellarium is confined to two thin lateral fields in the posterior half of the body. This corresponds with the situation in *E. alluaudi*, but differs from the more extensive lateral fields seen in *E. anterorchis* and *E. chauhani* and the distribution throughout the body as reported for *E. rajai*.

The characters established for *E. vanasi* n. sp. require minor amendment to the generic diagnosis of *Eupolystoma* revised by Tinsley (1978a): genital spine number ranges from four to nine within the genus.

Table 1. Characteristics of *Eupolystoma* spp.

	<i>E. vanasi</i> n. sp.	<i>E. alluaudi</i>	<i>E. alluaudi</i>	<i>E. alluaudi</i>	<i>E. alluaudi</i>	<i>E. anterorhithis</i>	<i>E. rejai</i>	<i>E. chauthani</i>
Reference	Present study	Salami-Cadoux, 1979	Beverley-Burton, 1962	Bourgat, Morere & Kulo, 1983	Bourgat, Morere & Kulo, 1983	Tinsley, 1978a	Kaw, 1950	Pandey, 1969
Host	<i>Schismaderma carens</i>	<i>Bufo regularis</i>	<i>Bufo gutturalis</i> <i>Pyxicephalus adspersus</i>	<i>Bufo xeros</i>	<i>Bufo pentoni</i>	<i>Bufo pantherinus</i>	<i>Rana</i> sp.	<i>Bufo</i> sp.
Country of origin	South Africa	Togo	Zimbabwe	Senegal	Togo	South Africa	India	India
Number of specimens	38	200	3	21	121	20	6	2
Body length	5,977 (3,775-7,733)	(1,530-3,100)	(2,700-3,850)	2,900 (1,500-3,500)	(4,000-4,730)	4,320 (3,390-4,760)	(2,400-3,200)	(3,240-4,250)
Maximum width	2356 (1505-3275)	(610-1,520)	(1,030-1,850)	1,300 (700-1,700)		1,720 (1,450-1,860)	(890-1,400)	(1,200-1,750)
Haptor length	836 (564-1,128)	(310-460)	(560-650)			790 (550-830)	(580-660)	(1,210-1,350)
Haptor width	1,447 (1,076-1,924)	(460-1,040)	(720-920)	800 (500-1,000)	(1,200-1,400)	1,310 (1,070-1,330)	(940-1,240)	(1,320-1,450)
Sucker diameter	226 (160-285)	(140-210)		216 (140-239)	(330-381)	260 (220-270)	(150-230)	
Length marginal hooklet	34.9 (33.5-36.0)	(17-23)				26	(22-27)	
Oral sucker width	222 (160-290)					440 (360-480)	(280-350)	(210-300)
Pharynx length	206 (165-270)					230 (200-240)	(120-170)	(80-90)
Pharynx width	166 (131-230)					210 (170-210)	150	(60-70)
Ovary length	250 (184-470)		(140-190)			470 (360-560)	280	
Ovary width	121 (100-150)					120 (90-160)	80	
Number of genital spines	4	(8-9)				8 (6-8)	8	6
Length of genital spines	31.8 (29.5-32.5)	20				24	27	
Egg length	129 (136-150)	(125-160)		140	(121-162)	(135-185)	(157-170)	(70-80)
Egg width	83 (79-89)	(48-80)		70	(70-101)	(80-105)	(81-99)	(20-30)

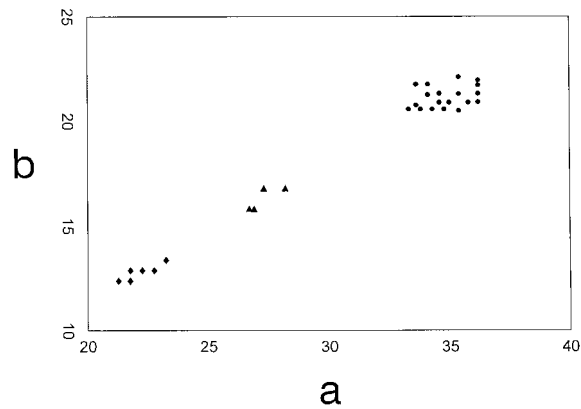


Figure 3. Scatter diagram of total length (a) against handle length (b) of marginal hooklets I for *Eupolystoma vanasi* n. sp. (black dots), *E. alluaudi* (black diamonds) and *E. anterorchis* (black triangles). Measurements for *E. alluaudi* taken from parasites found in *Bufo regularis* from Lomé (Togo) (preparation no. 978.173, Museum Geneva Collection); measurements for *E. anterorchis* taken from material in collection of Tinsley.

