STUDIES ON THE MONOGENEAN PARASITES OF ANURAN AMPHIBIA IN MEGHALAYA, INDIA - MORPHOLOGICAL, ECOLOGICAL AND BIOLOGICAL ASPECTS

ABSTRACT

MAMONI DUTTA

THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF
DOCTOR OF PHILOSOPHY IN ZOOLOGY

To

THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA
DECEMBER, 1995

1. The anuran amphibian fauna of Meghalaya was explored for the occurrence of monogenean parasites. Various localities of the State were surveyed for the host collection. The host species collected include *Rhacophorus nigropalmatus*, *Rhacophorus reinwardtii*, *Polypedates leucomystax*, *Rana mawphlangensis*, *Rana malabarica*, *Rana limnocharis*, *Rana cyanophlyctis*, and *Amolops afghanus*, tadpoles and adults of all of which were examined for the recovery of parasites. The monogenean parasites spectrum was found to be represented by only two polystomatid species, namely *Polystoma indicum* Diengdoh et Tandon, 1991 and *Parapolystoma bulliense* (Johnston, 1912) Ozaki,1935. The present investigation deals with a detail study of the morphology, biology and prevalence of these two species.

2. *Polystoma indicum* Diengdoh et Tandon,1991, was found restricted to *R. nigropalmatus* and *R. reinwardtii* from Cherrapunji, Mawsynram and Shillong.

(i) The present study supplements the original morphological description of this species. The presence of 16 microhooks in the opisthaptor of the adult fluke is established. Scanning electron microscopic studies of the adult fluke revealed that the surface is devoid of any spines or scales but is coarsely covered with tegumental folds, ridges and craters. Minute tubercules abounding all over the surface give it a beaded texture. Tuberculate elevations or tegumental vesicles on the ridges possess pitted apices. Domed papillae, some pitted at their apices, encircle the oral sucker. The general surface of both the forebody and opisthaptoral disc has a fine tuberculate texture.

(ii) The nervous system could be visualized in toto with the localization of non-specific esterases. It exhibits a similar basic pattern as that described for other polystomatid species. The nervous system is bilaterally symmetrical. Cerebral ganglia, which are connected by a thick dorsal commissure, are present dorsal to the pharynx. From the cerebral ganglia arise five anterior pairs and four posterior pairs of nerve trunks. The anterior nerves and/or their fine branches join to form a conspicuous circumoral nerve ring complex. The posterior nerves unite in the opisthaptoral region to form two main haptoral nerves, the branches from which innervate the components of the opisthaptor. Presence of nerve cells is also revealed in association with the main nerves, more predominantly in the vicinity of the opisthaptoral suckers.

(iii) 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 189 eggs could be harvested from a single fluke. Surface fine topography of the egg surface reveals a pitted texture, uneven with small tubercules dispersed all over.

No oncomiracidium was ever recovered during the present study. Unciliated larvae with 16 microhooks, 2 pairs of eye spots and a pair of hamuli primordia representing gyrodactyloid - I stage were the 1st 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 2,4 and then 6 opisthaptoral clamps, 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 remain almost the same from the 2-clamped 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 distinct guard and handle. Variations in the relative length and depth of the incision between the two hamuli exist even within the same specimen.

Surface fine topography of these larval stages reveals the presence of transverse folds and ridges giving the surface a honeycomb-like texture. However, in post gyrodactyloid-Ic stage tegumentary vesicles or microvilli and button-like papillae are unevenly distributed and striae of anastomosing lamellae are observed to from concentric round patches with a cilium within at several places. The tegument of the prohaptor in all the stages has domed papillae, the latter having apical pits in post-gyrodactyloid -Ia and Ic stages. The tegument of the opisthaptor and that covering the clamps appears spongy.

The larval and juvenile stages were always recovered from the internal gills, branchial chambers and urinary bladder of the metamorphosing tadpoles and rarely from the intestine. The larval migration to the final destination seems to follow an internal 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 even in the branchial chambers of the older ones. Neotenic development thus does not seem to occur in *P. indicum.*

(iv) The prevalence of *P. indicum* infections was found to be quite low. The intensity of infection of adult flukes in adult hosts is lower than that of the larval stages recovered from metamorphosing tadpoles. No tadpole mortality was observed as caused by parasite burden. *P. indicum* also exhibits host specificity and restricted geographical distribution. A predilection towards *R. nigropalmatus* amongst the three rhacophorid hosts is indicated.

3. *Parapolystoma bulliense* (Johnston,1912) Ozaki, 1935, has been redescribed and forms the first report of its occurrence from India. *Rana malabarica* from Mawsynram and Tura (Meghalaya) forms a new host record for the species.

(i) The morphological details of the adult fluke tally with the original description except that the intestinal caeca, which are described to be without diverticula, exhibit varying degree of diverticulation in different individuals under the present study.

Surface fine topography of the adult fluke reveals the presence of ridges and valleys, with a honeycomb-like network. Tegumental vesicles are distributed unevenly all over the body. Pits are observed on the antero-dorsal surface.

(ii) The developmental biology of *P. bulliense* presents differences from *Polystoma indicum*. The eggs of *P. bulliense* are brown in colour, operculate and with a sclerotin-like egg shell. Both embryonated and unembryonated eggs are present in the uterus of the same fluke. In vitro the egg laying period lasted 60 h when 271 to 476 eggs could be harvested per fluke. Surface fine topography of the egg surface reveals blebs of different shapes and sizes all over the egg shell.

No oncomiracidia and/or other larval stages were ever recovered from the tadpole hosts. Fully developed oncomiracidium is present in the embryonated eggs in utero. Non-embryonated eggs, when kept in vitro conditions, developed oncomiracidia within 26 days at room temperature. The oncomiracidium, with 16 microhooks and 2 hamuli primordia on its opisthaptoral disc, has a randomly ciliated body, cilia emerging singly and not arranged in discrete pattern of ciliary plates.

Juvenile flukes were also recovered along with mature flukes parasitizing adult frogs. These resemble the adult fluke in all respects except for the absence of the reproductive components.

No neotenic larval stages were ever recovered from tadpoles of *R. malabarica*. An internal vesicular cycle of development seems to be operative in *P. bulliense*.

(iii) The prevalence and intensity of infection were found to be quite low. Restriction of *P. bulliense* to only *R. malabarica* is suggestive of a narrow host specificity of this monogenean species, too.

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DECEMBER, 1995

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I certify that the thesis entitled "Studies on the Monogenean Parasites of Anuran Amphibia in Meghalaya, India – Morphological, Ecological and Biological aspects" submitted by Ms. Mamoni Dutta for the Degree of Doctor of Philosophy in Zoology of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by her under my supervision. She has duly registered and the thesis presented is worthy of being considered for the award of the Ph.D degree. This work has not been submitted for any degree of any other University.

Date : 19. 12. 1995.
Place : Shillong

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Mamoni Dutta

Mamoni Dutta

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Preface

PREFACE

Anurans are found all over the world except in some Pacific islands, deserts and snow-clad mountains. They are a group among amphibia which forms the largest living order of the vertebrate animals. Frogs are utilized as model animals for a variety of biological studies the world over. In North-East India frogs have acquired an added value in that tailless amphibians (anurans) form a delicious food item for a section of native populations in the region. The value of frogs in medicine and food has been known since ages (Cochran,1961). Among the native populations of Nagaland and Meghalaya, like in many far-eastern countries, frogs are used for the treatment of various ailments (Kiyasetuo,1986). Frogs also act as final host to a wide variety of parasites such as protozoans and helminths; they are also utilized as transfer hosts in the life cycle of several helminth parasitic species. Frogs are also involved in several zoonotic infections which are transmittable to man. Of these, the most significant ones are gnathosomiasis and sparganosis. Highly endemic forms of these types of zoonoses are found occurring among populations where treatment of wounds by using raw flesh of frogs is practised (Hoeden,1964).

The varied climate, vegetation and topographical conditions prevailing in India provide a great range of environments which lead to high faunal diversities. The amphibian fauna is represented by 181 species (Inger and

Dutta,1986). All the three orders of Amphibia occur in India: Caudata (salamanders) - 1 species, Gymnophiona (caecilians) - 15 species and Anura (frogs and toads) - 165 species. Frogs and toads make up 91% of the species of Indian amphibia. The Indian fauna has a high proportion of frogs of the families Raniidae and Rhacophoridae and low proportion of Pelobatidae and Microhylidae. The Indian amphibian fauna as a whole is quite distinct, having endemic genera of raniid and microhylid frogs and caecilians, and a large number of endemic species of several widespread Oriental genera viz., *Bufo, Rana, Philautus* and *Icthyophis.* The two known areas of high endemism (highest concentrations of species and genera) are the North-Eastern region and the Western Ghats of the West Peninsula. Endemic species constitute 62% of the Indian fauna; 84 endemics occur in the Western Ghats while 20 in North-East. The only exceptional overlap between the North east and North west faunas involves four swift water breeders typical of mountainous areas: *Rana blanfordi, R. liebigi* and two species of *Amolops.*

Of about 181 species of anuran amphibians known to occur in the Indian sub-continent, 55 species belonging to 18 genera and 6 families are represented in the North-East region. Of the North-Eastern states, Meghalaya shows the highest representation of 39 species, Assam 23, Arunachal Pradesh 22, Nagaland 7, Mizoram 14 and Tripura 8 (Chanda,1992). This state possessing a super-humid climate with moderate temperature and heavy rainfall throughout the year offers a congenial environment for perpetuation of several frog species, particularly of families Raniidae and Rhacophoridae. Since these families show high endemism in the North-East region, the present investigation was taken up for thorough exploration of anuran amphibian fauna coupled with the monogenean parasites specific to the State of Meghalaya.

Valuable surveys of parasites of amphibians both in the tropical and temperate regions of the world have been made by a number of workers like Johnston (1912), Beauchamp (1913), Ozaki (1935), Mazurmovich (1951), Williams (1960), Lees (1962), Walton (1967), Combes (1968,1976),Vande Vusse (1976), Vojtková (1982), Kok (1990), du Preez and Kok (1992), Kearn (1993) and Tinsley (1993). Prudhoe and Bray (1982) provided a review of platyhelminth parasites of amphibia. Many Indian workers have also contributed to studies regarding the helminth parasites of amphibia from various localities in the country. Most noted among these are Mehra (1926), Bhalerao (1932), Srivastava (1933,1934,1937), Pandey (1937), Lal (1942,1944), Kaw (1943,1950), Bhardwaj (1963), Dwivedi (1965,1968) [ref. in Yamaguti,1971], and Fotedar (1966). However, from the North-Eastern region of India, the first studies in this direction were carried out by Diengdoh (1989), which pertain to a survey of some amphibian hosts in Meghalaya and record the occurrence of several species of helminth parasites from them.

Looking into the diversity of helminth fauna in amphibia in India, trematodes emerge as the most prominent group, of which 18 species have hitherto been recorded. Others in the faunal list include 4 genera of nematodes, 3 cestodes, 2 monogenea and 1 of acanthocephala. Of these, the occurrence of *Polystoma indicum* species reported from Meghalaya (Diengdoh and Tandon, 1991), the first ever report of the genus from the Indian Subcontinent, seems quite significant since the species of the genus are known to exhibit restricted geographical distribution and immense host specificity (Combes and Channing, 1979). The only other polystomid genus represented in amphibian hosts so far from India is *Eupolystoma* with its species reported from the northern region of the country.

Thus, in view of the richness of the anuran amphibian fauna coupled with a variety of climatic conditions occurring in different parts of Meghalaya, a thorough exploration and further studies on the monogenean fauna of amphibia are called for. Such studies would reveal the full extent of the diversity and host range of this group in this region. Besides, in the light of the remarkable host specificity and restricted geographical distribution exhibited by polystomatid monogenea, a study on their biology might prove to be a valuable model for polystomatid species of the Oriental region. It is expected that further studies on monogenea parasite fauna would supplement the information pertaining to the diversity of amphibian parasites in the region. It is therefore, intended to explore the anuran amphibians occurring in the climatically varying geographical regions in Meghalaya.

The objectives of the present investigation are:

1. to explore the monogenean parasites prevailing in the anuran amphibian hosts in Meghalaya;
2. to study the distribution and host-wise species composition of monogenean parasites, environmental influences and variability in host susceptibility, so as to reveal host specificity, if any of the prevalent species;
3. to study the surface fine topography, and detailed morphology of these parasites; and
4. to investigate into the biology of *Polystoma indicum,* which is seemingly exclusive to this region, and other monogenean parasites prevailing in the amphibian hosts of the region.

ABBREVIATIONS USED IN FIGURES

CG	-	cerebral ganglion
DNT	-	dorsal nerve trunk
E	-	egg
GiC	-	genito-intestinal canal
HG	-	haptoral ganglion
HN	-	haptoral nerve
LN	-	lateral nerve
LNT	-	lateral nerve trunk
MGN	-	marginal nerve
MLN	-	most lateral nerve
MMN	-	most medial nerve
MN	-	medial nerve
N	-	nerves supplying the suckers
NC	-	nerve cell
Od	-	oviduct
Oo	-	ootype
Ov	-	ovary
PN	-	pharyngeal nerves
S	-	sucker
T	-	tendon
U	-	uterus
ViD	-	vitelline duct
ViR	-	vitelline reservoir
VN	-	ventral nerve
VNT	-	ventral nerve trunk

Monogenea of Anuran Amphibia

MONOGENEA OF ANURAN AMPHIBIA

INTRODUCTION

The monogenea of Amphibia are mainly parasites of Anura and are represented by two orders, Monopisthocotylea and Polyopisthocotylea. The Monopisthocotylea of Amphibia are represented by two families Gyrodactylidae and Iagotrematidae with one genus and one species each namely, *Gyrodactylus elegans* and *Euzetrema knoepffleri*, respectively (Prudhoe and Bray,1982). While the latter species has so far been recorded as parasitic in the urinary bladder of caudate amphibians from North America and Europe, the former is essentially a gill and skin parasites of fishes; its occurrence in amphibian tadpoles probably indicates their usage as auxilliary or paratenic hosts and has been reported so far from erstwhile USSR, and Corsica. The Polyopisthocotylea of Amphibia are included in two families; Polystomatidae essentially includes the parasites of the urinary bladder, sometimes of the buccal cavity of the Anura, while Sphyranuridae are mainly parasites on the external gills and skin of aquatic Caudata from North America with one representative in the European *Proteus*. The family Polystomatidae has members that are essentially endoparasites of urinary bladder of tailless amphibia and further includes two subfamilies, Diplorchiinae and Polystomatinae. The former subfamily in which the testes are in two compact masses, is represented by three genera, namely, *Pseudodiplorchis* (characterized by the absence of large hooks in the opisthaptor and recovered from North American toads), *Diplorchis* (with vitelline follicles along intestinal caeca and occurring in Oriental frogs) and *Neodiplorchis* with two compact groups of vitelline follicles and from North American toads). the subfamily Polystomatinae, characterized by the testis forming a reticular or follicular mass, includes six genera, namely, *Protopolystoma* (vaginal apertures absent, and parasitic in *Xenopus* toads from Africa), *Polystoma* (uterus anterior to ovary; occurs in Palaearctic, Ethiopian, Nearctic and Neotropic regions, parasitic in anurans), *Parapolystoma* (uterus extending posteriorly beyond ovary; parasitic in Australasia and Oriental frogs), *Pseudopolystoma* (absence of vaginal apertures and intestinal caeca not joined posteriorly; parasitic in Japanese caudates and Australian lung fish), *Riojatrema* (ovary in anterior third of body; reported from anurans of Nearctic region) and *Eupolystoma* (ovary occupying posterior half of body; parasites in Indian and African frogs and toads).

