J. Zool., Lond. (2000) **250**, 129–139 Printed in the United Kingdom

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Developmental pattern and larval stages of *Polystoma indicum* Diengdoh & Tandon, 1991 (Monogenea: Polystomatidae) in rhacophorid anurans

Mamoni Dutta and Veena Tandon*

Department of Zoology, North-Eastern Hill University, Shillong, 793022 Meghalaya, India

(Accepted 7 April 1999)

Abstract

The developmental pattern of *Polystoma indicum* Diengdoh & Tandon, 1991 occurring in the urinary bladder of the frogs *Rhacophorus nigropalmatus*, *R. reinwardtii* and *Polypedates leucomystax* from Meghalaya (north-east India) was studied. Non-ciliated larval stages were recovered in the gyrodactyloid-I and post-gyrodactyloid-I stage mostly from the branchial chambers of the tadpoles; only rarely were they found in the intestines. These stages possessed features of bladder-destined forms and showed a gradual acquisition of 2, 4 and then 6 opisthaptoral suckers, coupled with an increase in the size of the body and various structures. While a conspicuous increase was noticeable in the dimensions of the hamuli, the size of the microhooks remained almost the same from the 2-suckered larval stage to the adult. The larval migration to the final destination seems to follow an external route leading to the cloaca of the metamorphosing host. The prevalence data of both the larvae and the adult flukes indicate a preference towards *R. nigropalmatus* amongst the three rhacophorid host species. The gyrodactyloid-II, i.e. neotenic larvae were not encountered at all either on the external gills of the very young tadpoles or even in the branchial chambers of the older ones.

Key words: *Polystoma indicum*, development, larvae, host, anurans

INTRODUCTION

Studies on the biology of polystomatid monogeneans have revealed some interesting findings. In some polystomatids, the sexual cycle of the fluke is known to be synchronized with that of its host and its development is also influenced by the developmental status of the host (Prudhoe & Bray, 1982). Accordingly, the fluke larvae may either exhibit an accelerated development producing neotenic 'gill forms' (on the external gills of the tadpole host) or alternately undergo normal development giving rise to the normal 'bladder forms' (developing in the branchial chambers of the metamorphosing tadpole and finally migrating to the urinary bladder) (du Preez & Kok,1998). Other polystomatids, viz., *Polystoma grassei* and *Eupolystoma alluaudi* have a direct life cycle, with multiplication of the parasite in the urinary bladder of the host (Dupouy & Combes, 1977; Fournier & Combes, 1979). The neotenic and predestined bladder stages can be differentiated on the basis of their morphological features and establishment patterns (Kok & du Preez, 1987, 1989; Kok, 1990).

*All correspondence to: Professor Veena Tandon, Department of Zoology, North-Eastern Hill Univeristy, Shillong 793 022, India

Polystoma indicum is a monogenean parasite of rhacophorid anurans in the north-eastern part of India (Diengddoh & Tandon,1991). The seemingly strict host specificity and restricted geographical distribution of the fluke make it suitable for biological sutudy. We present here the results of an investigation of the developmental pattern and larval stages of *P. indicum* in rhacophorid anurans.

MATERIALS AND METHODS

Hosts collected or surveyed

The anuran host collections from Cherrapunji, Mawsynram and Shillong (East Khasi Hills District, Meghalaya) comprised predominantly the rhacophorids. Of these, adult frogs of *Rhacophorus nigropalmatus* Boulenger and *R. reinwardtii* Schlegel and tadpoles of both these species and also of *Polypedates leucomystax* Kuhl were found harbouring the adult and larval stages, respectively of *P. indicum*. Adults and tadpoles of *Amolops afghanus*, collected from the same localities did not reveal any infection by the monogenean parasite. Repeated collections were made of the available species from these localities in the morning or

Table 1. Prevalence of adult *P. indicum* in rhacophorid anurans

Host species	Locality	No. examined (no. infected)	% Prevalence	Adult flukes recovered (mean abundance[a])
R. nigropalmatus	Cherrpunji	369 (40)	10.89	40 (0.108)
	Mawsynram	62 (16)	25.80	16 (0.258)
R. reinwardtii	Cherrapunji	128 (22)	17.18	22 (0.171)
	Mawsynram	56 (9)	16.07	30 (0.535)
P. leucomystax	Cherrapunji	30 (0)	[b]	[b]
	Mawsynram	3 (0)	[b]	[b]

[a] Per host examined.
[b] No infection of Monogenea.

sometimes at nightfall by following the croaking sound of males or observing the nest constructed by the females. Most catches were possible between May and October, since the frogs tend to move to trees during this period. The frogs from different spots were kept separately and brought to the laboratory and maintained till autopsy.

Tadpoles in different stages of development were also collected from those localities where the monogenean infection in adult frogs was evident. While most of them were autopsied at the earliest possible moment following collection, some tadpoles were maintained in the aquaria to allow them to grow to the post metamorphosis stage.

For the recovery of larval forms, first the live tadpoles were observed under the stereoscopic binocular microscope, to see if any parasitic forms were present on the body or external gills and fins. Next they were killed, and their internal gills, branchial chambers, and the alimentary canal were immersed in 0.7% saline and examined. Live specimens were studied under the bright field and phase contrast microscopes and observations recorded. Data were maintained regarding the developmental status of the host and the number of parasites recovered per host.

Whole mount preparations

Live flukes recovered were narcotized with a few drops of 70% alcohol. For flattening them, the specimens were compressed gently between a glass side and a cover slip. 70% alcohol and Bouins were used as fixatives. Borax carmine and Gower's carmalum were used as general stains.

For determining the nature of the egg shell, the specimens fixed in 70% alcohol were stained following the method given by Smyth & Smyth (1980) for demonstration of sclerotin egg-shell precursors.

Light microscopy

Light microscopic observations were made under Wild MS APO stereoscopic and Leitz Orthlux-2 research microscopes. Measurements were taken with the help of ocular and stage micrometers and, unless and otherwise stated, are in mm and based, where possible, on 10 specimens in each case.

OBSERVATIONS

The intensity of infection by *P. indicum* adults was found to be quite low (Table 1), ranging from one to four flukes in a single individual host.

