

# A new species of *Polystoma* (Monogenea) parasitic in rhacophorid amphibians in Meghalaya, India

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Received April 22, 1991

## Summary

A new species of polystomatid monogenean, *Polystoma indicum* is described from *Rhacophorus nigropalmatus* in Meghalaya. This also forms the first record of the genus from India. Hamuli with entire base, opisthaptor considerably small compared to body dimensions, intestinal anastomoses few and random and numerous eggs in the uterus are the distinguishing features of the new species.

**Key words:** *Polystoma*; Monogenea; amphibian hosts; new species; India

## Introduction

The anuran Polystomatidae in India are represented by a single genus *Eupolystoma* Kaw, 1950 and the species *E. rajai* Kaw, 1950 and *E. chauhani* Pandey, 1969, the latter described from the anurans of the genera *Rana* and *Bufo*, respectively. The genus *Polystoma* Zeder, 1800 which is represented by more than 45 species is widespread over several zoogeographical regions incorporating both the old and new world (Combes, 1976). However, the sole representative of the genus in the Oriental region is *P. hakgalense* Crusz et Ching, 1975 from *Rhacophorus cruciger* eques in Ceylon.

During an exploratory survey of the helminth fauna of amphibians in Meghalaya (North-East India), the rhacophorid frogs were found to harbour a polystomatid monogenean which appears new to science. The present communication deals with the description of this new species of *Polystoma* which also forms the first record of the occurrence of the genus in the Indian Subcontinent.

## Material and Methods

Eight specimens of the monogenean were recovered from the urinary bladder and/or ureters of 7 out of 17 *Rhacophorus nigropalmatus* Boulenger during 1981 and 1982. The host specimens were collected during the spring months of March and April from Cherrapunji (25°18'N and 91°46'E. at 1337 m ASL) which is about 45 km south-west of Shillong, the state capital of Megha-

laya. The collections were made from temporary rain pools which are utilised by these anurans for breeding purposes.

The worms were fixed in Bouin or 10% neutral buffered formalin; some specimens were flattened under the pressure of a cover slip. For whole mount preparations, after staining with Gower's carmalum or borax carmine, the specimens were cleared in methyl benzoate and mounted in Canada balsam. With the use of catechol method following Johri and Smyth (1956), the distribution of vitellaria could be differentiated from that of the follicular testes.

The observations were made with the help of the Leitz Ortholux-2 research and Wild-M5APO stereoscopic microscopes. Description is based on measurements of 7 specimens, 3 of which possessed eggs in their uterus. The measurements, all in millimeters, are presented in Table 1.