A perusal of literature reveals that the anuran monogenea occur in all continents, except Antarctica. and are mainly parasites of Anura. *Polystoma* Zeder, 1800 is the most dominant and widespread genus having been recorded from Europe, Asia, Africa and South and North Americas. As per the published literature, this genus is represented by about 50 known species from anuran hosts the world over. Of these, only one, namely, *P. integerrimum* exhibits a wide ecological valency and is a polytypic species, widely distributed in several species of frogs in Europe (Prudhoe and Bray,1982; Vojtková,1989). A majority of species are reported from Central America and represent a '*Polystoma africanum*' species complex (Tinsley,1974), in which the species are differentiated on the basis of strict host specificity. The species of *Polystoma* are known to exhibit restricted geographical distribution and immense host specificity (Combes,1976; Combes and Channing, 1979; Kok and van Wyk,1986; Kok and Seaman,1987). *Polystoma hakgalense* Crusz et Ching,1975 from *Rhacophorus cruciger eques* in Ceylon emerged as the sole representative of the genus in the whole of the Oriental region. The occurrence of *Polystoma indicum* Diengdoh et Tandon, 1991 formed the first ever report of the genus from the Indian sub-continent. This species is so far found restricted to rhacophorid frogs inhabiting the humid montanous region of Meghalaya. Besides *Polystoma*, there are about 10 other polystomatid genera represented in anuran amphibia of the world. However, the only other polystomatid genus represented in amphibia so far from India is *Eupolystoma* with its species, *E. rajai* Kaw,1950 from *Rana* sp. and *E. chauhani* Pandey, 1969 from *Bufo* sp. in the Northern parts of the country. In addition to India, the occurrence of *Eupolystoma* in Africa seems significant since it is accepted that at one time these two regions were integral part of the same continent (Tinsley,1975).

The monogenean have been subject to numerous ultrastructural studies that cover more than 60 monopisthocotylean species and 70 polyopisthocotylean species. These studies pertain to external morphology and various organ systems and have provided valuable information on the fine surface topography, tegumentary receptors, digestive apparatus, protonephridia, nerve cells, reproductive ducts, oogenesis and eggs, vitellogenesis, spermiogenesis and spermatozoa (ref. reviews by Lyons,1973b, 1977; Rohde,1973; Halton,1982,; Threadgold, 1984; Ehlers, 1985; Gerasev and Yunchis, 1986; Galkin, 1988; Malmberg,1990; Rohde, 1990; Fried and Haseeb, 1991; Justine,1991a,b).

Electron microscopic studies pertaining to monogenean flukes are not many. However, significant contribution has been made in this line by several workers (Bychowsky, 1957; Llewellyn, 1963,1972; Kearn, 1967; Halton et al., 1968; Lyons, 1968, 1970a,b, 1971, 1972, 1973a; Morris and Halton, 1971; Bresciani, 1972; Erasmus,1972; Rohde, 1973; Tinsley, 1976, 1981; Lambert, 1981; Tinsley and Earle, 1983; du Preez and Kok, 1987). The bibliographical data on these aspects have been recently listed by Justine (1993). In respect of the surface fine topography, while information available for about 30 species of monopisthocotylean parasites, that pertaining to polyopisthocotylea covers almost the same or a little more number of species. However, among the polyopisthocotylea parasitizing the anuran amphibia only *Polystoma australis* and *P. integerrimum* adult forms and oncomiracidium of *P. australis* and a *Polystoma* sp. have been subject to this investigation (du Preez and Kok, 1987; Kok and du Preez, 1989; Mehlhorn et al., 1988; Beverly-Burton in Fried and Haseeb, 1991). Recently surface topographical details of both the bladder (normal) adult and branchial (neotenic) adult of *Polystoma integerrimum* have been provided by Williams and Mckenzie (1995).

Information pertaining to the nervous organization of monogenean parasites is available only for a few species. Andre (1910) provided the first morphological account of the nervous system of *Polystoma integerrimum.* Using histochemical localization of nonspecific esterases (NSE), Halton and Jennings (1962) described the nervous system of *Diplozoon paradoxum.* Utilizing histochemical techniques, nervous system in toto was also described for *Polystomoides* spp. and *Diclidophora merlangi* by Rohde (1968) and Halton and Morris (1962), respectively. Rahemo (1982) further worked out the nervous system of about 30 species of monogenea that included one polyopisthocotylean, using localization of cholinesterase in toto preparations. Rahemo and Gorgees (1987) also provided a detailed account of the nervous system of *P. integerrimum* using the same techniques. Advanced information is now available regarding the nature (serotoninergic, cholinergic and peptidergic) of the nervous components in *P. merlangi* (see Maule et al., 1990).

Majority of the trematodes possess a tough egg shell made up of resistant structural protein, sclerotin (a 'tanned' protein) but in amphistomes it is keratin. The protein chains in sclerotin are stabilized by disulphide bonds and these bonds are involved in quinone-tanned eggs. The egg shell precursors are mostly derived from vitellaria and minor quantity from Mehlis' gland. The egg shell precursors for sclerotin are phenols, protein and phenolase. The enzyme phenolase catalyzes the reaction (phenol $\xrightarrow{\text{phenolase}}$ quinone) and the quinone formed combines with the basic proteins to tan it and form sclerotin. Phenolic material in the newly formed egg shell is oxidised enzymatically to quinone which then forms cross-linkages between the adjacent protein molecules. With the use of diazo technique for phenolic substances and catechol technique for phenolase, the egg shell precursors have been identified in respect of monogenean parasites, viz., *Gastrocotyle trachuri* (see Freeman and Llewellyn, (1958), *Entobdella soleae* and *Diclidophora luscae* (see Llewellyn, 1965), and *Oögyrodactylus farlowellae* (see Harris,1983). Phenolase exists as an inactive precursor (prophenolase) in the vitellaria of the monogeneans, *Pricea* and *Protomicrocotyle* (see Ramalingam, 1970, 1971, 1973).

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 (ref. Prudhoe and 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). Other polystomids, viz., *Polystoma grassei* and *Eupolystoma alluaudi* exhibit an internal direct cycle, with multiplication of the parasite in the urinary bladder of the host (Dupouy and Combes, 1977; Fournier and Combes, 1979). The neotenic and pre-destined bladder stages are differentiable on the basis of their morphological features and establishment patterns (Kok and du Preez, 1987,1989; Kok, 1990).

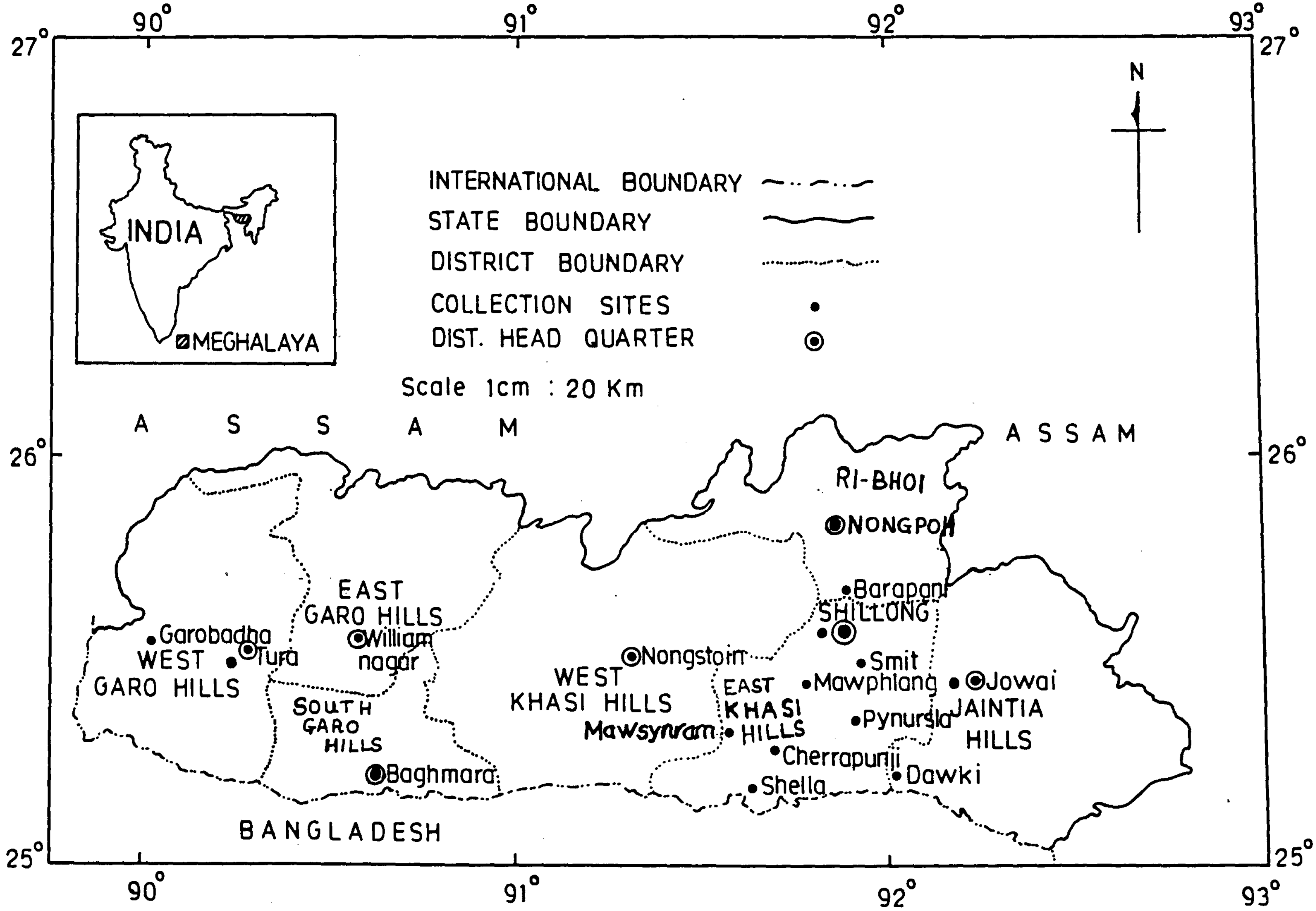
Material and Methods

MATERIAL AND METHODS

STUDY AREA

The study area includes several localities falling under the Khasi, Jaintia and Garo Hills of the State of Meghalaya which is situated between 25°10'N - 26°12'N and 80°49'E - 92°45'E (Fig.1). The State of Meghalaya, formed in 1972, signifies `the abode of clouds' lying in between the plains of Assam in the north and Surma in the south and being cornered by the districts, Dhubri, Goalpara and Kamrupa of Assam; by North Cachar and Karbi Anglong (Mikir Hills) in the east and by Bangladesh on the west and south, the southern boundary forming the international border of the country. Basing on the physio-geographical divisions, Meghalaya falls into three main regions, viz., (1) The Western Meghalaya (Garo Hills), (2) The Central Meghalaya (Khasi Hills) and (3) The Eastern Meghalaya (Jaintia Hills). Politically, Meghalaya has seven districts viz., the East Khasi Hills, the West Khasi Hills, the Jaintia Hills, Ri-bhoi (=Bhoilymbong), East Garo Hills, West Garo Hills and South Garo Hills. Meghalaya is also endowed with several mountain ranges (Tura range, Arbela range, Marangksih range and Barail range), plateaus (Shillong or Ri Khasi), peaks (Shillong peak, 1967 mASL; Nokrek peak, 1418 mASL), and ridges varying between 1050 and 1350 mASL. The hills of Meghalaya took shape during the Mesozoic and Tertiary times, representing just a small segment of the old ‘Gondwana land’ of

Fig.1 : Map of Meghalaya indicating the collection sites and localities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Nongstoin
EAST GARO HILLS
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SOUTH GARO HILLS
Baghmara
BANGLADESH

1

the pre-Cambrian period. Rocks composed of different minerals clothe the central plateau, but most of them are not of much economical values. Some of the important minerals with much potentiality are coal, limestone, silimanite and mineral oil (Marwein,1992). The flora of Meghalaya represented by forests accounts to about 8.5 lakh hectares of the total area of the state. About 7.5 lakh hectares account for the unclassified forests. Reserved forests, protected forests and National parks account for another 1 lakh hectares. Higher elevated areas are covered by pine and chestnut trees, while low lying regions, by mixed types of moderate and tropical forests.

Meghalaya experiences varied climatic conditions throughout the year influenced by the seasonal winds of the south west monsoon and north east winter winds. Varied climatic conditions prevail throughout the year. Different months undergo different seasons viz., March and April experience spring, May to September rainy season, October to November autumn and December to February withstand a winter season. The whole region of the North-East, especially Meghalaya, is the meeting place of the two rain bearing winds from the Bay of Bengal and the Arabian Sea, making the whole region a place of heavy rain. Due to this fact Cherrapunji, locally known as Sohra, and Mawsynram record the highest rainfall in the world. Both the villages are situated in

East Khasi Hills adjacent to each other on the southern platform overlooking the plains of Bangladesh. The average annual rainfall at Cherrapunji normally varies from 400 to 700 inches or 12000mm to 20000mm. The weather from October to April is mostly dry, while December and January are the coldest months of the year with heavy frost and fog at several places in the central upland region. Spring and Autumn are normally pleasant except that it is dry and windy before the onset of the monsoons.

The capital of Meghalaya, Shillong is situated at an altitude of 1500.2m (4908ft) above sea level. In winter the temperature goes below 10°C and in warm season goes up to 26°C. The annual average rainfall recorded is around 2000mm or 79 inches/annum because of its being situated in a rain shadow area.

HOSTS COLLECTED/SURVEYED

Of the anuran hosts, while mostly the members of the family Raniidae were abundantly available in Tura, Jowai, Shillong and its suburbs, the host collections from Cherrapunji and Mawsynram comprised predominantly the rhacophorids. During the study period repeated collections were made of the available species, both adults and tadpoles, from the mentioned localities. The host animals were collected from different spots in the same locality in morning hours or sometimes even at nightfall either with nets or with hands following the croaking sound of males or the nest constructed by the females (Cochran,1961) as in the case of *Rhacophorus* spp. In respect of rhacophorids, a major number of catches was possible between May and October, since they 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 spots where monogenean infection in adult frogs was evident. While most of them were autopsied at the earliest possible following collection, some tadpoles were also maintained in the aquaria and pond to allow them to grow to post metamorphosis stage in order to facilitate correct identification of the host species.