Of the several specimens collected, only 4 were found to be egg-containing adult flukes. Three of the flukes contained only a single egg. One specimen having several eggs (10–12) *in utero*, which was observed under laboratory conditions, laid 41 eggs within the first 24 h after recovery, followed by 112 eggs in the next 24 h and on the third day 29 eggs, after which the parasite died with seven eggs still retained in the uterus. Eggs maintained under laboratory conditions at 23 ± 1 °C showed no sign of further development.

The morphology of the developmental stages of Polystoma indicum

Egg

The eggs are reddish brown, oval, slightly tapering towards one end and unembryonated while *in utero*. The operculum is in the form of a depression towards one end. The egg surface has a porous appearance. As revealed by the positive staining for phenolase and phenolic precursors, the egg shell exhibits a proteinaceous, sclerotin-like nature.

Larval stages

The larval and juvenile polystomatids were recovered from the branchial chambers or internal gills, urinary bladder and rarely from the intestine of the metamorphosing tadpoles retaining a tail vestige. These unciliated larvae were distinguishable as representing four stages of development on the basis of their opisthaptor. The latter showed gradual development from no-suckered opisthaptoral disc to the one

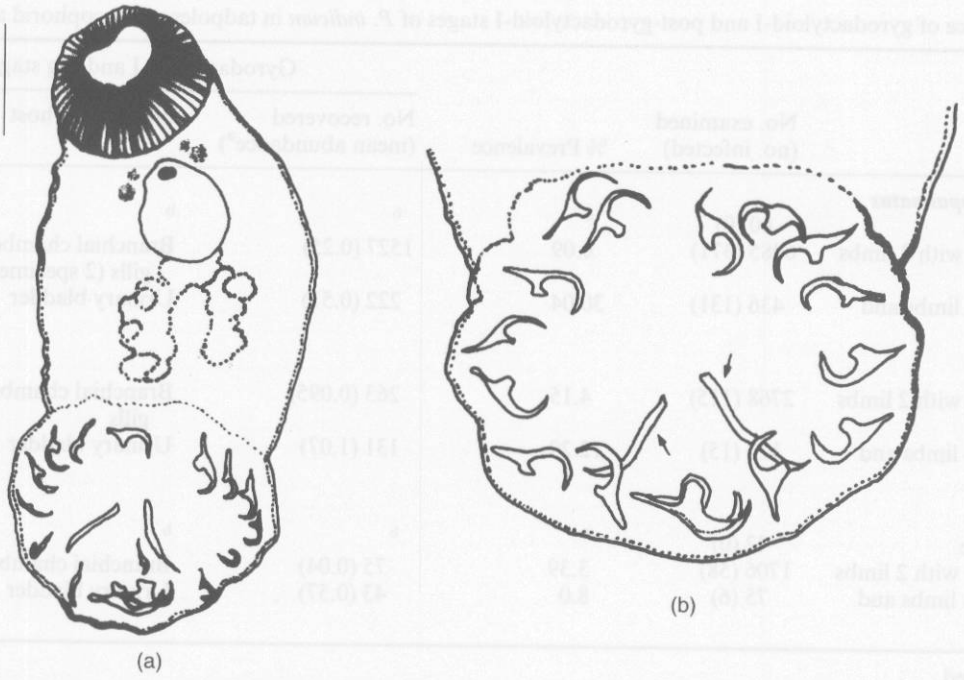

Fig. 1. *Polystoma indicum*: Gyrodactyloid-I larva, whole mount, (a) Larva, ventral view. (b) Opisthaptoral disc with its armature of microhooks and hamuli primordia (arrows), enlarged. Scale bar = 0.05 mm.