## *Polystoma indicum*

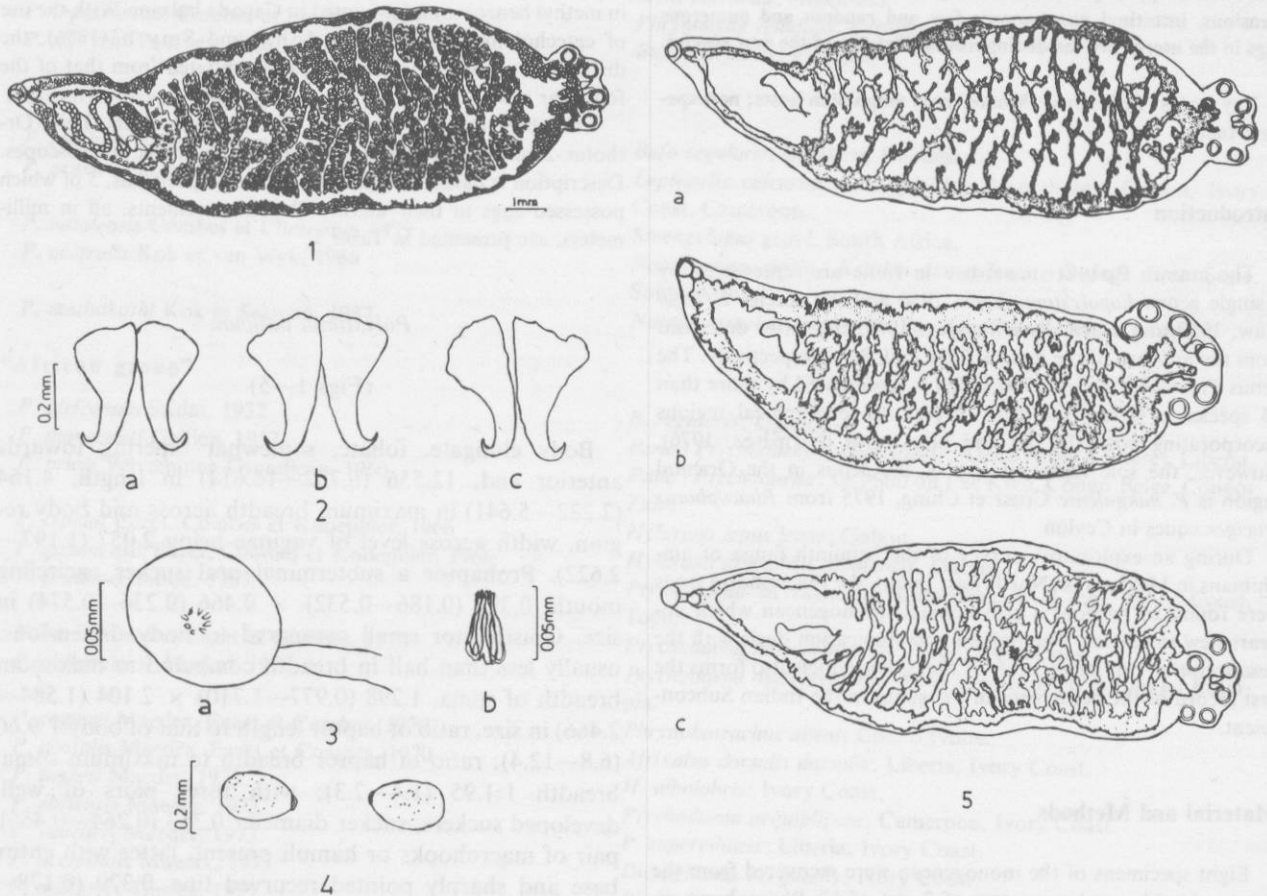
(Figs 1—5)

Body elongate, foliate, somewhat tapering towards anterior end, 12.556 (6.732—16.614) in length, 4.164 (2.222—5.641) in maximum breadth across mid body region, width across level of vaginae being 2.057 (1.197—2.622). Prohaptor a subterminal oral sucker encircling mouth, 0.390 (0.186—0.532) × 0.466 (0.236—0.574) in size. Opisthaptor small compared to body dimensions, usually less than half in breadth compared to maximum breadth of soma, 1.298 (0.977—1.710) × 2.104 (1.584—2.466) in size, ratio of haptor length to that of body 1:9.60 (6.8—12.4); ratio of haptor breadth to maximum somal breadth 1:1.95 (1.4—2.3); with three pairs of well-developed suckers, sucker diameter 0.353 (0.264—0.465), pair of macrohooks or hamuli present, latter with entire base and sharply pointed recurved tips, 0.320 (0.178—0.414) in length; microhooks not traceable.

Pharynx prominent, muscular 0.291 (0.164—0.395) × 0.249 (0.164—0.349) in size, leading to bifurcate intestine. Intestinal caeca indented at irregular intervals on lateral

Table 1. Morphometric measurements of *P. indicum* sp.n.

Characters		Range (mm)	Mean ( $\bar{x}$ )	$\pm$ S.D.
Length of the body		6.732—16.614	12.556	3.513
Maximum breadth of the body		2.222—5.641	4.164	1.061
Oral sucker or buccal funnel:	Length	0.186—0.532	0.390	0.111
	Breadth	0.236—0.574	0.466	0.126
Width at vaginae		1.197—2.622	2.057	0.464
	Opisthaptor:			
Haptor length: Body length	Length	0.977—1.710	1.298	0.264
	Breadth	1.584—2.466	2.104	0.316
Haptor breadth: Maximum somal breadth		1:6.8—1:12.4	1:9.60	—
Hamulus length		1:1.4—1:2.3	1:1.95	—
Opisthaptor sucker diameter		0.178—0.414	0.320	0.094
Pharynx:	Length	0.264—0.465	0.353	0.065
	Breadth	0.164—0.395	0.291	0.070
Ovary:	Length	0.164—0.349	0.249	0.061
	Breadth	0.504—1.368	1.049	0.294
Distance of genital pore from anterior end		0.288—0.630	0.499	0.120
Genital spine length		0.632—1.682	1.101	0.385
Distance of vaginae from anterior end		0.038—0.040	0.039	0.001
Egg size:	Length	1.100—2.860	2.206	0.654
	Breadth	0.190—0.214	0.205	0.008
		0.125—0.140	0.132	0.006