The host species, both adults and tadpoles, surveyed and their locality are listed in Table I. The sites and localities of collections are depicted in Fig.1.

Ecological Notes

Members of the genera *Rhacophorus* and *Polypedates*, all of which are arboreal, nocturnal frogs, live on bamboo and banana plants; though closely associated with water, they are seldom found in water. During the breeding season male croak loudly and select nest sites and spawning occurs in ditches or temporary

TABLE 1

Host species surveyed for monogenean parasites

Host species	Locality (mASL) of host collection	No.examined	Status of (monogenean) infections
ADULT FROG:			
Rhacophorus nigropalmatus, Boulenger	**Cherrapunji** (1341.02):		
	Mawsmai falls	244	+
	Mawblang	12	+
	Khoh-ramhah	1	–
	Nohkalikai falls	71	+
	Laitryngew	7	–
	Sohrarim	3	–
	Dewlieh	5	–
	Mawkdok	2	–
	Mawmihthied	3	–
	Ladmawphlang	4	–
	Mawlong	7	+
	Thangkharang	3	+
	Wahlong	5	–
	Mawsynram (1305.0):	17	+
	Phlangwanbroi	33	+
	Balat road	12	–
	Tura (1412.0):		
	Dakopgiri	3	–
	Denakgre	3	–
	Nehru Park	2	–
	Jowai (1369.14):		
	Mission Compound	7	–
Rhacophorus reinwardtii, Schlegel	**Shillong** (1500.20):		
	Nongthymmai	3	+
	Cherrapunji:		
	Mawsmai falls	81	+
	Mawblang	4	+
	Laitryngew	5	–
	Wahlong	12	+
	Nohkalikai falls	26	+
	Mawsynram:		
	Phlangwanbroi	56	+
Polypedates leucomystax, Kuhl (=*Rhacophorus leucomystax*)	**Cherrapunji**:		
	Mawsmai falls	18	–
	Nohkalikai falls	10	–

cont.

Host species	Locality (mASL) of host collection	No.examined	Status of (monogenean) infections
	Mawsynram:		
	Phlangwanbroi	3	–
Rana mawphlangensis Pillai and Chanda	**Cherrapunji**:		
	Mawsmai falls	25	–
Rana limnocharis Weigmann	**Shillong**:		
	Lalchand Basti	171	–
	Polo ground	12	–
	Golf link	21	–
	Nongthymmai	18	–
	Madanryting	10	–
	Happy Valley	21	–
	Ward's Lake	16	–
	Umpling	7	–
	Smit	83	–
	Cherrapunji:		
	Mawsmai falls	196	–
	Shella	340	–
	Nohkalikai falls	18	–
	Dawki	36	–
	Mawsynram:		
	Phlangwanbroi	56	–
	Tura:	85	–
	Jowai	34	–
Rana cyanophlyctis Schneider	**Shillong**:		
	Lalchand Basti	186	–
	Pologround	13	–
	Golf link	32	–
	Ward's Lake	21	–
	Smit	33	–
	Barapani	27	–
	Cherrapunji:		
	Mawsmai falls	138	–
	Mawsmai caves	12	–
	Nohkalikai falls	51	–
	Khoh-ramhah	31	–
	Umtyngar	61	–
	Dawki	14	–
	Jowai:	82	–

Cont.

Host species	Locality (mASL) of host collection	No.examined	Status of (monogenean) infections
	Tura:	107	–
Rana malabarica Tschudi,1838	**Shillong**:		
	Barapani	50	–
	Mawsynram:		
	Phlangwanbroi	4	–
	Tura:		
	Danakgre	119	–
Amolops afghanus Gunther	**Cherapunji**:		
	Mawsmai falls	217	–
	Mawmluh	12	–
	Nohkalikai falls	15	–
	Mawblang	8	–
	Laitryngew	9	–
	Wahlong	11	–
	Khoh-ramhah	17	–
	Mawsynram:	36	–
TADPOLES:			
R. nigropalmatus	**Cherrapunji**:		
	Mawsmai falls	2973	+
	Circuit House area	1038	+
	Mawblang	501	+
	Khoh-ramhah	226	–
	Nohkalikai falls	817	+
	Laitryngew	7	–
	Mawsynram:		
	Umjakoid	73	+
	Phlangwanbroi	412	+
	Shillong:	45	–
R. reinwardtii	**Cherrapunji**:		
	Mawsmai falls	201	+
	Circuit House area	75	–
	Mawblang	73	+
	Nohkalikai falls	137	+
	Mawsynram:		
	Phlangwanbroi	1646	+
	Balat road	711	–

cont.

Host species	Locality (mASL) of host collection	No.examined	Status of (monogenean) infections
Polypedates leucomystax	**Cherrapunji**:		
	Mawsmai falls	1189	+
	Nohkalikai falls	176	+
	Mawsynram:		
	Phlangwanbroi	341	-
Rana mawphlangensis	**Cherrapunji**:		
	Mawsmai falls	65	-
R. limnocharis	**Cherrapunji**:		
	Nohkalikai falls	177	-
	Mawsynram:		
	Phlangwanbroi	234	-
	Jowai:	253	-
	Tura:	131	-
R. cyanophlyctis	**Cherrapunji**:		
	Dawki	213	-
	Shella	371	-
	Jowai:	441	-
	Tura:	183	-
R. malabarica	**Shillong**:		
	Barapani	81	-
	Mawsynram:		
	Phlangwanbroi	171	-
	Tura:		
	Danakgre	25	-
Amolops afghanus	**Cherrapunji**:		
	Mawsmai falls	341	-
	Nohkalikai falls	118	-
	Circuit House area	151	-
	Mawsynram:		
	Phlangwanbroi	174	-

rain pools. Eggs are small, pigmented, deposited in a foamy nest on grassy edges, branches overhanging water surfaces, or on rocks 60-90 cm away from the water bodies. The development of eggs after hatching is directly dependent upon rain which flushes the eggs into the pool of water after which tadpoles develop within a month. During the later part of breeding season increased number of frogs are on the trees and decreased numbers in the water bodies; with advancing monsoon they move to trees and below the covering sheaths of banana stems. The onset of winter and dry season make them hide in hollow trees; the frogs start entering the ground litter and finally hibernate till the winter season is off and spring brings in a new start which causes them to move to bamboo holes and crevices in trees (Kiyasetuo,1986).

Of the raniid species, *Rana limnocharis* is usually found in paddy fields, pools or lotic waters throughout the year. *Rana cyanophlyctis* occupies the warmer places in paddy fields, ditches, and pools during summer, autumn and winter. It is a non-hibernating species. *Rana mawphlangensis* is found in swift stream waterfalls during autumn. *Rana malabarica* occupies marshy places near ponds and swift flowing waters. Spawning takes place in summer before the rains, whereby the eggs are deposited on the grass masses where the water level is low enough to cover only the roots. By mid-summer the tadpoles develop to juvenile froglets which hide underneath the grass and aquatic plants or stones in close contact with water. With the approach of winter, they hide in holes and under forest litter. They are nocturnal dwellers; in dark they come out to the land and males start croaking aloud. At night during summer the catches are maximum, as they are found sitting on the land, stones and shrubs adjacent to the waterpool. Catches are not possible during day time, as the frogs hide underneath the plants and soil to protect themselves from the scorching rays of the sun.

RECOVERY OF PARASITES

Anurans under confined conditions do not feed and at the same time they tend to get rid of their worm burden (Smyth and Smyth,1980). Therefore, immediately on returning to the laboratory the hosts were autopsied for the recovery of parasites. The host animals were either pithed or narcotized with chloroform and left for sometime until they became fully unconscious. Various organs, viz., buccal cavity, lungs, intestine, urinary bladder, ureter, gall bladder, mesentries and muscles of limbs and abdomen of the adult hosts were thoroughly examined. The parasites obtained were washed thoroughly in 0.7% saline solution and processed as detailed vide infra.

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

METHODS OF PROCESSING

(i) Whole mount preparations

Live specimens recovered were narcotized with a few drops of 70% alcohol. For flattening them, the specimens were tied gently between a glass slide and a cover slip. 70% alcohol and Bouins were used as fixatives. Borax carmine and Gower's carmalum were used as general stains. After their differentiations in 70% acid alcohol, the stained specimens were dehydrated through graded series of alcohol, cleared in methyl benzoate and mounted in Canada Balsam or DPX.

Specific staining methods were used for visualizing vitellaria, egg shell precursors and nervous system as follows :

a) Vitellaria : The catechol technique following Johri and Smyth (1956) was used. Flattened specimens, fixed in 70%

ethanol for 30 min to 12 h., were placed in a solution of 0.1% freshly prepared catechol for 15 min to 1 h. Brown tanning of the phenolase sites in the vitellaria revealed the extent of their distribution in the parasite.

b) Egg shell precursors : Following the technique after Smyth and Smyth (1980) the specimens fixed in 70% alcohol were placed in 1% freshly prepared Fast Red Salt B for several (3 to 15) minutes. The presence of phenolic shell precursors in the vitelline cells, indicated by yellow or red colour, helped in distinguishing the vitellaria from the testicular follicles.

c) Nervous system : The technique followed was basically that of Holt and Withers (1952). The specimens were fixed in cold 10% neutral buffered formalin for 6 h to overnight. After washing them in cold distilled water several times the specimens were incubated at room temperature and/or 37°C for 12 - 18 h in the standard bromo-indoxyl acetate incubating medium (0-acetyl-5-bromo indoxyl- 1.3 mg, dissolved in 0.1 ml ethanol and mixed with 2.0 ml of 0.1M tris, 1.0 ml of 0.05M potassium ferricyanide, 1.0M calcium chloride, and final volume made to 10 ml with distilled water) at pH between 5.0 and 5.5. Stained specimens were dehydrated with ascending ethyl alcohol grades and mounted in Canada Balsam. Various nervous elements were observed in whole mounts with the localization of NSE by their deep indigo blue colour. The terminology has been used according to Rahemo and Gorgees (1987).

(ii) Histological sections :

The Bouins-fixed material was processed for paraffin embedding. Serial sections cut at a thickness of 6-8um were stained with double staining technique using haematoxylin and eosin.

(iii) Scanning electron microscopy

Live specimens, after thorough washing in 0.7% saline were fixed in 4% cold neutral phosphate buffered formalin for 12-18 h. Following fixation, the specimens were washed in phosphate buffer and dehydrated with ascending grades of ethanol and ethanol-amyl acetate mixtures to pure amyl acetate. After their final treatment in dry amyl acetate, the specimens were critical point dried (CPD) using liquid carbon dioxide. Besides CPD, the specimens after washing in phosphate buffer were dehydrated in ascending grades of acetone and treated with Tetramethylsilane [TMS- $(CCH_3)_4$ Si, boiling point 26.3°C, surface tension 10.3 dynes/cm at 20°C] for 10 min and dried off TMS at 25°C following the method of Dey et al. (1989). The CPD or TMS-dried samples were placed horizontally on brass stubs with double-sided sticky tapes, metal coated with gold in a fine coat Ion Sputter JFC-

1100 (JEOL). Observations were then made with scanning electron microscope JSM 35CF (JEOL) under an electron accelerating voltage ranging between 10 and 20 kv.

Light microscopic observations were made under Wild M5 APO stereoscopic and Leitz Ortholux-2 research microscopes. Measurements were taken with the help of occular and stage micrometers and, unless and otherwise stated, are in mm and based, to the extent possible, on 10 specimens in each case.

The following formula was utilized for finding the standard deviation of the different morphometric measurements

$$\text{Standard Deviation} = \frac{X^2 - N\,\bar{x}^2}{N - 1}$$

where X = sum of measurements

N = number of observations

and $\bar{x}$ = mean

(iv) Culture of eggs :

During incubation, the parasites' eggs were maintained in tap water in petridishes at a temperature of 23°C(±1°C). Eggs hatched within the 10th or 11th day under normal conditions. Those eggs which did not hatch after the stipulated time were discarded after 20 days if hatching did not take place spontaneously.

Polystoma indicum Diengdoh et Tandon, 1991

Polystoma indicum Diengdoh et Tandon, 1991

Polystoma indicum Diengdoh et Tandon,1991, was originally reported from the rhacophorid frog, *Rhacophorus nigropalmatus*, from Cherrapunji, East Khasi Hills District of Meghalaya state,India. In an exploratory survey conducted for monogenean parasites of anuran amphibia of the State, immature and mature adults of *P. indicum* were found to parasitize another species of rhacophorid frogs, *Rhacophorus reinwardtii*, inhabiting the humid montanous areas of the region such as Cherrapunji, Mawsynram and Shillong, and their adjoining areas. The present study supplements the original morphological account of the species and incorporates observations pertaining to the surface topographical details and biology of the parasite.

Order - Polyopisthocotylea Odhner,1912

Family - Polystomatidae Carus,1863 emended Gamble,1896

Genus - *Polystoma* Zeder,1800
Polystoma indicum Diengdoh et Tandon,1991

TABLE 2

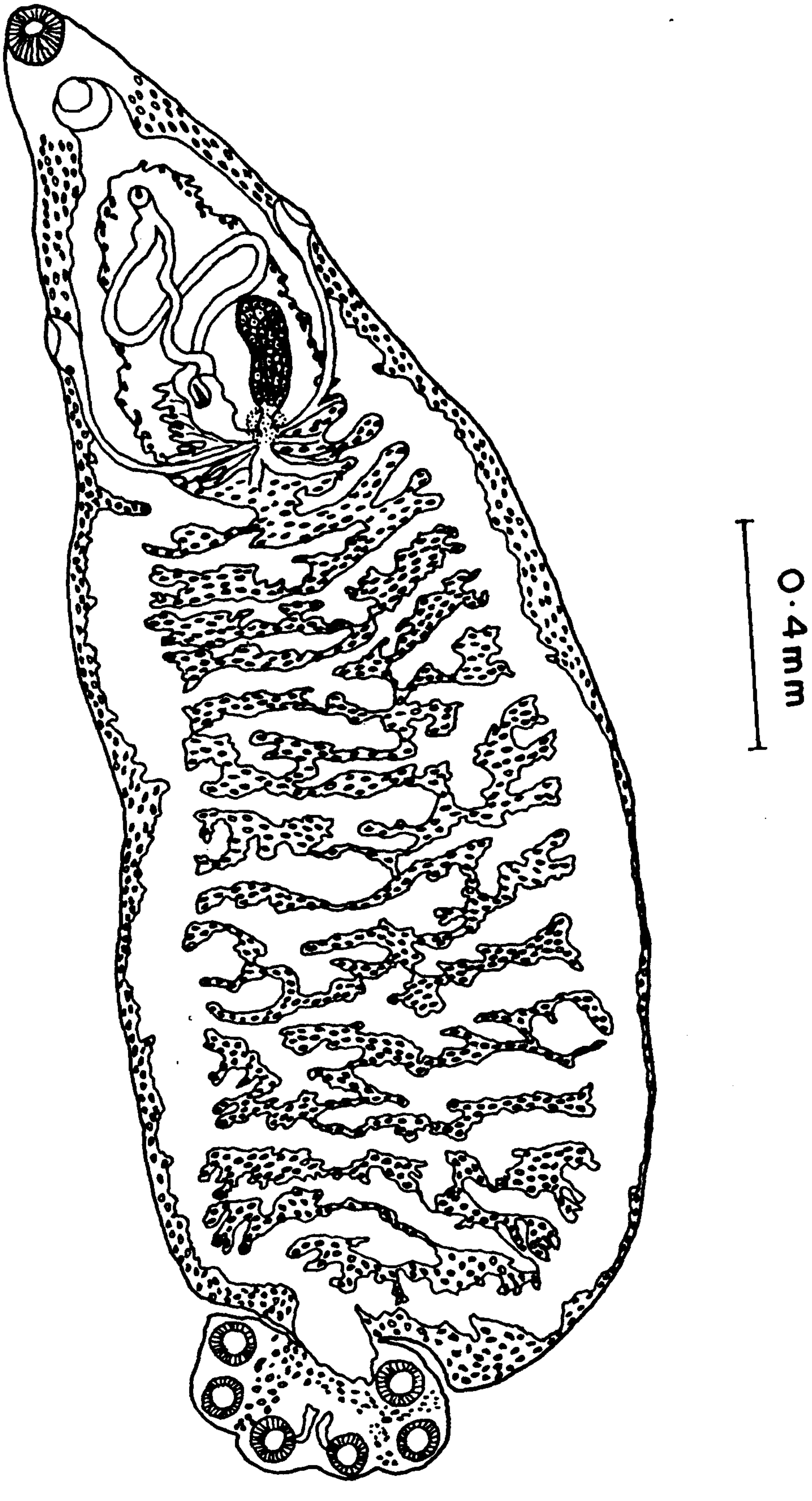
Morphometric measurements (in mm) of adult *Polystoma indicum* Diengdoh et Tandon,1991