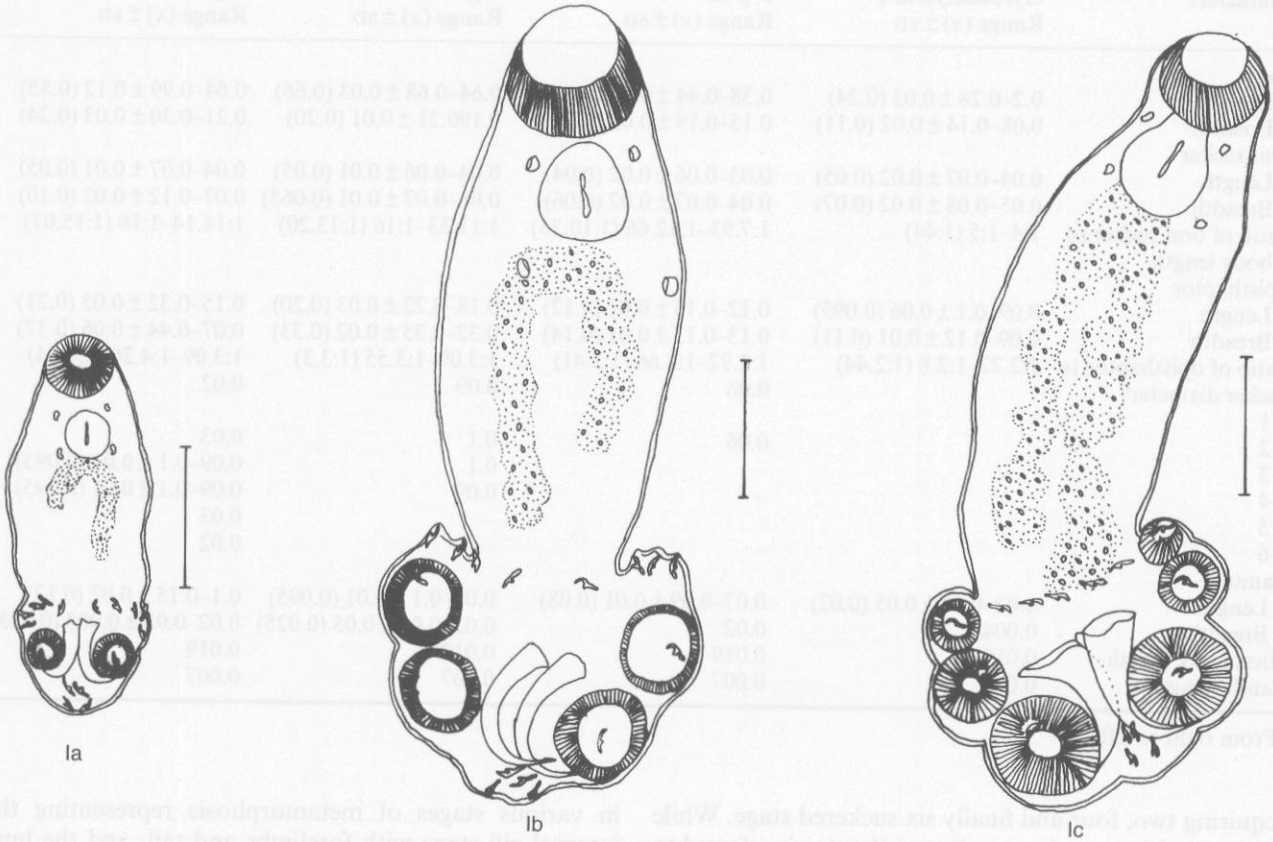

Fig. 2. *Polystoma indicum*: Post-gyrodactyloid-Ia, Ib and Ic stages, whole mounts. Scale bar = 0.5 mm.

Table 2. Prevalence of gyrodactyloid-I and post-gyrodactyloid-I stages of *P. indicum* in tadpoles of rhacophorid anurans

Host species	No. examined (no. infected)	% Prevalence	Gyrodactyloid-I and P-g stages	
			No. recovered (mean abundance[a])	Location in host
Rhacophorus nigropalmatus				
External-gill stage	60 (0)	[b]	[b]	[b]
Internal-gill stage with 2 limbs and tail	6085 (371)	6.09	1527 (0.25)	Branchial chamber, internal gills (2 specimens in intestine)
Lung stage with 4 limbs and vestige of tail	436 (131)	30.04	222 (0.51)	Urinary bladder
R. reinwardtii				
Internal-gill stage with 2 limbs and tail	2768 (115)	4.15	263 (0.095)	Branchial chamber, internal gills
Lung stage with 4 limbs and vestige of tail	122 (15)	12.29	131 (1.07)	Urinary bladder
P. leucomystax				
External-gill stage	42 (0)	[b]	[b]	[b]
Internal-gill stage with 2 limbs	1706 (58)	3.39	75 (0.04)	Branchial chamber, internal gills
Lung stage with 4 limbs and vestige of tail	75 (6)	8.0	43 (0.57)	Urinary bladder

[a] Per host examined.
[b] No infection.

Table 3. Measurements (in mm) of gyrodactyloid-I and post-gyrodactyloid (p-g) larvae of *P. indicum*

Characters	Gyrodactyloid-I Range (*x*) ± SD	P-g-Ia Range (*x*) ± SD	P-g-Ib Range (*x*) ± SD	P-g-Ic Range (*x*) ± SD
Body				
Length	0.2–0.28 ± 0.03 (0.24)	0.38–0.44 ± 0.44 (0.41)	0.64–0.68 ± 0.03 (0.66)	0.64–0.99 ± 0.12 (0.85)
Breadth	0.08–0.14 ± 0.02 (0.11)	0.15–0.19 ± 0.03 (0.17)	0.190.21 ± 0.01 (0.20)	0.21–0.30 ± 0.03 (0.24)
Oral sucker				
Length	0.04–0.07 ± 0.02 (0.05)	0.03–0.06 ± 0.02 (0.04)	0.04–0.06 ± 0.01 (0.05)	0.04–0.07 ± 0.01 (0.05)
Breadth	0.05–0.08 ± 0.02 (0.07)	0.04–0.07 ± 0.02 (0.06)	0.06–0.07 ± 0.01 (0.065)	0.07–0.12 ± 0.02 (0.10)
Ratio of oral sucker to body length	1:4–1:5 (1:44)	1:7.93–1:12.66 (1:10.25)	1:11.33–1:16 (1:13.20)	1:14.14–1:16 (1:15.07)
Opisthaptor				
Length	0.09–0.1 ± 0.06 (0.095)	0.12–0.13 ± 0.01 (0.12)	0.18–0.22 ± 0.03 (0.20)	0.15–0.32 ± 0.05 (0.21)
Breadth	0.09–0.12 ± 0.01 (0.11)	0.13–0.15 ± 0.01 (0.14)	0.32–0.35 ± 0.02 (0.33)	0.07–0.44 ± 0.06 (0.37)
Ratio of opisthaptor to	1:2.22–1:2.8 (1:2.44)	1:2.92–1:3.66 (1:3.41)	1:3.09–1:3.55 (1:3.3)	1:3.09–1:4.26 (1:4.04)
Sucker diameter[a]				
1		0.06	0.09	0.02
2		0.06	0.1	0.03
3		–	0.1	0.09–0.1 ± 0.01 (0.095)
4		–	0.09	0.09–0.1 ± 0.01 (0.095)
5		–	–	0.03
6		–	–	0.02
Hamuli				
Length	0.02–0.03 ± 0.05 (0.02)	0.07–0.09 ± 0.01 (0.08)	0.09–0.1 ± 0.01 (0.095)	0.1–0.15 ± 0.02 (0.12)
Breadth	0.004	0.02	0.02–0.03 ± 0.05 (0.025)	0.02–0.03 ± 0.003 (0.025)
Microhook length	0.019	0.019	0.019	0.019
Handle length	0.007	0.007	0.007	0.007

[a] From right to left.

acquiring two, four and finally six suckered stage. While the stage with no suckers on its opisthaptor is referred to as gyrodactyloid-I (Fig.1) following Prudhoe & Bray (1982), the suckered stages are referred herein as post-gyrodactyloid-Ia, -b and -c, respectively (Fig. 2).