Experimental infection and host-specificity

Immediately after transfer of gravid parasites from the urinary bladder to water, fully embryonated eggs were released *en masse* and actively swimming oncomiracidia began to hatch within seconds. Some oncomiracidia lost their cilia and ability to swim shortly after hatching. Hatched oncomiracidia were also observed *in utero*; this was probably caused by water entering the uterus.

Three of the four *S. carens* used in experimental infections were found infected 14 days p.i. (at 20–25 °C) with two, six and six worms in the urinary bladder; these had a body length of about 850 μm with one pair of suckers developed. In the cross-infection trial, no parasites were recovered from the *Bufo garipeensis*, *B. gutturalis* and *Breviceps mossambicus* after the same 14 day post-exposure period.

Discussion

Eupolystoma is characterised by a lack of features whose variation has been typically employed in polystomatid taxonomy, including the absence of hamuli and of intestinal branching (Tinsley, 1978a). This is likely to obscure species differentiation. Nevertheless, *E. vanasi* n. sp. is clearly distinguished from other known representatives of the genus.

The ratio of handle length to total length of the marginal hooklets separates *E. vanasi* n. sp. from other

known African species. The value of this character emphasises the need for comprehensive documentation of marginal hooklet morphology throughout the distribution of *Eupolystoma* species (and indeed in other polystomatids). The marginal hooklets of *Eupolystoma* species, are all of the same size and shape. This contrasts with the usual situation in *Polystoma* species, where the posteriormost pair of marginal hooklets is significantly enlarged and more robust (see, for instance, Tinsley, 1973, 1974). Although, the genera *Polystoma* and *Eupolystoma* appear closely related, this difference in their marginal hooklet organisation may reflect a fundamental division between the genera. There is also a small difference in the distribution of ciliated cells in the oncomiracidial tegument: in the cephalic group, the three mid-dorsal cells form a triad on each side of the midline in *Polystoma* and *Metapolystoma*; however, in the African *Eupolystoma* species (now including *E. vanasi* n. sp.) these cells form a single transverse row across the mid-line (Tinsley, 1978b). Combes et al. (1978) also noted that, in comparison with *Polystoma* species, the medio-anterior cells of *E. alluaudi* form a more angular group adjoining the posterior cells of the cephalic group. It is likely that oncomiracidial cell patterns represent a very sensitive indicator of phylogenetic affinities within polystomatid monogeneans (Tinsley, 1981).

The majority of records of *E. alluaudi* are based on small sample sizes, even single specimens, from a given host species. These have permitted little indication of geographical or host-related variation. Tinsley (1978a) tentatively assigned representatives of *Eupolystoma* from *Nectophrynoides* to the type-species, but the material, from preserved hosts in museum collections, did not permit detailed assessment of all taxonomic characters. There is also insufficient detail in most of the other records of *E. alluaudi* to assess species status. With the increasing knowledge of the complexity of polystomatid speciation (see, for instance, Tinsley & Jackson, 1998), it now seems more appropriate to regard these isolated records from non-type hosts as *species inquirendae*, pending future analysis of larger population samples.

The relationships of the African and Indian species of *Eupolystoma* are entirely unresolved. A congeneric affinity could be supported by the wider faunal relationships between the continents and could point to an ancient origin for the group. On the other hand, the Indian species are poorly documented with apparent errors in important characters, including the description of 18 marginal hooklets in *E. chauhani* (see Pandey,

1969; Tinsley, 1978a). The morphological similarity, based on a highly specialised reproductive system within a relatively simplified body plan, could reflect convergent evolution between distinct polystomatid clades. Tinsley (1983) considered that a series of different body plans within the anuran polystomatids, all characterised by a major extension of the uterus that facilitates deposition of immediately-infective larvae, may have evolved independently as an adaptation for instantaneous transmission within breeding aggregations of adult anuran hosts. The superficial similarity between African and Indian polystomatids could therefore reflect independent adaptation to equivalent life-cycle constraints rather than close affinity. Molecular studies of relationships would be invaluable for distinguishing these alternatives.