Characters		Present observations		Observations according to Diengdoh and Tandon 1991	
		Range (Mean)	S.D	Range (Mean)	S.D
Body	Length	6.74 - 19.20(12.68)	±5.04	6.732 - 16.614(12.556)	±3.513
	Breadth (maximum across mid-body region)	1.66 - 6.24(4.49)	±1.73	2.222 - 5.641(4.164)	±1.061
Oral sucker	Length	0.29 - 0.53(0.39)	±0.08	0.186 - 0.532(0.390)	±0.11
	Breadth	0.39 - 0.64(0.47)	±0.07	0.236 - 0.574(0.466)	±0.126
Ratio of oral sucker to body length		1:23.24 - 1:36.22 (1:32.51)	-	1:31.229 - 1:36.193 (1:32.194)	-
Opisthaptor	Length	0.50 - 1.68(1.19)	±0.43	0.977 - 1.710(1.298)	±0.264
	Breadth	1.28 - 2.47(1.87)	±0.50	1.584 - 2.466(2.104)	±0.316
Ratio of opisthaptor to body length		1:11.42 - 1:13.48 (1:10.65)	-	1:6.89 - 1:9.715 (1:8.30)	-
Clamps	Length	0.22 - 0.54(0.39)	±0.11	0.264 - 0.465(0.353)	±0.065
	Breadth	0.22 - 0.54(0.39)	±0.11	-	-
	Length	0.13 - 0.41(0.27)	±0.09	0.178 - 0.414(0.320)	±0.094

cont.

Hamuli (microhooks)					
	Breadth	0.09 – 0.21(0.16)	±0.03	–	–
	Handle length (x)	0.26 – 0.46(0.35)	–	–	–
	Guard length (y)	0.23 – 0.40(0.296)	–	–	–
	$^{x}/_{y}$ ratio	$^{0.35}/_{0.296}$ ≑1.182	–	–	–
Microhooks	Length	0.02 –	–	–	–
	Breadth	0.003 –	–	–	–
Egg	Length	0.18 –	–	0.190 – 0.214(0.205)	±0.008
	Breadth	0.1 –	–	0.125 – 0.140(0.132)	±0.006
Host		*Rhacophorus nigropalmatus,* *Rhacophorus reinwardtii*		*Rhacophorus nigropalmatus*	
Locality		Cherrapunji (25°18'N:91°46'E)		Cherrapunji	
		Mawsynram (25°10'S:91°40'W)			
		Shillong (25°35'N:91°53'E)			

Fig.2 : Adult form of *Polystoma indicum* - whole mount



2

1. ADULT WORM

(i) Morphology and Surface Topography (Figs. 2 - 23)

Description (based on 1 egg-containing and 20 without eggs flukes). Body flat, elongated, leaf like, broader in the middle, tapering towards anterior region. Mouth subterminal, surrounded by prohaptor in the form of well developed oral sucker. Pharynx muscular; intestinal caeca consisting of number of indentations on outer lateral sides and branched diverticula on inner side, caeca uniting near region of opisthaptor. Opisthaptor with 6 prominent clamps or suckers, 16 microhooks and a pair of hamuli; the shape and size of hamuli in the adult fluke is varied with a smooth base in some, with slight incisions or deep incision at the base of the hamulus in others; of 16 microhooks, 1 in each clamp, 2 pairs below hamuli, 3 pairs in anterior region of opisthaptoral disc. Testis in form of numerous follicles forming a mass in post ovarian region; cirrus sac small. Genital pore just behind bifurcation of caeca, surrounded by a circlet of 8 small hooklets. Ovary elongate, Mehlis' gland complex clear, uterus running anteriorly in zig-zag course, only one intra-uterine egg observed; vaginal opening on either lateral sides; vitellaria extensive, lateral to caeca from level of pharynx to that of ovary, in post-ovarian region occupying intercaecal zone, also interspersed with testis (as revealed by the specific staining technique followed), extending into opisthaptoral disc. Genito-intestinal canal present. (Figs. 2-7).

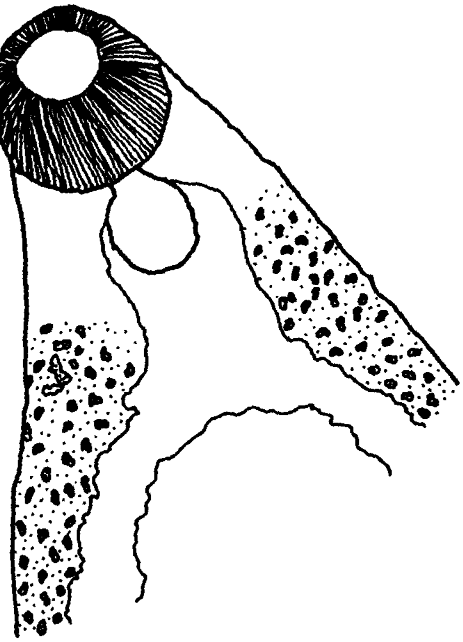
Figs.3-6 : *Polystoma indicum* - whole mount preparations

3 : Enlarged view of anterior region

4 : Mid-body region, showing the various organs in the reproductive system

5 : Opisthaptor with 6 well-developed clamps with one microhook in each, and 2 large hamuli

6 : Crown of genital hooklets



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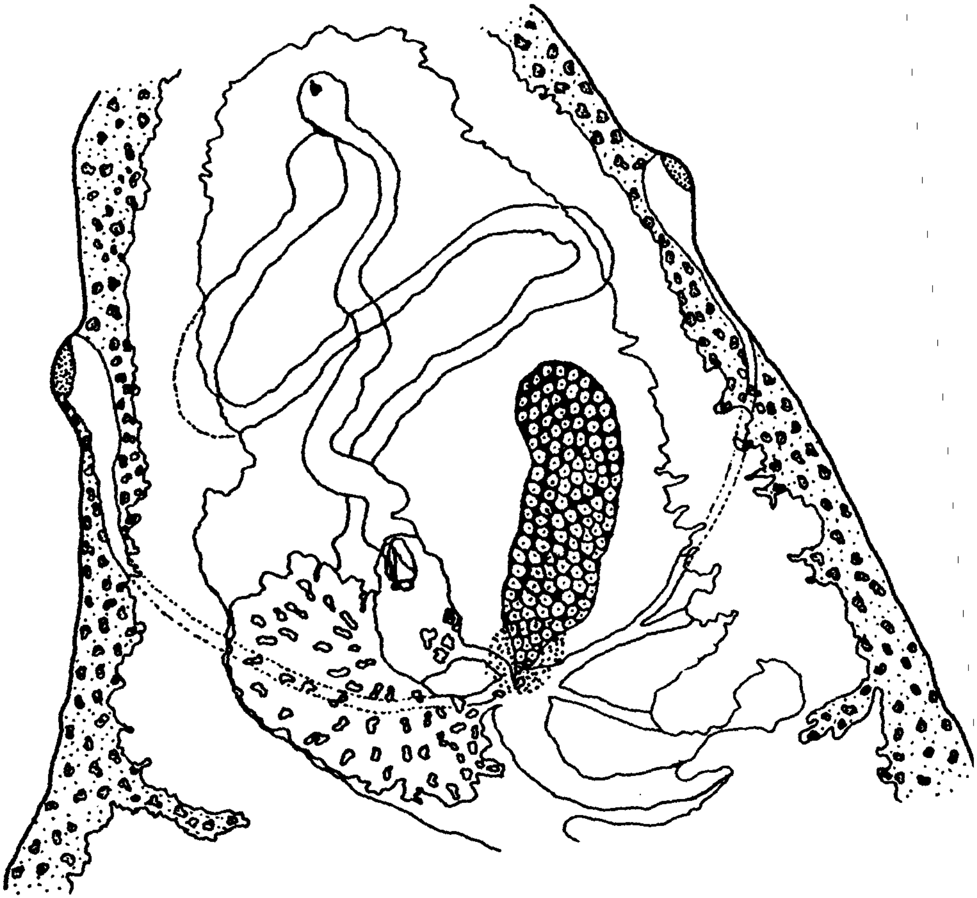

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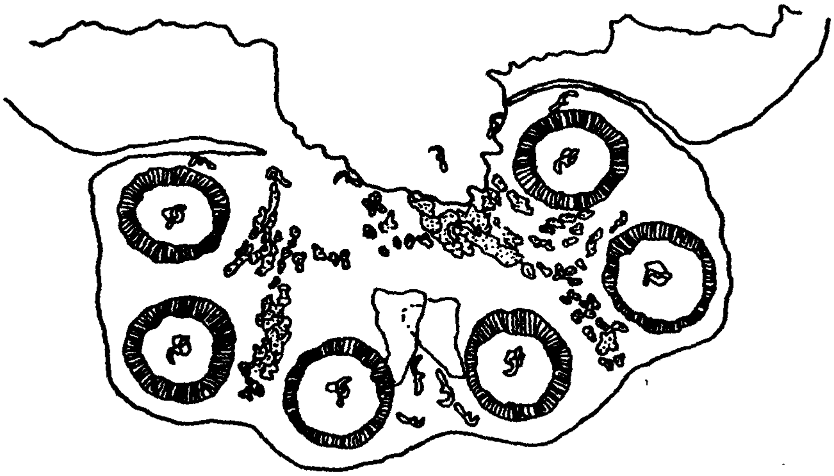

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6

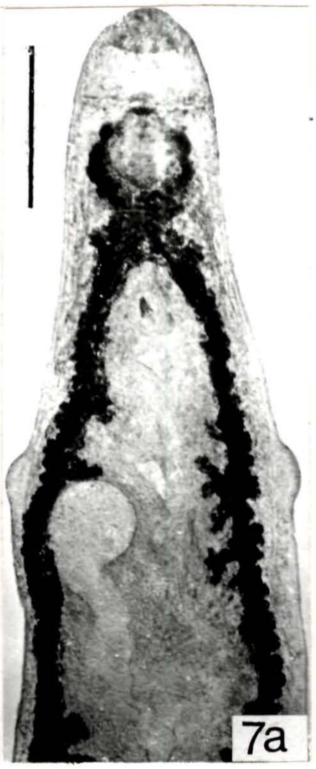
Fig.7 *Polystoma indicum* : photomicrographs

a : Anterior one third of the fluke (scale bar 1 mm)

b,c: Mid and posterior regions of the body, showing diverticulation of the intestinal caeca (scale bar 1 mm)

d : Anterior end, an enlarged view (scale bar 0.05 mm)