The prevalence data of the larval stages is presented in Table 2. These were found to occur in the tadpoles in various stages of metamorphosis representing the internal-gill stage with forelimbs and tail, and the lung stage with four limbs and vestige of diminishing tail (the last stage being at the verge of completing metamorphosis). The larvae inhabited the internal gills, branchial chamber and urinary bladder of the metamorphosing tadpoles, though on two occasions one

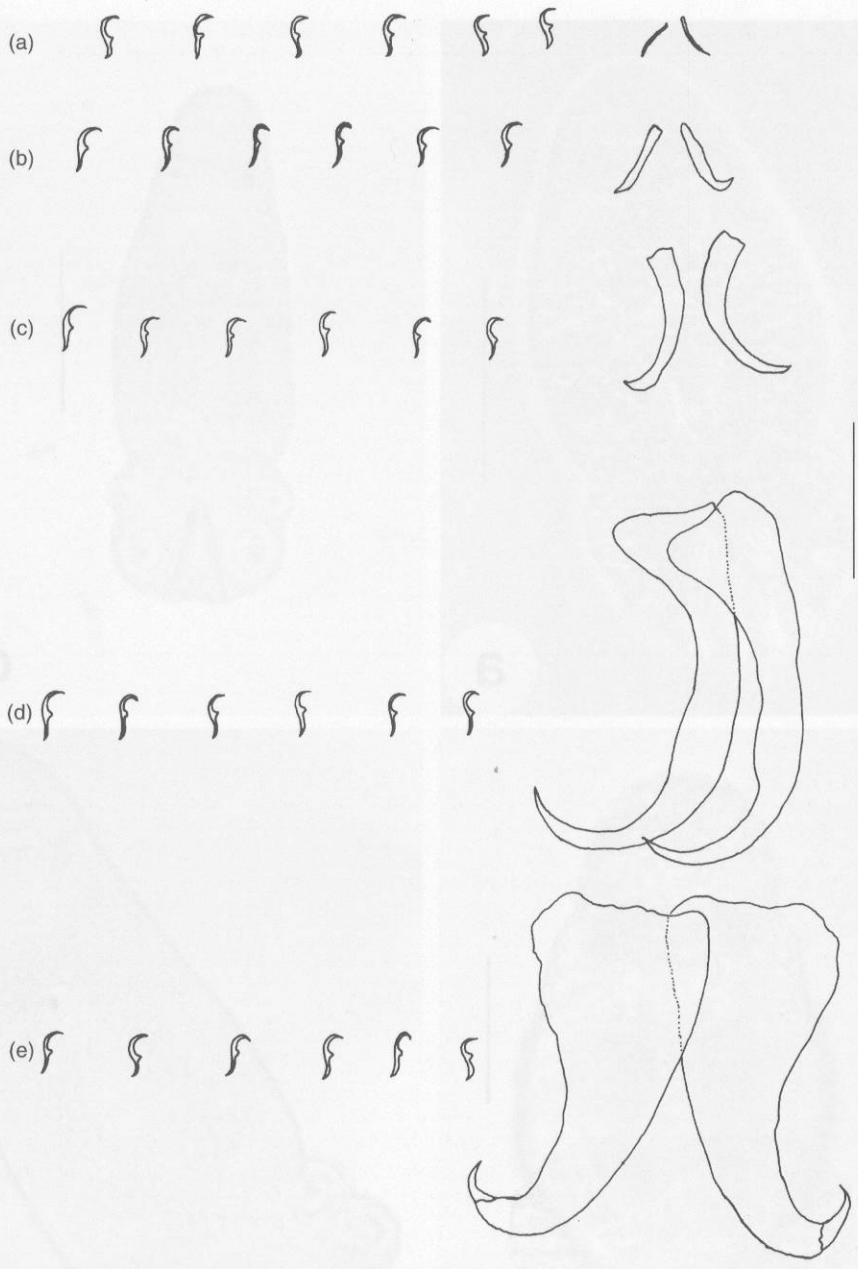

Fig. 3. *Polystoma indicum*: microhooks (which are associated with the suckers) and hamuli of (a) gyrodactyloid-I, (b) post-gyrodactyloid-Ia, (c) post-gyrodactyloid-Ib, (d) post-gyrodactyloid-Ic, and (e) adult fluke. Scale bar = 0.1 mm.

specimen was recovered from the intestine as well. In tadpoles in the late stage of metamorphosis, the larval forms representing all the four stages were recovered from the urinary bladder. Not a single larval specimen was ever recovered from the external-gill stage tadpoles during the present study. The intensity of infection in a single host ranged between one and 15 larvae in the internal-gill stage and 20-25 in the lung stage. In *P. leucomystax* also, no infection was found in the external gill stages and no larvae were recovered from the intestine of the tadpoles.

Measurements of the body and various structures of these stages are presented in Table 3.

Gyrodactyloid-I stage

This is the first and the earliest form of development recovered from the amphibian host in the present study. It has minute non-ciliated, oval or somewhat cylindrical body, 0.20–0.28 mm in length, with a terminal oral opening in the form of a prohaptor. The opisthaptor boundary is not constricted off from the main body but the posterior quarter is made up by the rounded opisthaptoral disc. The haptoral disc has 16 microhooks, and a pair of hamuli primordia. Of the microhooks, three pairs are anterior to the hamuli primordia, two pairs are in the region posterior to the