The great majority of anuran polystomatids are characterised by very low prevalence and intensity of infection, predominantly fewer than five worms/host (Tinsley, 1993). Records for African *Eupolystoma* are clearly distinct with maximum intensities around 2,000 worms/host for both *E. alluaudi* and *E. anterorchis* (see Combes et al., 1973b; Tinsley, 1978b, 1993). Two factors are involved. Firstly, there may be repeated infection of adult hosts at each mating assembly (contrasting with *Polystoma* species, where there is typically only a single period of invasion, during the tadpole stage). Secondly, there may be an internal cycle of autoinfection in which infective larvae attach to the bladder wall immediately after release from the parent parasite, without leaving the host. Combes et al. (1973b) and Fournier & Combes (1979) have demonstrated that, uniquely, there appears to be a population regulation mechanism, whereby parasites produce two different types of oncomiracidia, one ciliated and destined for release into the external environment for transmission to new hosts and the other unciliated and destined to remain within the original host to boost existing infrapopulations. Field and laboratory observations on *E. vanasi* n. sp. suggest that the same processes may also occur in this new representative. Intensities up to 130 worms/host recorded in the field sample of 27 toads examined in this study (mean 37 worms/host) are entirely atypical of *Polystoma* but typical of *Eupolystoma* populations. The present records of up to five different size classes of *E. vanasi* n. sp. within host individuals, comprising both immature and mature worms, confirm repeated infection of adult toads. The finding that some oncomiracidia shed their cilia soon after hatching suggests the potential for an internal cycle of auto-infection as in

E. alluaudi. However, Fournier & Combes (1979) demonstrated that the larvae of *E. alluaudi* destined for the internal cycle never developed ciliated cells during embryo development. It is possible, therefore, that the initial development but rapid post-hatching loss of ciliated cells in *E. vanasi* n. sp. indicates an earlier stage in the evolution of this unique adaptation: it may be that the life cycle route (external or internal infection) is not pre-determined in the embryo of *E. vanasi*, as in *E. alluaudi*, but at the point of hatching. Further experimental life-cycle studies are now required. Despite the potential in many polystomatid life-cycles for the build up of very high infection levels, there is evidence that worm burdens in anurans are highly regulated (Tinsley, 1995, 1999). Experimental confirmation that this control is mediated by a host immune response has now been provided for *Protopolystoma xenopodis* (Price, 1943) in *Xenopus laevis* (see Jackson & Tinsley, 2001). Development of acquired immunity might explain the paradox that, although *Eupolystoma* species are potentially capable of a massive internal increase in infrapopulations, the majority of field records, including those in this study, comprise relatively few individuals: half of the toads found infected by *E. vanasi* n. sp. harboured only one to five worms.

The majority of field and experimental evidence suggests that anuran polystomatids are strictly host specific (Tinsley, 1981; Du Preez & Kok, 1997; Tinsley & Jackson, 1998). The recording of *E. alluaudi* from at least seven anuran species representing four genera and two families is therefore anomalous. Bourgat et al. (1983) referred to this apparent lack of host-specificity in the case of *E. alluaudi*; support was provided by Salami-Cadoux (1979) who experimentally infected *Bufo maculatus* with *E. alluaudi* usually infecting *B. regularis* in Togo. The present pilot studies of host-specificity of *E. vanasi* n. sp., involving cross-infection of three sympatric anuran species, were unsuccessful (whereas controls employing exposure of *S. carens* under the same conditions led to infection). This outcome, on its own, is of limited significance, but it highlights the need for critical, experimental tests. Du Preez & Kok (1997) postulated that in *Polystoma* species the basis of host-specificity lies with larval recognition of the host species tadpole. In *Eupolystoma*, where the oncomiracidium infects the adult host rather than the tadpole, host-specificity may be reduced. However, species of *Protopolystoma* and *Pseudodiplorchis* also infect only post-metamorphic stages of their respective hosts (and show no attrac-

tion to the tadpoles), but experimental studies show that these too are highly host species specific; in these cases, rejection of parasites in foreign hosts occurs in the sites of juvenile development, in the kidneys and lungs respectively (Tinsley, 1995; Jackson & Tinsley, 1998; Tinsley & Jackson, 1998). More comprehensive studies of host specificity are now required to establish the species diversity of *Eupolystoma*, especially within the poorly-defined '*E. alluaudi*'.

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