e : A portion of the opisthaptor enlarged, showing 2 clamps and hamuli (scale bar 0.05 mm)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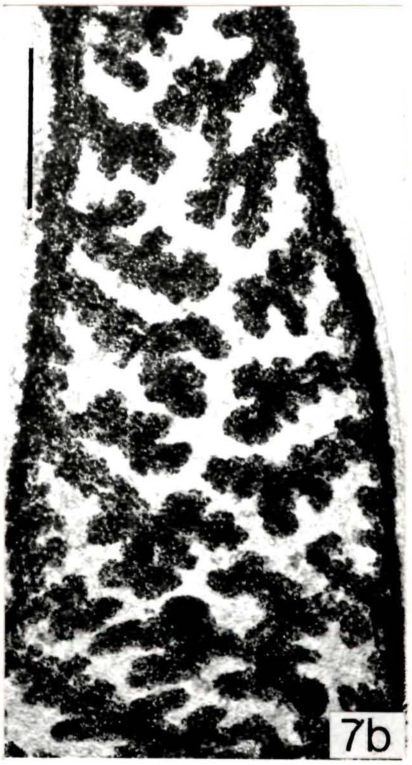
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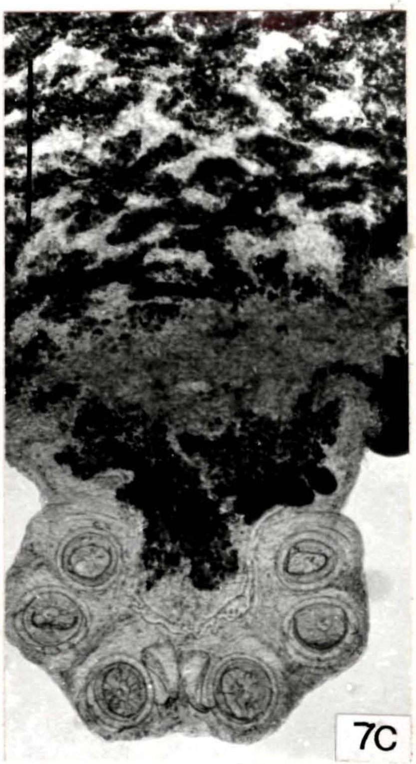
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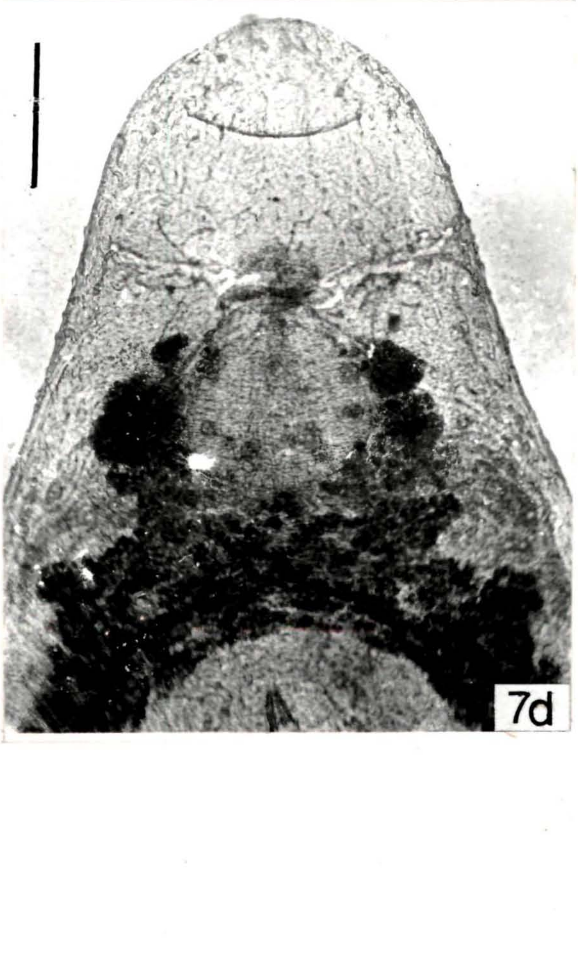
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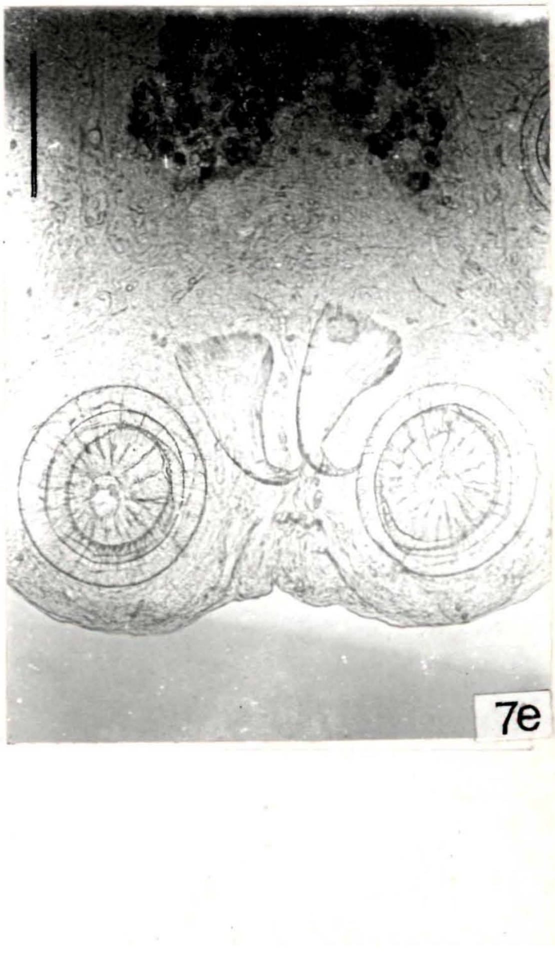

7e

The most important measurements of the body and organs and their comparison with the original description are given in Table 2.

Surface Fine Topography

Scanning electron microscopic observations on the adult fluke reveal two distinct parts of the body: the dorsoventrally flattened and bluntly tapering anteriad forebody and the suctorial disc-like hindbody, the opisthaptor (Fig.8). The prominence of this division is partly dependent on the individual size and also on the degree of muscular contraction in the forebody and opisthaptor. The tegument of the forebody is thrown into fine transverse ripple-like folds with ridges and craters, though the general surface appears smooth, devoid of spines, hooks or scales (Fig.9). However, at higher resolution, the surface covering both the ridges and the craters is revealed to have minute tubercules giving it a finely beaded texture (Fig.10). Tiny elevated structures are coarsely scattered here and there on the ridged areas of the tegument and at higher resolution they are revealed to possess pitted apices (Fig.11). However, there seems to be no localized or discrete pattern of distribution of these elevated structures.

The prohaptor in the form of a subterminal oral sucker encircles the antero-ventrally directed mouth (Fig.12). The

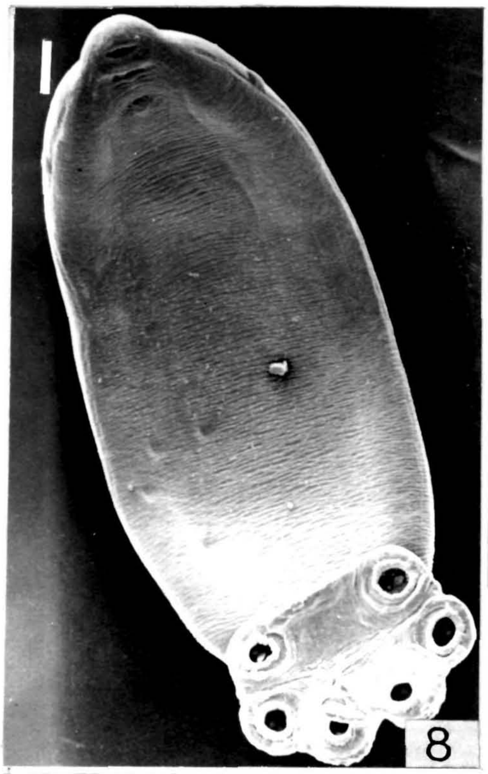
Figs.8-11 : *Polystoma indicum* : Scanning electron micrographs

8 : Whole worm in ventral view (scale bar 100 µm)

9 : Tegument of the general body surface (scale bar 10 µm)

10: Tegumental ridges and valleys (scale bar 10 µm)

11: Domed papillae on tegumental surface (scale bar 10 µm)



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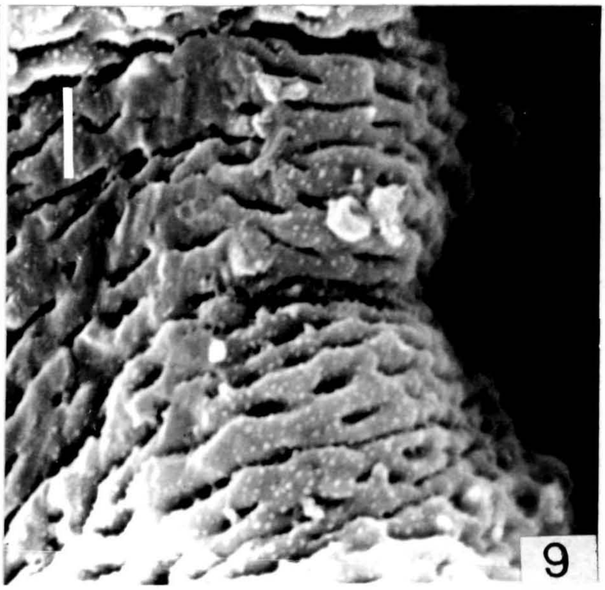

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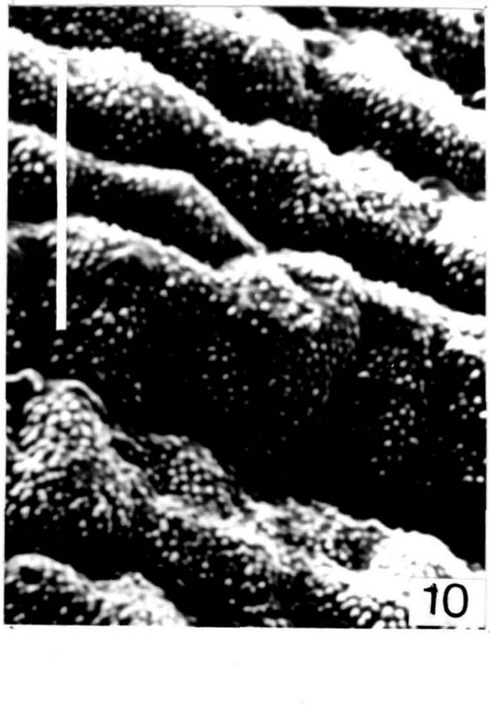

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11

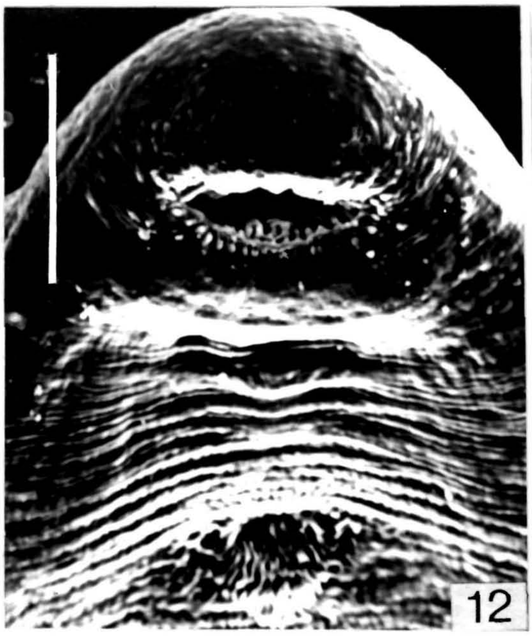
Figs.12-15 : *Polystoma indicum* : Scanning micrographs

12 : Anterior region, showing prohaptor and genital opening (arrows) (scale bar 100 um)

13 : A portion of the oral sucker enlarged, showing the random distribution of oral papillae (scale bar 10 um)

14 : Oral papillae under high resolution (scale bar 1 um)

15 : Genital opening (scale bar 10 um)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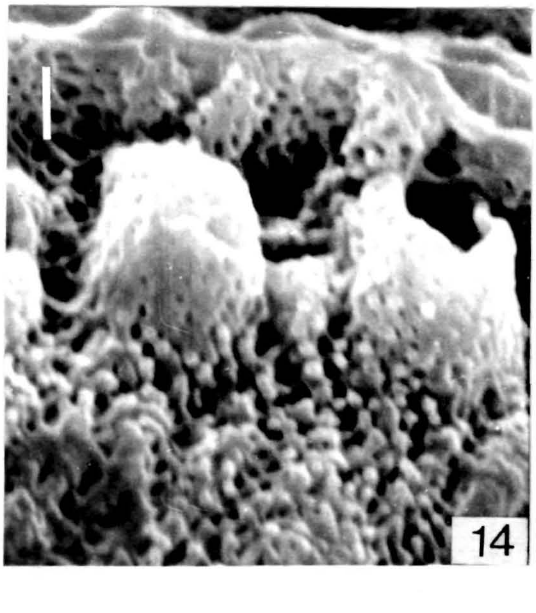
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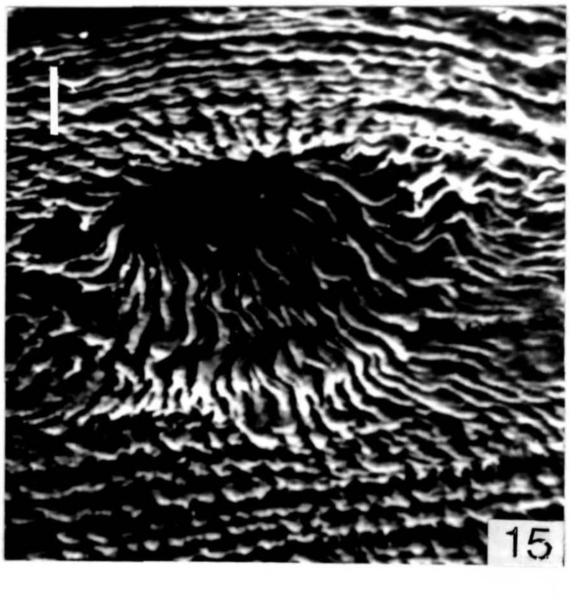
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circumoral tegument has irregular radial folds and prominent domed papillae are clustered along the rim of the sucker (Fig.13). Some of these domed papillae are seen to possess depressions or pits at their apices. Each papilla appears to be a tegumental protrusion with a granular surface covered with densely packed tegumental vesicles (Fig.14).

The tegument in the region of the genital pore has a distinct pattern of folds (Figs.15,16). The inner facet of the genital pore has radially directed tegumental folds devoid of spines, pits or papillae. At a higher resolution the tegumental texture of this region resembles that of the general body tegument (Fig.17).

The vaginae appear as protrusions along the lateral margins in the anterior middle-third of the body with an apical depression, the vaginal pore. The transverse folds of the general body continue over the vaginal protrusions (Fig.18). A clear view of the tegument here appears similar to that of the midbody surface, possessing domed papillae with apical pits.

The hind body comprises a prominent six-lobed opisthaptoral disc (Fig.19) with six haptoral clamps, a pair of hamuli and 16 microhooks. The general tegument of the opisthaptoral disc which is not occupied by the clamps presents a

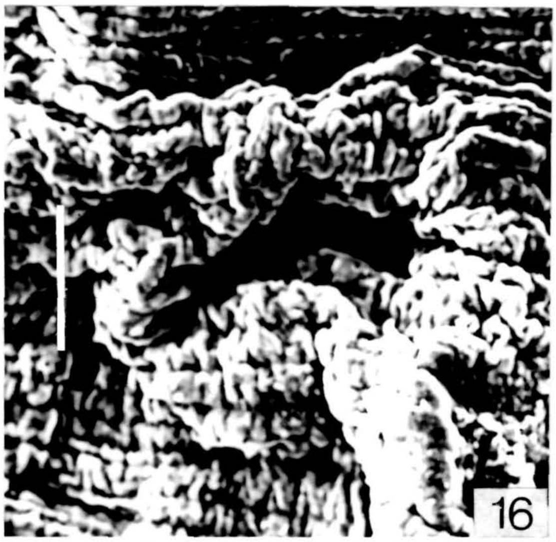
Figs.16-19 : *Polystoma indicum* : Scanning electron micrographs

16 : Genital opening in another specimen (scale bar 10 µm)

17 : A portion of the genital pore enlarged, showing inner (arrows) and outer walls (scale bar 10 µm)

18 : Vaginal protrusion on lateral margin of the fluke (scale bar 10 µm)