Figs 1—5. *Polystoma indicum* sp. n.: 1). Whole mount; vitellaria overlapping intestinal caeca and diverticula are not shown; 2. a, b, c). Hamuli in different specimens; 3. a). Genital crown, apical view, b). Genital hooks in side view; 4). Eggs; 5). Intestinal silhouette, showing: a — single prehaptor anastomosis, b — single prehaptor anastomosis without terminal opisthaptor union of caeca in an immature specimen and c — a number of prehaptor anastomoses

aspects, sending out 10—15 prominent diverticula from their inner facet, which in turn give off secondary branches, interspersed with 6—10 smaller unbranched diverticula; caeca usually uniting in immediate prehypertoral region, may extend into opisthypertoral disc; diverticula also randomly anastomosing medially, number of prehypertoral anastomoses 1—2 in most specimens, often more, diverticulation almost absent in region of caecal union.

Testes numerous, forming insurmountable mass in postovarian intercaecal zone, commencing just behind vitellovaginal ducts; vas deferens arising in region of junction of female genital ducts, running anteriorly slightly to side of median line; cirrus sac small, cirrus tipped with genital crown of 8 hooklets, latter 0.039 (0.038—0.040) in length; male terminal duct opening in front of female genital pore into common genital sinus; latter opening to exterior close behind intestinal bifurcation, 1.101 (0.632—1.682) from anterior extremity.

Ovary retort shaped, on left or right of median line, 1.049 (0.504—1.368) in length, 0.499 (0.288—0.630) in breadth; Mehlis' gland complex opposite to base of ovary; uterus emerging from junction of oviduct to vitelline reservoir, proceeding anteriorly in serpentine course, containing numerous eggs (up to 40); egg size 0.205 (0.190—0.214) × 0.132 (0.125—0.140); genito-intestinal canal given off from same junction as uterus, opening into caecum of ovarian side. Vaginae on either lateral margin of body between genital pore and ovary, at distance of 2.206 (1.100—2.860) from anterior end. Vitellaria follicular, extensive, commencing from pharyngeal level, posteriorly extending into opisthypertoral zone, intruding in spaces between suckers, overlapping intestinal diverticula, confined only to lateral sides (though still overlapping caeca) in ovarian and uterine regions.

Host: *Rhacophorus nigropalmatus* Boulenger, 1895.

Location: Urinary bladder, Ureter.

Locality: Cherrapunji (25°18'N: 91°46'E), East Khasi Hills District, Meghalaya State, India.

Deposition of specimens:

Holotype (No.  $\frac{IV}{ERS/ZSI}$  304), types (No.  $\frac{IV}{ERS/ZSI}$  305)

deposited in Eastern Regional Station, Zoological Survey of India; other paratypes No. NEHU/Z-MA/1) in helminthological collection of Department of Zoology, NEHU, Shillong, India.

Etymology: Named after the country.

### Discussion

On account of the distinct morphological features (i.e., opisthypertor with one pair of hamuli; testes numerous, postovarian in intercaecal field; uterus preovarian; vaginae present) the present form can be undoubtedly relegated to

the genus *Polystoma* Zeder, 1800. The species known so far under the genus are listed in Table 2, primarily on the basis of zoogeographical distribution of species as provided by Combes (1976).

Most workers have considered of diagnostic value the characters namely, ratio of opisthypertor length to total body length, intestinal diverticulation and mean number of intestinal medial anastomoses in the prehypertoral region, nature of hamuli (with entire or incised base), mean hamulus length, and dimensions of larval hooklets.

In view of the high degree of strict host specificity demonstrated among anuran monogeneans, host specificity and geographical distribution have also been assigned significant importance in species differentiation within the genus *Polystoma* (see Maeder *et al.*, 1970; Combes and Knoepffler, 1977; Dupouy, 1978; Combes and Channing, 1979; Murith, 1981; Kok and van Wyk, 1986; Kok and Seaman, 1987). However, the extent to which host specificity could be depended upon as differential character is controversial (Prudhoe and Bray, 1982).

On a comparison based on the morphological features with the known species of *Polystoma*, the present form is found to share some characters with a few species. In *P. gallieni*, *P. naevius*, *P. palancai*, *P. ivindoi*, *P. ebriensis*, *P. borellii*, *P. viridis*, *P. praecox*, *P. andinum* and *P. guevarai* the hamuli are or may be with entire or uncleft base. However, the different pattern of intestinal diverticulation and anastomoses, besides the difference of host, sets them apart from the present form.