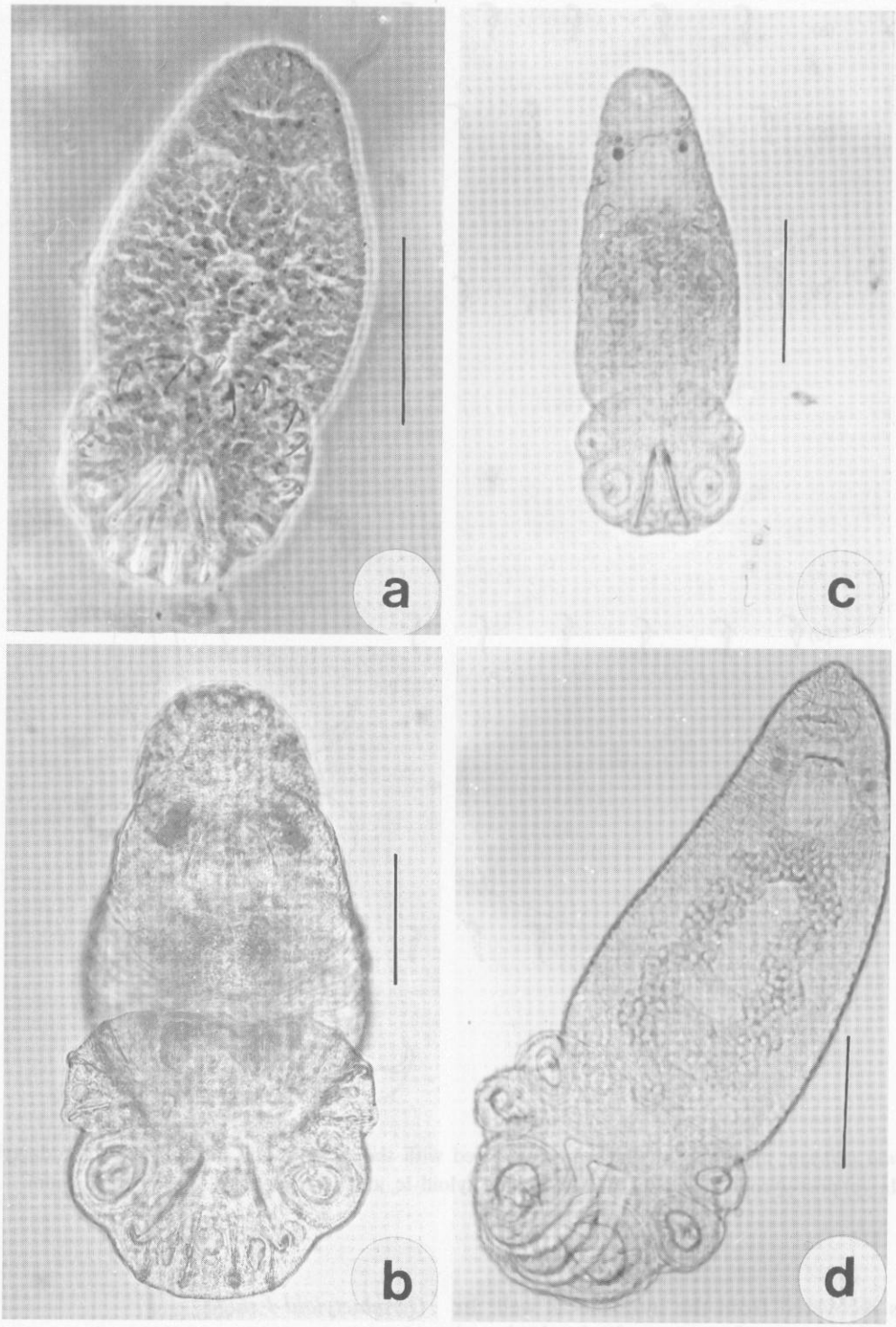

Fig. 4. *Polystoma indicum* larvae, phase contrast microscopy. (a) Gyrodactyloid-I larva, showing microhooks and hamuli primordia. Sclae bar = 0.5 mm. (b) Post-gyrodactyloid-Ia, showing two distinct suckers on the opisthaptor and two pairs of eye spots. Scale bar = 0.05 mm. (c) Post-gyrodactyloid-Ib; eye spots are distinct. Scale bar = 1 mm. (d) Post-gyrodactyloid-Ic, retaining the eyespots. Scale bar = 0.05 mm.

latter and six microhooks are lodged singly in the prospective positions where future six suckers would develop. A well-developed pharynx follows the prohaptor and immediately behind it is the bifurcated intestine. The intestinal caeca are simple, thin walled and unequal in length, with one caecum extending posteriorly up to a little behind the equatorial region of the body, while the other falling much short of it. Two pairs of eye spots, one on either side of the pharynx, are present. (Figs 1, 3b, 4a & 5a).