19 : Opisthaptor of adult worm (scale bar 100 µm)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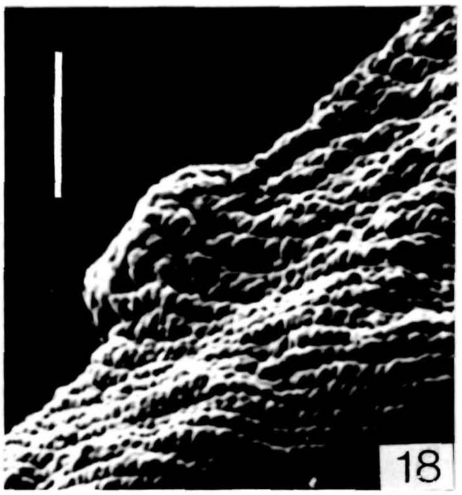
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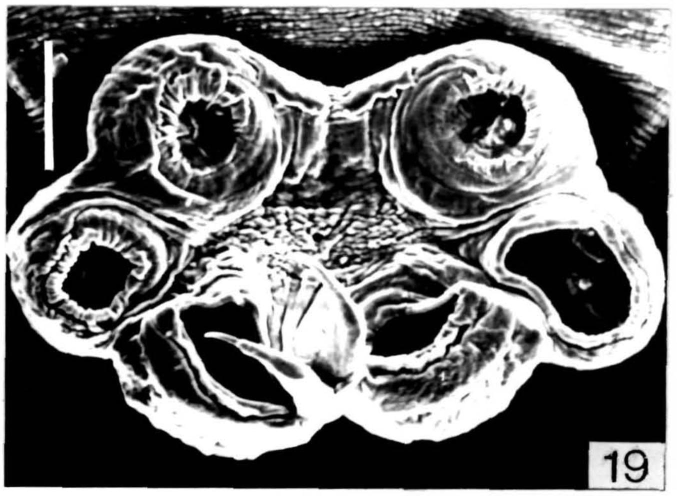
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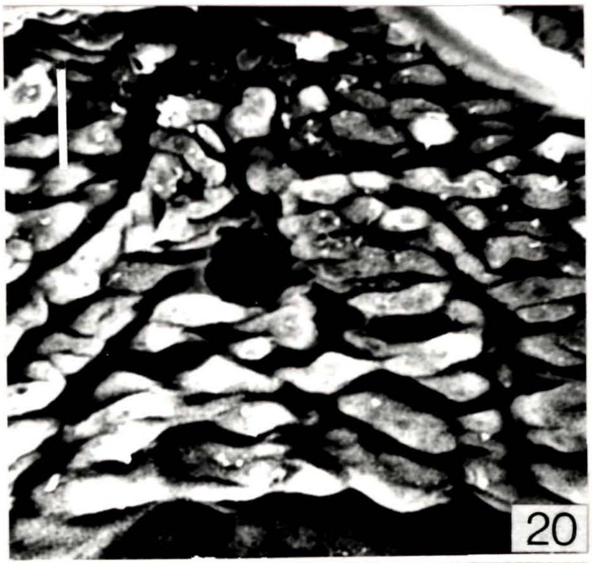
Figs.20-24 : *Polystoma indicum* : Scanning electron micrographs

20 : Tegument of the opisthaptoral region surrounded by the 6 clamps (scale bar 10 µm)

21-22 : A single opisthaptoral clamp showing different tegumental texture (scale bar 10 µm)

23-24 : A closer view of the opisthaptoral clamp with inner and outer surface magnified (scale bar 10 µm)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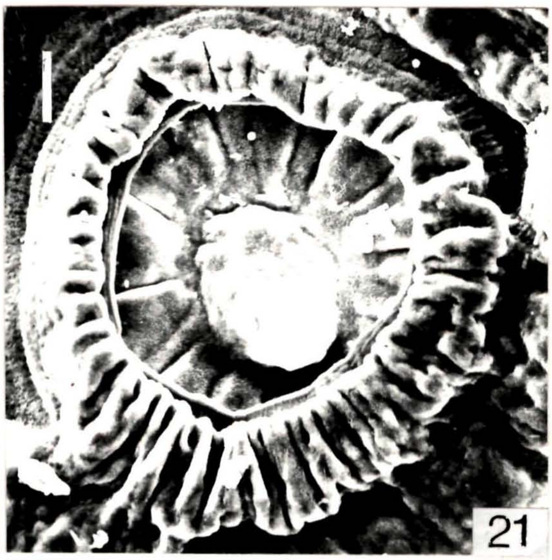
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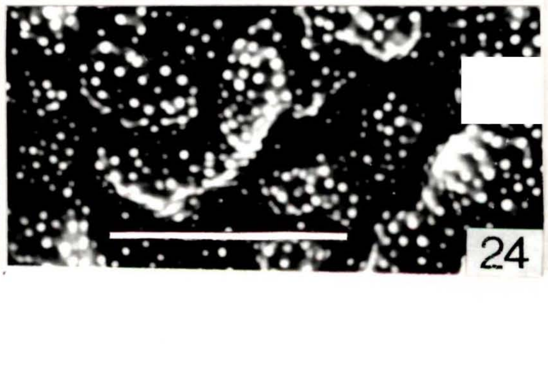
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corrugated appearance with irregular folds (Fig.20) but at higher resolution is observed to have a similar beaded texture as that of the forebody. Each individual clamp is conspicuously delimited from its neighbour with its rim having prominent radially folded tegument (Figs.21,22). The fine tuberculate texture of the general tegument continues over the tegument of the clamp floor as well as that surrounding the clamps (Figs.23,24).

Host - *Rhacophorus nigropalmatus, R.reinwardtii*
Location - Urinary bladder, ureter, cloacal chamber
Locality - Cherrapunji, Mawsynram, Shillong

(ii) **Nervous system**

The bilaterally symmetrical nervous system of *Polystoma indicum* could be revealed in toto. On the antero-lateral sides of the pharynx there are two prominent cerebral ganglia connected by a thick dorsal commissure. From the cerebral ganglia arise five anterior pairs of short nerves and four posterior pairs of distinct nerves (Figs.25,27-29).

Among the anterior nerves (Figs.25,27-29), there is a pair of most medial nerves, each of which arises from the anterior facet of the cerebral ganglion of either side and

Fig.25 : Nervous system of *Polystoma indicum* - anterior region, diagrammatic representation

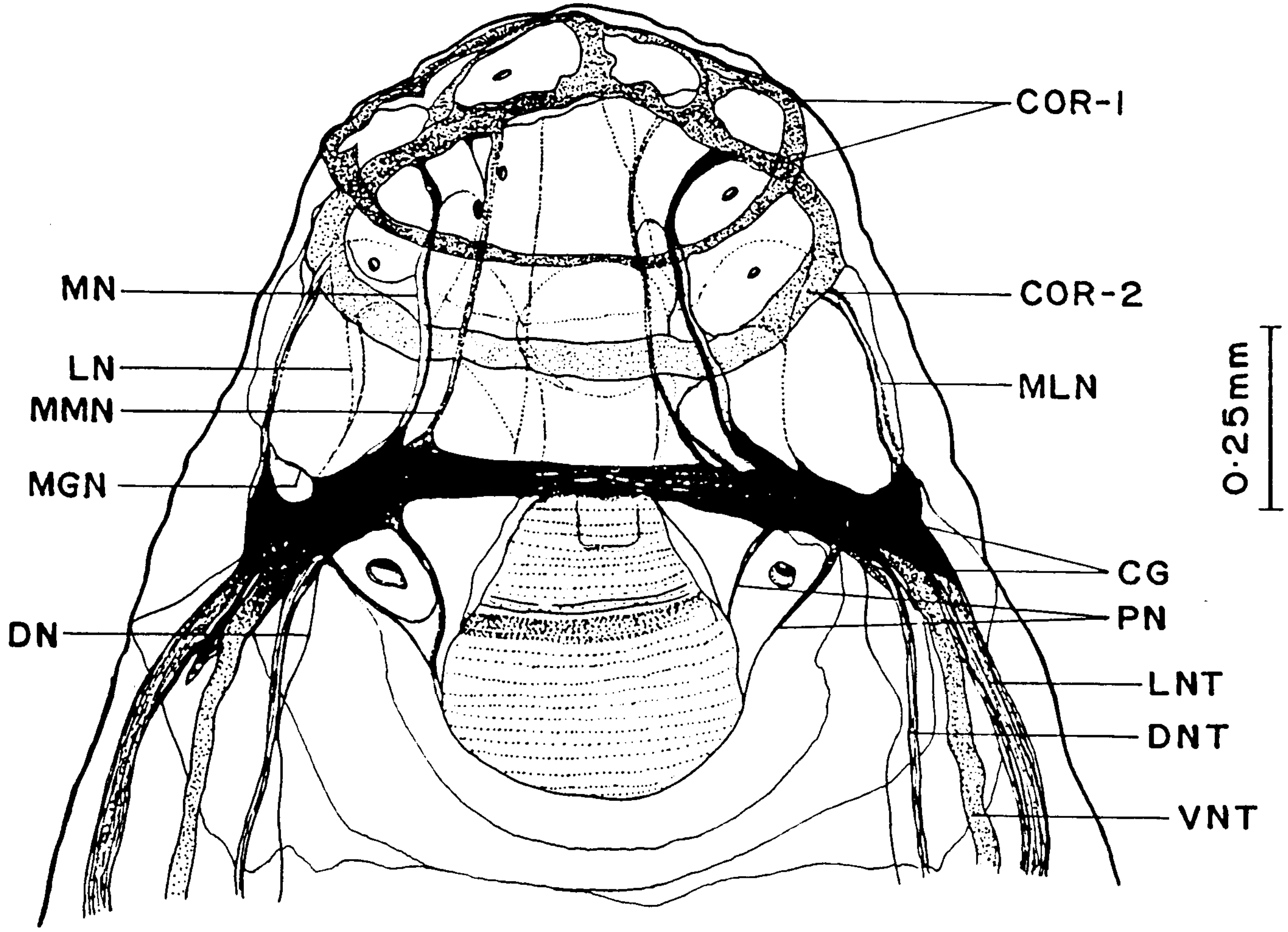

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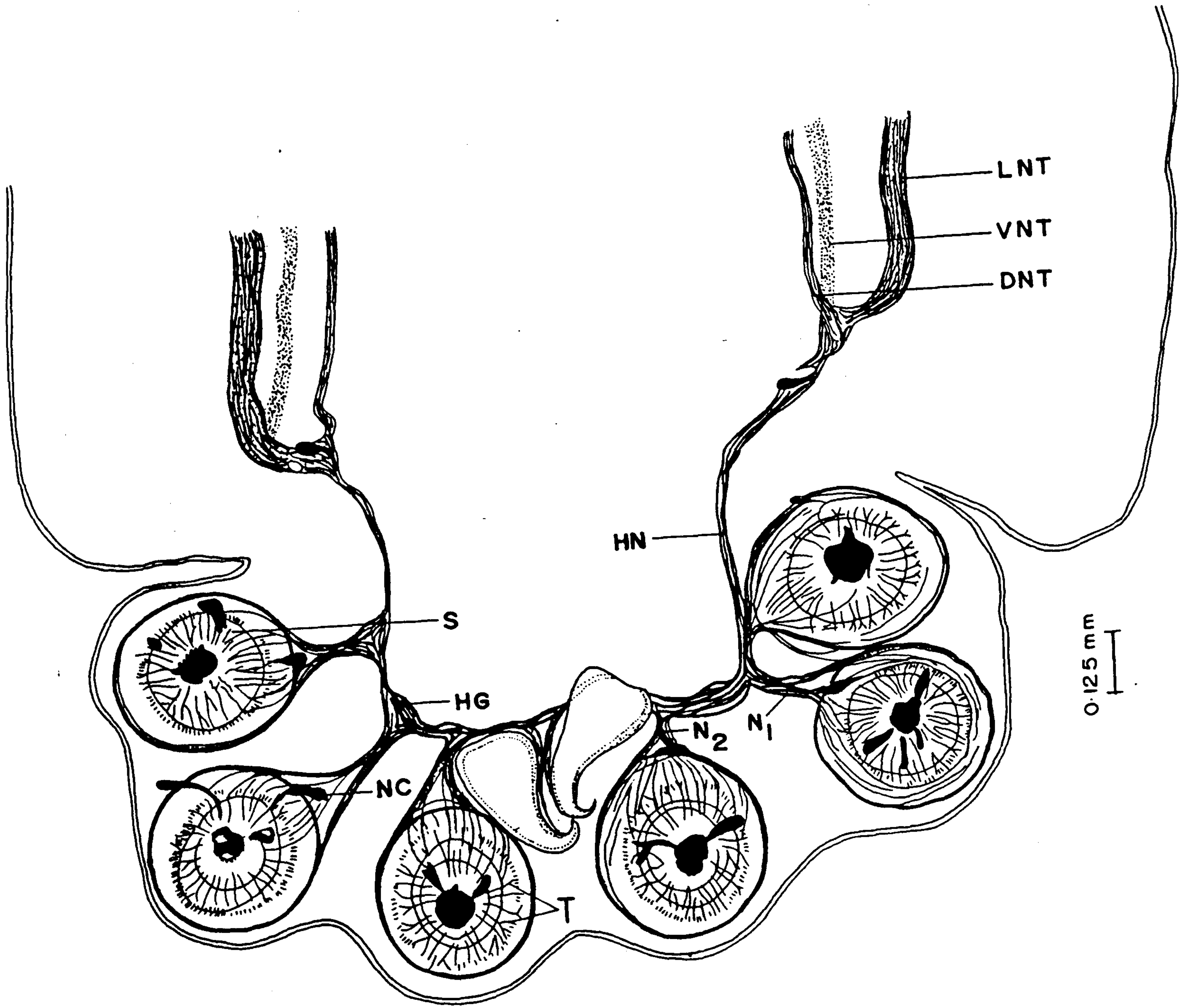
Fig.26 : Nervous system of *Polystoma indicum* - nerve arrangement in the opisthaptoral disc, diagrammatic representation



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innervates the oral sucker. These are short and slender nerves occupying the most medial region. The other anterior nerves, in accordance with their position, include a pair each of thick and stout medial nerves, dorsal in position; slightly less distinct lateral nerves that arise ventrally one on either side from the middle part of the cerebral ganglion, branching into two nerves distally; thick most lateral nerves, arising from the most lateral side of each cerebral ganglion and stout like the most medial nerves; and marginal nerves which are very slender and least conspicuous of all the nerves. All these nerves and their fine branches join to form circumoral complex comprising two conspicuous circumoral rings. While the most medial nerves, the medial nerves and their tributaries participate to form the anterior circumoral ring that lies in close approximation of the oral opening, the lateral nerves, the most lateral nerves and the marginal nerves contribute to the formation of the second circumoral ring, just a little posterior to the anterior one.

Among the posterior nerves (Figs.25,26,28), there are two dorsal nerve trunks, each arising from the cerebral ganglion. These nerves run posteriad somewhat parallel to each other until they combine with the other posterior nerve trunks in the region just in front of the opisthaptor. An additional pair of dorsal nerves are the innermost of all the posterior long nerves, which are very slender and run parallel to the dorsal nerve trunks.

The ventral nerve trunks are also a pair of stout nerves that arise from the postero-ventral sides of the cerebral ganglia (Fig.25). These long prominent nerves run parallel to the dorsal nerve trunks. In the posterior haptoral region they form two main haptoral nerves as a result of their combination with the other trunks.

The lateral trunks, two in number, also arise from the lateral sides of the cerebral ganglia, being marginal in position and much stouter than the dorsal nerve trunks but less prominent than the ventral nerve trunks (Fig.25). These nerves also join the other nerve trunks in the haptoral region.

A pair of thin and short pharyngeal nerves arises from the inner aspects of the cerebral ganglion on either side. The four pharyngeal nerves innervate the pharynx.

All the nerve trunks are connected along their length by numerous transverse commissures. While commissures present in the vicinity of the phraynx and in the post pharyngeal region (Fig.25) are quite prominent, others cannot be traced exactly as they are very fine and thin.