Of the hitherto recorded species of *Polystoma*, only two have been reported from the rhacophorid anurans. These are *P. rhacophori* and *P. hakgalense*, the latter being the only representative of the genus recorded from the Oriental region. The breadth of the opisthypertoral disc (i.e. 1.016—1.509 mm) almost equalling that of the main body or the soma (0.8—1.79 mm), random and reticulate anastomoses of intestinal diverticula, and vitellaria not extending in between the opisthypertoral suckers in *P. hakgalense* are the characters which deviate the present form from the latter species.

Though the present form closely resembles *P. rhacophori* in having a somewhat similar pattern of intestinal diverticulation (only a few transverse anastomoses present) and hamuli with uncleft base, it deviates from *P. rhacophori* conspicuously in the ratio of soma vs opisthypertor and the number of eggs contained in the uterus; in the latter species the opisthypertor is definitely quite broad (1.1—1.6 mm) relative to somal breadth (1.0—1.9 mm), and the uterus contains usually one, seldom more than 10 eggs. Besides, in *P. rhacophori* two pairs of eye spots are present which are lacking in the form described herein.

In view of the foregoing differences from all the hitherto described species of *Polystoma* and also of the strict host specificity existing among amphibian polystomids, the present form is considered a new species.

Table 2. Known\* species under genus *Polystoma* and their host and locality records

**Palaeartic region**

*P. integerrimum* (Fröhlich, 1791) Rud., 1808  
*P. uncinatum* Mace, 1880  
*P. rhacophori* Yamaguti, 1936  
*P. ozakii* Price, 1939  
*P. gallieni* Price, 1939  
*P. palancai* Lopez-Neyra, 1952  
*P. pelobatis* (Euzet et Combes, 1966) Combes, 1968  
*P. viridis* Euzet, Combes et Batchvarov, 1974  
*P. mazurmovici* Buchvarov, 1980  
*P. combesi* Buchvarov, 1982  
*P. skrjabini* Buchvarov, 1984

*Rana temporaria*, *Bufo* spp., *Pelobates cultripipes*, *Hyla arborea*; Europe, Japan, Korea, Bulgaria (in Japan and Korea).  
*R. temporaria*, *Rana viridis*; Italy.  
*Rhacophorus schlegeli* var. *arborea*, *H. arborea japonica*; Japan.  
*R. temporaria ornativentris*; Japan, USSR, Korea.  
*H. arborea* var. *meridionalis*; Europe.  
*H. arborea*; Spain.  
*P. cultripipes*; France.  
*Bufo viridis*; France.  
*Rana dalmatina*; Bulgaria.  
*Rana graeca*; Bulgaria.  
*H. arborea*; Bulgaria.

**Nearctic region**

*P. nearcticum* (Paul, 1935) Price, 1939  
*P. naevius* Caballero et Cerecero, 1941  
*P. stellai* Perez Viguera, 1955

*Hyla versicolor*, *Hyla squirella*; North America.  
*Hyla baudinii*; Mexico.  
*Hyla septentrionalis*; Cuba.

**Neotropical region**

*P. borellii* Combes et Laurent, 1974  
*P. praecox* Combes et Laurent, 1978  
*P. andinum* Combes et Laurent, 1978  
*P. guevarai* Combes et Laurent, 1979  
*P. lopezromani* Combes et Laurent, 1979  
*P. diptychi* Vaucher, 1986

*Pleurodema borelli*; Argentina.  
*Telmatobius oxycephalus*; Argentina.  
*Melanophryniscus rubriventris*; Argentina.  
*Hyla pulchella*; Argentina.  
*Phrynohyas venulosa*; Paraguay.  
*Bufo diptychus*; Paraguay.

**Palaeotropical region**

*P. mashoni* Beverley-Burton, 1962  
*P. grassei* Euzet, Combes et Knoepffler, 1966  
*P. natalensis* Combes et Channing, 1979  
*P. australis* Kok et van Wyk, 1986  
*P. umthakathi* Kok et Seaman, 1987

*Bufo regularis*; Southern Rhodesia.  
*Leptopelis calcaratus*, *L. hylodes*, *L. ocellatus*; Gabon, Ivory Coast, Cameroon.  
*Strongylopus grayi*; South Africa.  
*Notokassina wealii*, *Kassina senegalensis*; Orange Free State, South Africa.  
*Natalobatrachus bonebergi*; Southern Natal, South Africa.