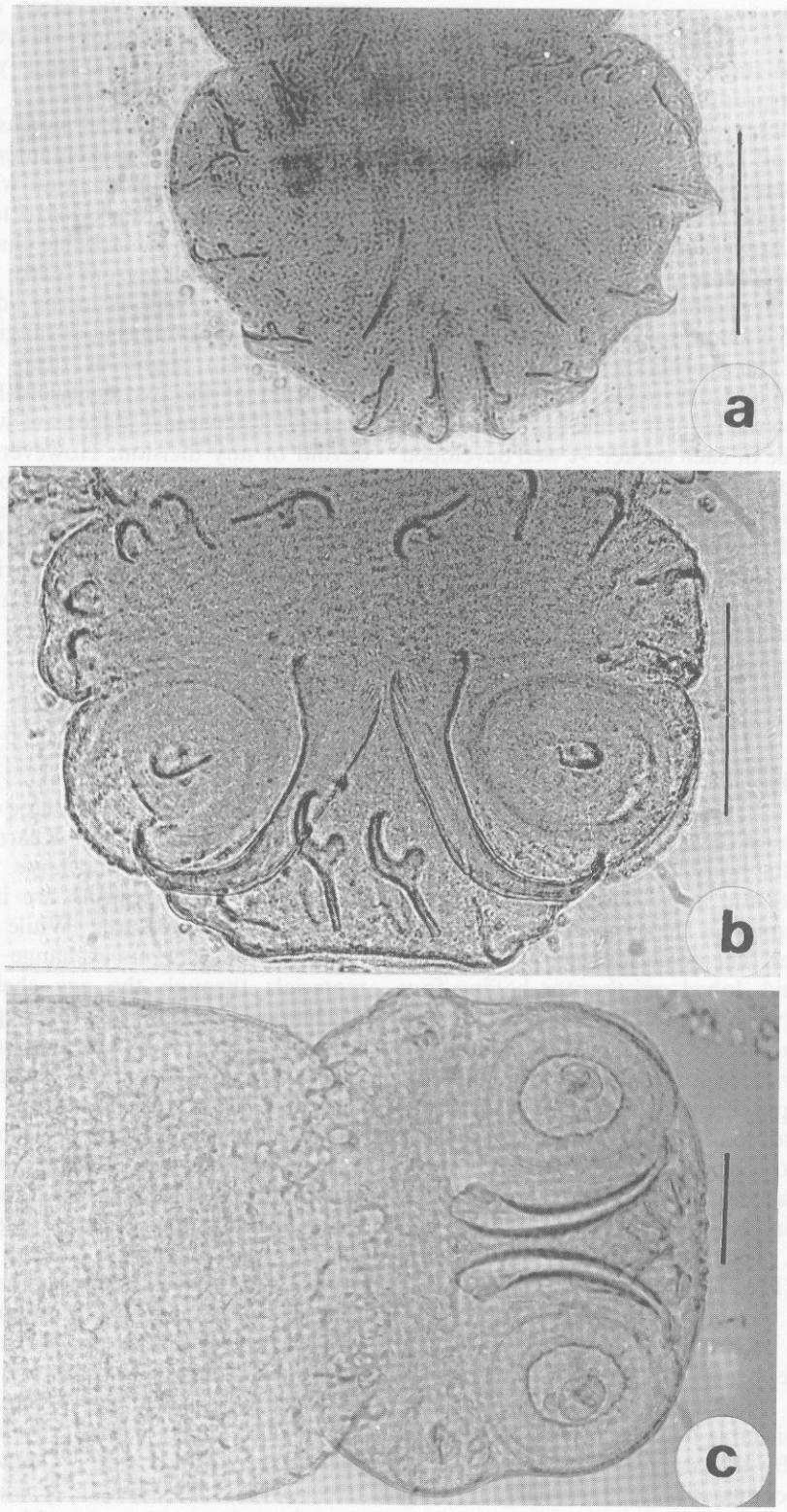

Fig. 5. *Polystoma indicum* larvae, phase contrast microscopy. (a) Gyrodactyloid-I stage, opisthaptoral end showing microhooks and hamuli primordia. Scale bar = 0.5 mm. (b) Post-gyrodactyloid-Ia stage, opisthaptor disc showing its full complement of suckers, hamuli microhooks. Scale bar = 0.05 mm. (c) Post-gyrodactyloid-Ib stage, with four suckers (two prominent and two developing), a pair of hamuli and microhooks. Scale bar = 0.025 mm.

Post-gyrodactyloid-Ia

This being the second stage, has a minute body, 0.30–0.44 mm long, with a prominent terminal prohaptor in the form of an oral sucker. The opisthaptor boundary is not fully constricted off from the main body; however, the disc for attachment is more complex than in the earlier stage, its fore-limit from the forebody is perceptible because of the distribution of the microhooks. It is endowed with two suckers and a pair of well defined, though small, hamuli in addition to 16 microhooks. The hamuli have acquired a shape somewhat similar to that in the adult fluke. Of the microhooks five pairs are just anterior to the hamuli and the suckers, two pairs are in the region posterior to the hamuli, while one microhook is present in the middle of each sucker. A well developed pharynx follows the oral sucker and immediately behind it is the bifurcated intestine. The intestinal caeca are simple, undiverticulated, much longer than those of the preceding stage and extend posteriad further up to the level a little behind the equatorial region of the body and close to the opisthaptor. Two pairs of eyespots, one on each side of the pharynx, are prominent (Figs 2, 3(ii), 4b & 5b).

Post-gyrodactyloid-Ib

This is the third stage of development recovered during the present study. Its body is moderately large and cylindrical, 0.64–0.68 mm long. The anterior terminal end carries the prohaptor in the form of a conspicuous prominent oral sucker. The posterior region is provided with a distinct discoid, lobed opisthaptor having four prominent suckers, two hamuli and 16 microhooks; of the microhooks, two pairs are post hamuli, one microhook is associated with each sucker and four pairs are arranged somewhat parallel to the anterior limits of the opisthaptor. The intestinal caeca are better developed than in the previous two stages; their extent is still asymmetrical, one caecum being longer than the other and they are beginning to diverticulate, as indicated by their wavy contour. Two pairs of eyespots are also present in this stage (Figs 2, 3c, 4c & 5c).

Post-gyrodactyloid-Ic

This stage is distinguishable by the presence of 6 suckers in the opisthaptor. By this stage the larvae acquire an elongated and more compact body, 0.64–0.99 mm long, with an adult-like opisthaptor. With the incorporation of one microhook nuclating each of the six suckers of the opisthaptor, the number of free microhooks is reduced to 10, of which three pairs are located anterior to the hamuli, and two pairs posterior to them. The muscular nature of the oral sucker and pharynx is prominent in this stage and so is the caecal bifurcation. The intestinal caeca show irregular indentation and extend posteriorly up to the level of the opisthaptor; in some specimens the two caeca are seen joining each other posteriorly. Two pairs of eyespots are still retained in this stage. However, there is yet no trace of genital primordia (Figs 2, 3d & 4d).

While there is a gradual increase in the size of several structures, viz., oral sucker, intestinal caeca, opisthaptoral disc, suckers and hamuli with the increase in the body size of the gyrodactyloid-I to post-gyrodactyloid-I stages, there is no remarkable change in the dimensions of the microhooks from early to the late stage of development as well as in the adult (Figs 3 & 6). The hamuli show varying shapes ranging from smooth based to those with slight or deep incision. The hamuli primordia of the gyrodactyloid-I stage and hamuli of post-gyrodactyloid-I stages are with smooth bases and devoid of any incision. However, in mature adults the hamuli are with smooth base or with slight or deep incision at the base whereby a guard and a handle could be differentiated in the hamulus. It was evident from the observations that there are variations in the relative length and width of the guard and handle and also in the depth of the incision between the two hamuli even in the same specimen.

DISCUSSION

Conforming to the general shape of polyopisthocotylean eggs (Baer & Euzet, 1961; Kearn, 1986), in *P. indicum* the eggs are fusiform and large. The shape and size of the eggs of monogeneans are influenced by different environmental pressures. While the shape of the egg influences the gaseous exchange and transport of other materials through the egg shell, it also affects the mechanical strength of the egg (Kearn, 1986). The fusiform shape of the egg may help in providing higher surface area/volume ratio compared to the spherical eggs and may be advantageous to the parasite which occupies the urinary bladder as its habitat.