The haptoral nerves (Figs.26,30-32,35-37) are formed by the merger of the dorsal, ventral and lateral nerve trunks of

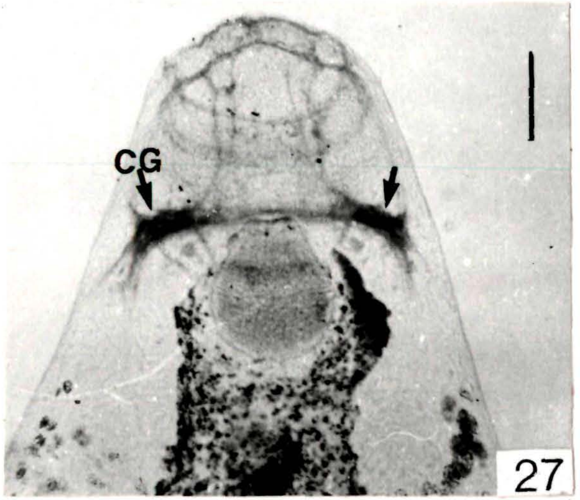
Figs.27-30 : Nervous system of *Polystoma indicum* - photomicrographs

27 : Anterior region, showing the cerebral ganglia with commissure and anterior nerves (scale bar 0.2 mm)

28 : Magnified view of the anterior region, showing the cerebral ganglion and 4 pairs of nerves posteriorly (scale bar 0.125 mm)

29 : Anterior region, showing circumoral rings, 5 pairs of anterior nerves (scale bar 0.125 mm)

30 : Opisthaptor region, showing the 6 suckers, the ventral haptoral nerve, haptoral ganlion and sucker ganglion (scale bar 0.2 mm)



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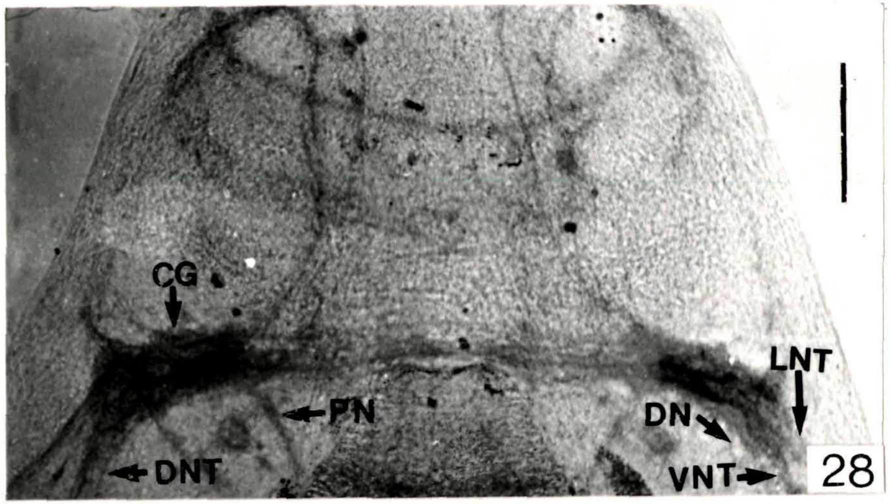

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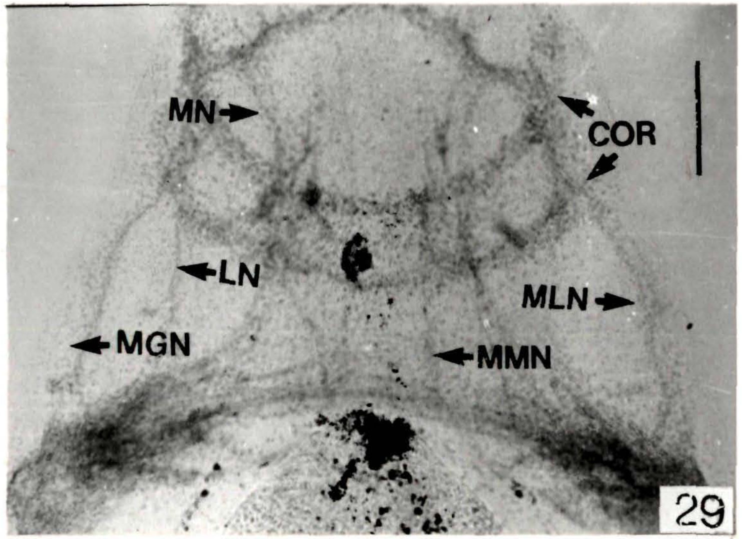

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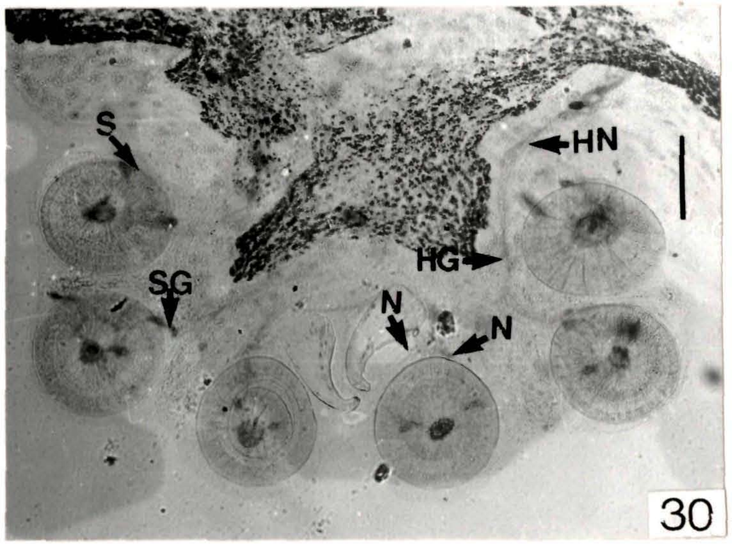

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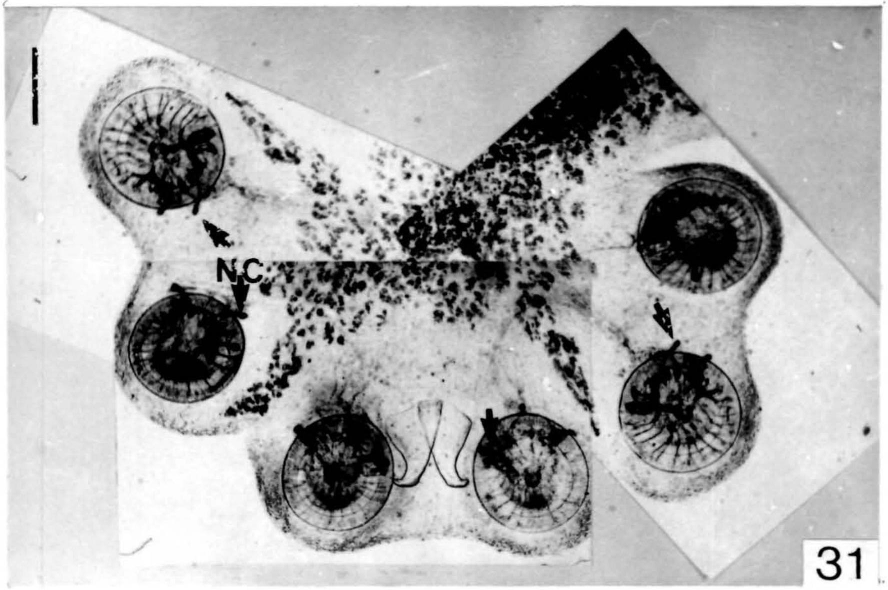
Fig31 : Nervous system of *Polystoma indicum* - photomicrographs

31 : Opisthaptor magnified and reconstructed from three micrographs, showing innervation to suckers and the nerve cells (arrows) (scale bar 0.125 mm)



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either side just prior to the opisthaptoral region and extend further into the opisthaptor. They run inner to the opisthaptoral clamps or suckers and join medially, thus forming a U-shaped commissure. Along the course of this ‘U’ of the haptoral commissure, there occurs a thickening confronting each of the opisthaptoral clamps. These thickenings or ‘haptoral ganglia’ are the points from each of which arise two or more fine nerves leading to the corresponding sucker. These nerves run divergently from each other but along the periphery of the sucker, thus forming a ring by eventually joining each other. These nerves innervate the suckers from their dorsal and ventral facets and give out very fine tributaries in the tissue of the suckers. A pair of fine hamuli nerves is given out from the `U' loop of the haptoral nerves in the region between the posterior pair of the opisthaptoral suckers; these nerves innervate the hamuli from their basal part.

Of the organs innervated by fine nerves arising from the commissures and also from the main nerve trunks are ovary, ootype, uterus, cirrus sac and testis. The pharynx and the digestive tract receive nerves from the pharyngeal nerves and the various transverse commissures.

Many dark staining nerve cells were observed in association with the cerebral ganglia, anterior nerves and the

Figs.32-37 : Nervous system of *Polystoma indicum* : photomicrographs

32 : Opisthaptoral sucker, showing enzymatic reactivity, (arrows) (scale bar, 0.125 mm)

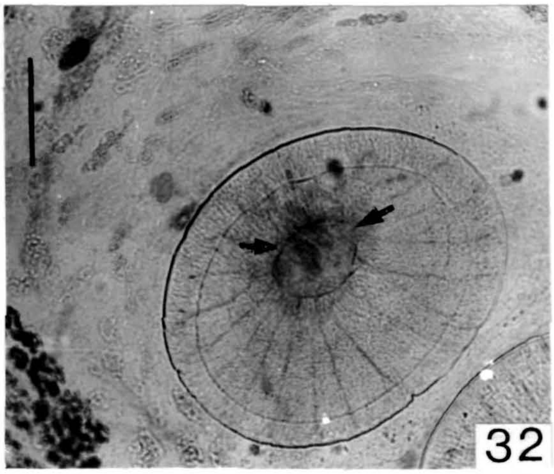
33 : A magnified view of the same, showing intense enzymatic reactivity, (arrows) (scale bar 0.025 mm)

34 : A nerve cell associated with an opisthaptoral sucker, showing intense nonspecific esterase activity, (arrow) (scale bar 0.025 mm)

35 : A closer view of the nerve innervating the sucker, (arrows) (scale bar 0.025 mm)

36 : Another opisthaptoral sucker in a magnified view; several nerve cells are evident, (arrows) (scale bar 0.05 mm)

37 : Innervation of the ventral region of the sucker in Fig.36, (arrows) (scale bar 0.025 mm)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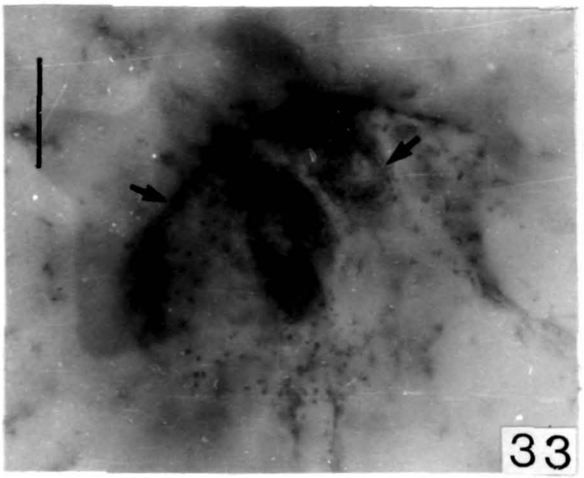
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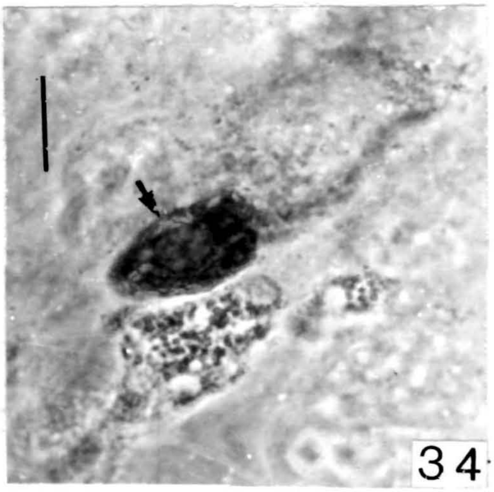
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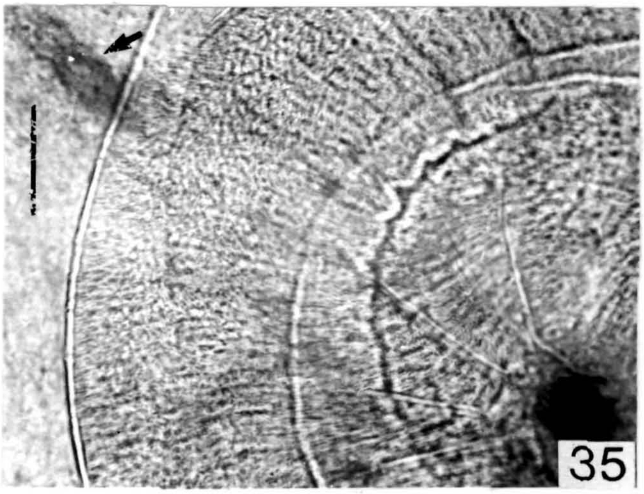
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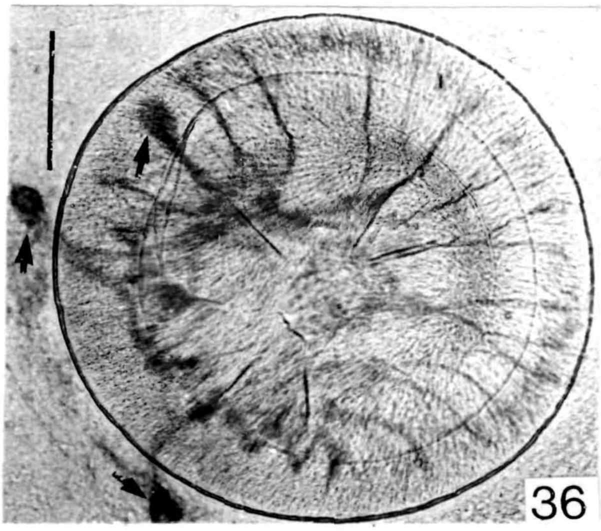
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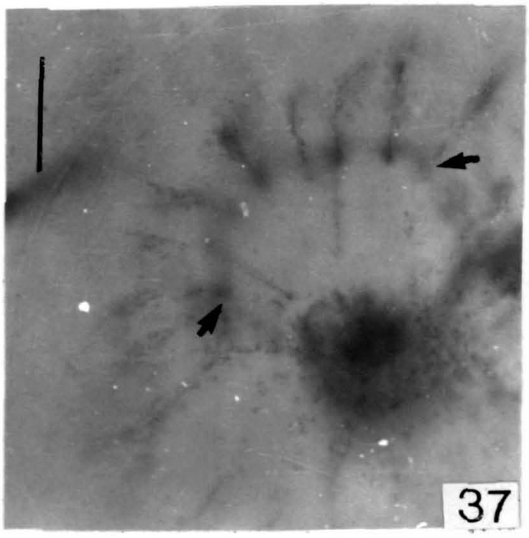
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main nerve trunks and also with the fine branches more predominantly of the nerves supplying to the opisthaptoral suckers. The ones associated with the latter are mostly unipolar small club-shaped cells, with their cytoplasm showing intense staining reactivity (Figs.33,34,37).