**"African group"**

*P. africanum* Szidat, 1932  
*P. manganoti* Gallien, 1957  
*P. pricei* Vercammen-Grandjean, 1960  
*P. ivindoi* Euzet, Combes et Knoepffler, 1966  
*P. gabonensis* Euzet, Combes et Knoepffler, 1966  
*P. prudhoei* Saoud, 1967  
*P. baeri* Maeder, Euzet et Combes, 1970  
*P. aethiopiense* Meskal, 1970  
*P. ragnari* Maeder, Euzet et Combes, 1970  
*P. dorsalis* Maeder, Euzet et Combes, 1970  
*P. perreti* Maeder, 1973  
*P. ebriensis* Maeder, 1973  
*P. vaucheri* Maeder, 1973  
*P. occipitalis* Maeder, 1973  
*P. makereri* Tinsley, 1973  
*P. galamensis* Euzet, Bourgat et Salami-Cadoux, 1974  
*P. batchvarovi* Euzet, Combes et Knoepffler, 1974  
*P. hlewellyni* Euzet, Combes et Knoepffler, 1974

*B. regularis*; Uganda.  
*Rana (Ptychadaena) superciliaris*; Africa (Cote d'Ivoire), Liberia.  
*Rana (Ptychadaena) sp.*; Sud du Lac Kivu, Congo-Belge, Congo-Zaire.  
*Hylarana lepus lepus*; Gabon.  
*Hylarana albolabris albolabris*; Gabon.  
*Ptychadaena oxyrhynchus*; Cameroon, R. C. A., Ivory Coast, Togo.  
*Ptychadaena macCarthyensis*; Togo, Ivory Coast.  
*Ptychadaena mascareniensis*, *Rana angolensis*, *B. regularis*; Ethiopia.  
*Phrynobatrachus alleni*; Cote d'Ivoire.  
*Afraxalus dorsalis dorsalis*; Liberia, Ivory Coast.  
*H. albolabris*; Ivory Coast.  
*Ptychadaena aequiplicata*; Cameroon, Ivory Coast.  
*P. superciliaris*; Liberia, Ivory Coast.  
*Dicroglossus occipitalis*; Ivory Coast.  
*Phrynobatrachus sp.*; East Africa (Uganda).  
*Rana galamensis*; Togo.  
*Hyperolius tuberculatus*; Republic of Central Africa.  
*Afraxalus fulvovittatus leptosoma*; Republic of Central Africa.

*r. assoutinet* Bourgat, 1975  
*P. togoensis* Bourgat, 1977  
*P. chiromantis* Dupouy et Knoepffler, 1978  
*P. lamottei* Bourgat et Murith, 1980  
*P. aeschlimanni* Bourgat et Murith, 1980

### Oriental region

*P. hakgalense* Cruz et Ching, 1975

*Ptychadaena huguettae*; Togo.  
*Ptychadaena hylaea*; Togo.  
*Chiromantis rufescens*; Cameroon, Gabon, Central Africa.  
*Ptychadaena pumilio*; Togo.  
*P. pumilio*; Togo.

*Rhacophorus cruciger eques*; Ceylon (Sri Lanka).

\* *P. bulliense* Johnston, 1912, *P. alluaudi*, Beauchamp, 1913, *P. xenopi* Price, 1943, *P. dendriticum* Ozaki, 1948, *P. cachani* Gallien, 1957, and *P. brygoonis* Euzet et Combes, 1964 have been removed from the genus and are now transferred to *Parapolyostoma*, *Eupolyostoma*, *Protopolyostoma*, *Pseudopolyostoma* and the latter two to *Metapolyostoma*, respectively.

Specific diagnosis — Polystomatidae: Polystomatinae. Body 6.73–16.61 mm in length, 2.22–5.64 mm in maximum breadth; eye spots lacking, opisthaptor considerably small compared to body dimensions; hamuli with uncleft, entire base; intestinal anastomoses few and random, not reticulate. Eggs numerous (upto 40) in uterus in egg-producing stage, 0.190–0.214 × 0.125–0.132 mm.

### Acknowledgements

The authors wish to thank Prof. C. Combes, Department of Animal Biology, University of Perpignan, France and Dr. R. A. Bray, British Museum (Natural History), London for their critical comments. The investigation was supported in part by a UGC Teacher Fellowship to CD Laboratory facilities provided by the Head, Department of Zoology, NEHU are thankfully acknowledged.

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