Eggs of *P. indicum* contained in the uterus are reddish brown. Specific staining technique revealed the vitellaria to be positive for sclerotin egg shell precursors, i.e., phenol and phenolase. These results indicate the presence of a basic protein as the constituent of the egg shell. Based on the same techniques, the same precursors and the sclerotin-like protein nature of the egg shell have been reported in several Monogenea such as *Gastrocotyle trachuri* (see Freeman & Llewellyn, 1958), *Entobdella soleae* and *Diclidophora luscae* (see Llewellyn, 1965) and *Oögyrodactylus farlowellae* (see Harris, 1983). Smyth & Clegg (1959) pointed out that the red colour produced by incubation in catechol is localized in the shell globules, indicating that phenolase actually occurs in the same globule as its substrate. Some sort of blocking system may be present preventing premature sclerotization in the droplets before they coalesce to form the shell. According to Ramalingam (1970, 1971, 1973), phenolase exists as an inactive precursor (prophenolase) in the vitellaria of the monogeneans belonging to the genera *Pricea* and

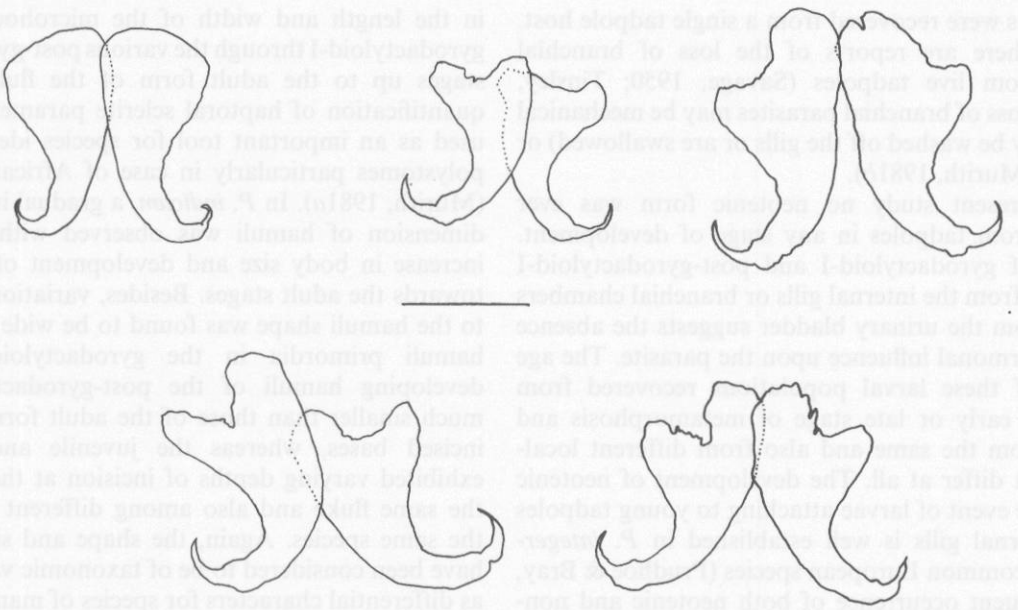

Fig. 6. *Polystoma indicum*: variation in incision in hamuli, showing guard and handle, in mature adults. Scale bar = 0.2 mm.

Protomicrocotyle. A similar claim was made for *Dionchus remorae* by Ramasamy (1984). Kearn (1986) suggested that the egg shell of *E. soleae* is homogeneous and in this respect it resembles the egg shell of the digenean *Fasciola hepatica*.

The eggs in *P. indicum* are operculate as in many other monogeneans (Llewellyn, 1957) and it seems that hatching could occur through the opercular opening by the splitting open of the operculum by the mobile larva enclosed within. Few monogeneans, for example *Eupolystoma anterorchis*, may have non-operculate eggs; in the absence of an operculum hatching takes place by longitudinal splitting of the egg shell (Tinsley,1978). In the present study the number of eggs observed in the uterus of the adult worm varied from one to 12. The egg-laying period and survival of *P. indicum* under laboratory conditions lasted for 48 h after its detachment from the host during which time a total of 182 eggs could be harvested and seven were still retained in the uterus when the fluke died. Tinsley (1983) generalized that most Monogenea deposit fewer than 100 eggs per fluke per 24 h. However, Combes (1972) recovered 1000–2500 eggs in 1 day by a single *P. integerrimum* parasitizing an isolated frog. Other polystomatids, i.e. *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopodis*, are reported to produce much fewer eggs, their annual output not exceeding 250 eggs (Tinsley, 1983), though estimates for other species indicate 10–20 eggs/parasite/24 h (Maeda, 1973; Tinsley & Owen, 1975). In the present observations the eggs *in utero* or after release by the parasites were unembryonated, indicating that the parasites are oviparous. Monogenean parasites with a short period of breeding are reported to adapt to ovoviviparity (Tinsley, 1983; Tinsley & Earle, 1983).

During the present investigation no oncomiracidium of *P. indicum* was encountered, although it is supposed to be the first larval stage in the ontogeny of the monogenean flukes. The larval stages were always recovered from the internal gills, branchial chambers and urinary bladder of the metamorphosing tadpoles. The earliest of these larvae recovered were distinguishable by the presence of four eyespots and 16 microhooks on the opisthaptor, characteristic of gyrodactyloid-I stage which were not neotenic but bladder-destined forms (Kok, 1990); they undertake migration from the internal gills of the tadpole host to finally reach the cloacal chamber or urinary bladder where sexual maturation is accomplished (Prudhoe & Bray, 1982). The neotenic or gyrodactyloid-II stages are characterized by the presence of a well developed opisthaptor and bulbous testis, and the absence of vagina, uterus and genito-intestinal canal (Prudhoe & Bray, 1982) and undergo accelerated development to become egg-laying adults on the external gills of the host.

Of the several hundreds of gyrodactyloid-I larvae recovered during the present study only two were found in the intestine. This rare occurrence of larval polystomes in the intestine, as also observed by other workers (Gallien, 1935; Williams, 1961; Combes, 1968) seems to support their observations that the migration of young polystomes occurs not through the gut but by moving over the ventral surface of the body towards the cloacal aperture, through which the parasites gain entry into the cloaca, and in turn, the bladder. Probably, the larvae unable to reach the exterior after the gill slits of the metamorphosing tadpole have closed would migrate through the gut. The intensity of infection was found to be lower in adult hosts as compared with that in tadpoles, probably as a result of high loss of larval parasites at the time of migration. No tadpole mortality as caused by parasite burden in the case of *P. indicum* was observed, although in certain instances more than

20 specimens were recovered from a single tadpole host. However, there are reports of the loss of branchial parasites from live tadpoles (Savage, 1950; Tinsley, 1983). The loss of branchial parasites may be mechanical (as they may be washed off the gills or are swallowed) or accidental (Murith, 1981*b*).

In the present study no neotenic form was ever recovered from tadpoles in any stage of development. Recovery of gyrodactyloid-I and post-gyrodactyloid-I stages both from the internal gills or branchial chambers and also from the urinary bladder suggests the absence of hosts' hormonal influence upon the parasite. The age structure of these larval populations recovered from tadpoles in early or late stage of metamorphosis and collected from the same and also from different localities did not differ at all. The development of neotenic forms in the event of larvae attaching to young tadpoles having external gills is well established in *P. integerrimum*, the common European species (Prudhoe & Bray, 1982). Frequent occurrence of both neotenic and non-neotenic forms together in the same tadpole has been reported in *Polystoma umthakathi* by Kok (1990) and Kok & du Preez (1998). It seems, therefore, that larval stages of different polystome species may differ in their response to the stimuli associated with the metamorphic changes in the host. Within anuran polystomatid parasites the intimate adaptation of the parasites' life cycle to the ecology of the hosts is well known (Tinsley, 1978, 1983; Murith, 1981*b*, 1982) Polystomatid parasites of anurans with a more terrestrial life style may reproduce by either a neotenic cycle or by a vesicular cycle within the urinary bladder (Murith, 1981*b*). In *Metapolystoma cachani* parasitizing *Ptychadena longiriostris* the life-cycle involves both a vesicular cycle in the adult amphibian and neotenic reproduction on the gills of the tadpole, thereby representing a pattern which could be considered a link between the Monogenea of aquatic hosts and those which are adapted to terrestrial hosts (Murith, Vaucher & Coombes, 1977) . The case in *P. africanum* which can multiply as egg-laying neotenics on the tadpoles appears similar (Combes, Bourgat & Salami-Cadoux, 1976). In contrast, *Eupolystoma alluaudi* and *Polystoma grassei* exhibit an internal direct cycle in the urinary bladder of their adult amphibian host, *Bufo regularis* and *Leptopelis ocellatus* (ref. Combes,1973; Combes *et al.*, 1976, Dupouy & Combes,1977; Fournier & Combes, 1979). Usually in aquatic hosts parasite reproduction is reported to be continuous but punctuated in the parasites of terrestrial hosts, ultimately leading to ovoviviparity in certain polystomatids (Tinsley, 1983). The rhacophorid hosts, i.e. *R. nigropalmatus*, *R. reinwardtii* and *P. leucomystax*, of *P. indicum* adults and larval stages are all arboreal, nocturnal frogs which live on bamboo and banana plants and though closely associated with water bodies are seldom found in them (Kiyasetuo, 1986). An intensive study would be required to determine whether the strategy of neoteny has no role in the life-cycle of *P. indicum*.

As observed in the present study there was no change in the length and width of the microhooks from the gyrodactyloid-I through the various post-gyrodactyloid-I stages up to the adult form of the fluke. However, quantification of haptoral sclerite parameters has been used as an important tool for species identification of polystomes particularly in case of African polystomes (Murith, 1981*a*). In *P. indicum*, a gradual increase in the dimension of hamuli was observed with the gradual increase in body size and development of the parasite towards the adult stages. Besides, variation with regard to the hamuli shape was found to be wide ranging. The hamuli primordia in the gyrodactyloid-I and the developing hamuli of the post-gyrodactyloid-I were much smaller than those of the adult form and lacked incised bases, whereas the juvenile and the adults exhibited varying depths of incision at the base within the same fluke and also among different specimens of the same species. Again, the shape and size of hamuli have been considered to be of taxonomic value and used as differential characters for species of many polystomes (Kok & van Wyk, 1986). In view of the variation observed in this regard in the present study the mere shape of the hamuli base and size should not be considered as authentic differential characters in respect of polystome taxonomy.

SUMMARY

The developmental pattern of *P. indicum* has been studied. The eggs of *P. indicum* are reddish brown in colour and operculate and the egg shell consists of a protein, sclerotin in nature. The egg-laying period under laboratory conditions lasted for 48 h during which time as many as 182 eggs could be harvested from a single fluke before it died. No oncomiracidium was recovered during the present study. Unciliated larvae with 16 microhooks, two pairs of eyespots and a pair of hamuli primordia representing gyrodactyloid-I stage were the first stage to be encountered from internal gills and/or urinary bladder of the tadpole host. The post-gyrodactyloid-I stages possess the features of bladder-destined forms and show a gradual acquisition of two, four and six opisthaptoral suckers, coupled with an increase in the size of the body and other structures. While a conspicuous increase is noticeable in the dimensions of the hamuli, the size of the microhooks remains almost the same from the two-suckered larval stage to the adult. The shape of the hamuli of larval and adult parasites may vary from smooth based to that with slight or deep incision at the base forming a distinct guard and handle. Variations in the length and depth of the incision between the two hamuli exist even within the same specimen. The larval and juvenile stages were always recovered from the internal gills, branchial chambers and urinary bladder of metamorphosing tadpoles and rarely from the intestine. The larval migration to the final destination seems to follow an external route leading to the cloaca of the metamorphosing host. The gyrodactyloid-II (i.e. neotenic larvae) were not

encountered at all either on the external gills of the very young tadpoles or in the branchial chambers of the older ones.

Acknowledgements

This work was supported by a grant to VT from the Department of Environment and Forest, Ministry of Environment, Government of India and partly by the DRS programme of the University Grants Commission, to the Department of Zoology, NEHU.

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