2. DEVELOPMENTAL STAGES : Morphology And Surface Topography

Of the several host specimens collected, only 3, 2 of *R. nigropalmatus* and one of *R. reinwardtii* were found to harbour 4 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 condition, 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 7 eggs still retained in the uterus. Eggs maintained under laboratory condition at 23°C(±1°C) showed no sign of further development.

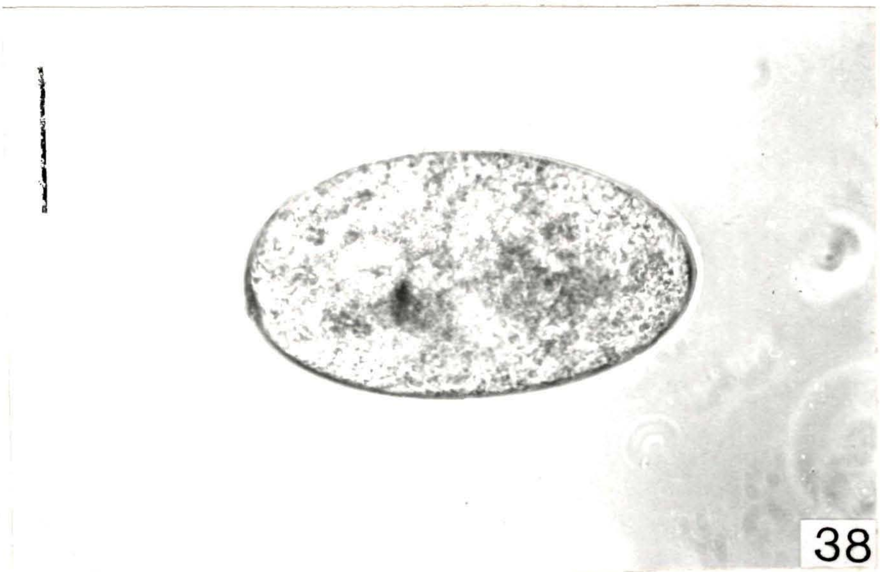
(i) **Egg** (Figs.38,39)

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 exhibits a smooth and porous appearance. As revealed by the positive staining for phenolase and phenolic precursors, the egg shell exhibits a proteinaceous, sclerotin-like nature.

Figs. 38,39 : *P. indicum* : Egg, photomicrographs

38 : Egg in utero; light microscopy (scale bar 0.05 mm)

39 : Surface view as revealed under scanning electron microscope (scale bar 10 um)



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Scanning electron microscopic observations revealed the egg surface as having a pitted texture, uneven, with small tubercules dispersed all over. The operculum appears as a depression at one pole of the egg, which splits open at the time when the oncomiracidium becomes mobile and emerges out of it (Fig.39).

(ii) **Larval Stages**

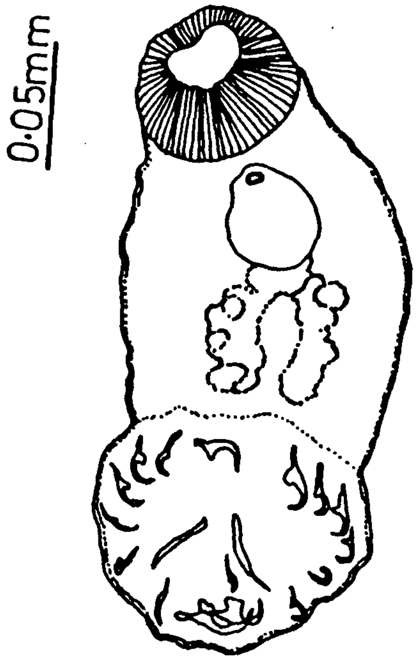
The larval and juvenile polystomids were always recovered from the branchial chambers or internal gills, urinary bladder and rarely from the intestine of the metamorphosing tadpoles with tall rudiments. These unciliated larvae were distinguishable as representing 4 stages of development on the basis of their opisthaptor. The latter showed gradual development from no-suckered opisthaptoral disc to the one acquiring 2,4 and finally 6 clamped stage. While the stage with no clamps on its opisthaptor is referred to as Gyrodactyloid-I (Fig.40) following Prudhoe and Bray (1982), the clamped stages are referred herein as post-gyrodactyloid Ia,b, and c, respectively (Fig.48).

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

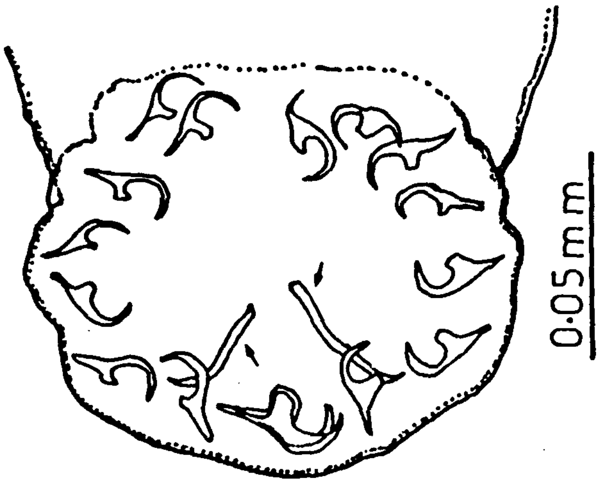
Fig.40 : *P. indicum* : Gyrodactyloid -I larva, Diagrammatic representation based on whole mounts

(i) : Larva, ventral view

(ii): Opisthaptoral disc with its armature, enlarged



(i)



(ii)

Figs.41-43 : *P. indicum* : Gyrodactyloid -I, photomicrographs

41 : Larva, whole mounts; microhooks and hamuli primordia are clearly seen under phase contrast (scale bar 0.5 mm)

42,43 : Anterior and posterior extremities respectively, enlarged view (scale bar 0.05 mm)

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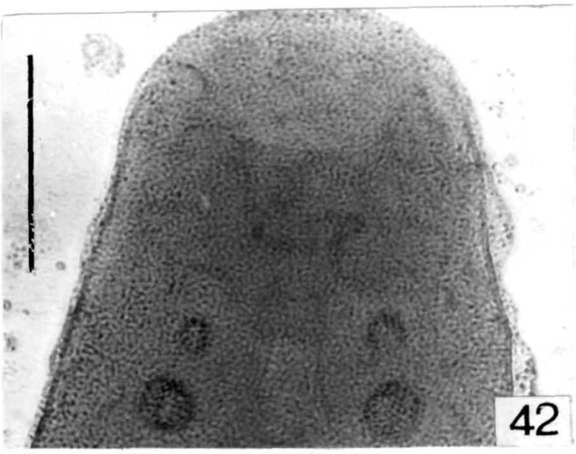

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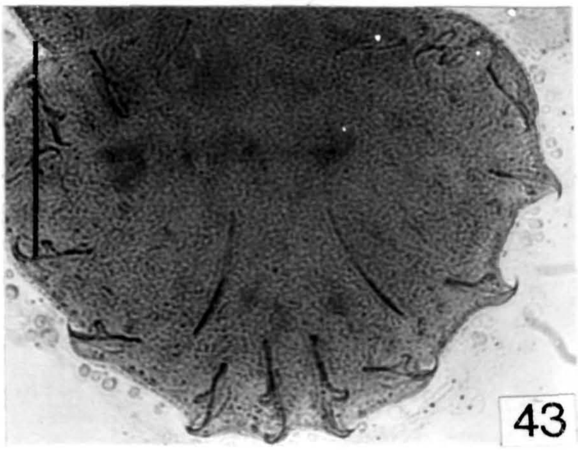

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TABLE 3

Morphometric measurements (in mm) of Gyrodactyloid -I and Post-gyrodactyloid larvae of *P. indicum*

Characters		Gyrodactyloid -I		Post-gyrodactyloid-Ia		Post-gyrodactyloid-Ib		Post-gyrodactyloid-Ic	
		Range $(\bar{x})$	S.D	Range $(\bar{x})$	S.D	Range $(\bar{x})$	S.D	Range $(\bar{x})$	S.D
Body	Length	0.20 - 0.28 (0.24)	±0.03	0.38 - 0.44 (0.41)	±0.44	0.64 - 0.68 (0.66)	±0.03	0.64 - 0.99 (0.85)	±0.12
	Breadth	0.08 - 0.14 (0.11)	±0.02	0.15 - 0.19 (0.17)	±0.03	0.19 - 0.21 (0.20)	±0.01	0.21 - 0.30 (0.24)	±0.03
Oral sucker	Length	0.04 - 0.07 (0.05)	±0.01	0.03 - 0.06 (0.04)	±0.02	0.04 - 0.06 (0.05)	±0.01	0.04 - 0.07 (0.05)	±0.01
	Breadth	0.05 - 0.08 (0.07)	±0.09	0.04 - 0.07 (0.06)	±0.02	0.06 - 0.07 (0.065)	±0.01	0.07 - 0.12 (0.10)	±0.02
Ratio of oral sucker to body length		1:4 - 1:5 (1:4.4)	-	1:7.93-1:12.66 (1:10.25)	-	1:11.33-1:16.0 (1:13.20)	-	1:14.14-1:16 (1:15.07)	-
Opisthaptor	Length	0.09 - 0.1 (0.095)	±0.06	0.12 - 0.13 (0.12)	±0.01	0.18 - 0.22 (0.20)	±0.03	0.15 - 0.32 (0.21)	±0.05
	Breadth	0.09 - 0.12 (0.11)	±0.01	0.13 - 0.15 (0.14)	±0.01	0.32 - 0.35 (0.33)	±0.02	0.07 - 0.44 (0.37)	±0.06
Ratio of opisthaptor to body length		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)	-
Clamps (diameter)	1			*0.06		0.09		0.02	
	2			0.06		0.1		0.03	

cont.

*Clamp no.from left to right	3			-		0.1		0.09 - 0.1 (0.095)	±0.01
	4			-		0.09		0.09 - 0.1 (0.095)	±0.01
	5			-		-		0.03	
	6			-		-		0.02	
Hamuli	Length	0.02 - 0.03 (0.02)	±0.05	0.07 - 0.09 (0.08)	±0.01	0.09 - 0.1 (0.095)	±0.01	0.1 - 0.15 (0.12)	±0.02
	Breadth	0.004	-	0.02	-	0.02 - 0.03 (0.025)	±0.05	0.02 - 0.03 (0.025)	±0.003
Microhook	Length	0.019	-	0.019	-	0.019	-	0.019	-
	Breadth	0.003	-	0.003	-	0.003	-	0.003	-
Handle length		0.007	-	0.007	-	0.007	-	0.007	

$\bar{x}$ = Mean

Gyrodactyloid-I stage (Figs.40-47).

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, 3 pairs are anterior to the hamuli primordia, 2 pairs are in the region posterior to the latter and 6 microhooks are lodged singly in the prospective positions where future 6 clamps 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. 40 - 43).

As observed under the scanning microscope the tegument covering the gyrodactyloid-I stage has transverse folds and ridges extending the whole length of the body (Fig.44). The ridges are separated from each other by deep grooves or valleys and are seen to have a network-like texture with chains of

Figs.44-47 : *P. indicum* : Gyrodactyloid -I, scanning electron micrographs

44 : Larva in toto as seen under scanning electron microscope (scale bar 10 µm)

45 : Surface tegument of the midbody region (scale bar 1 µm)

46 : Oral opening with uneven and corrugated surface (scale bar 10 µm)

47 : Opisthaptoral disc, showing the placement of microhooks (small arrows) and hamuli (big arrows) (scale bar 10 µm)

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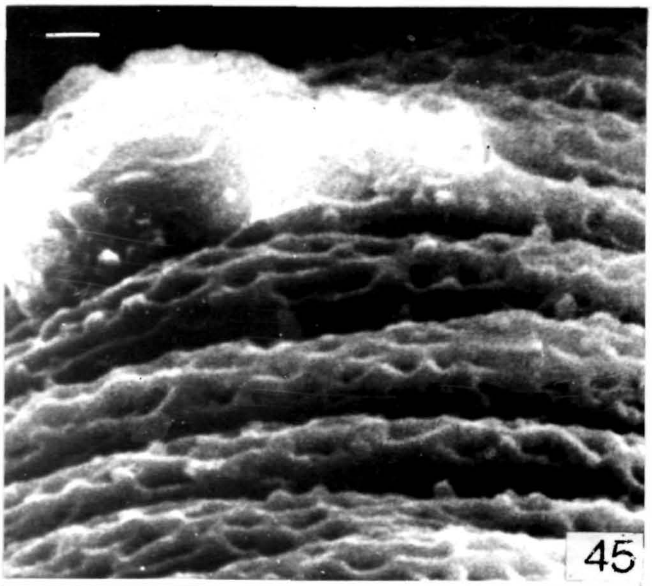

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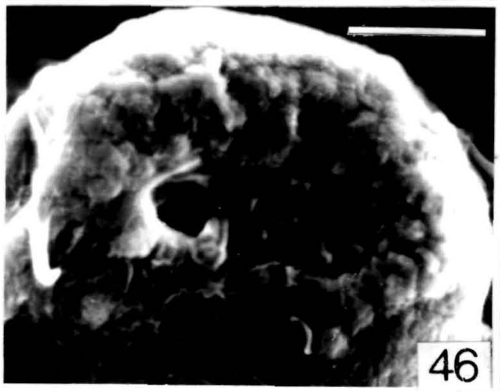

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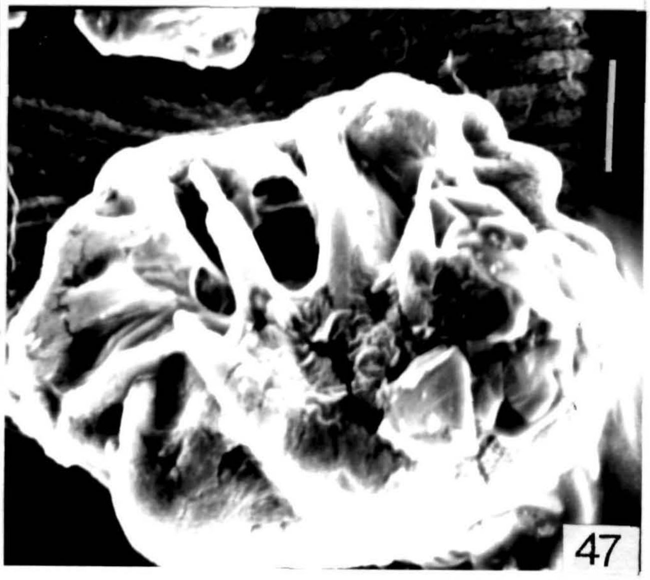

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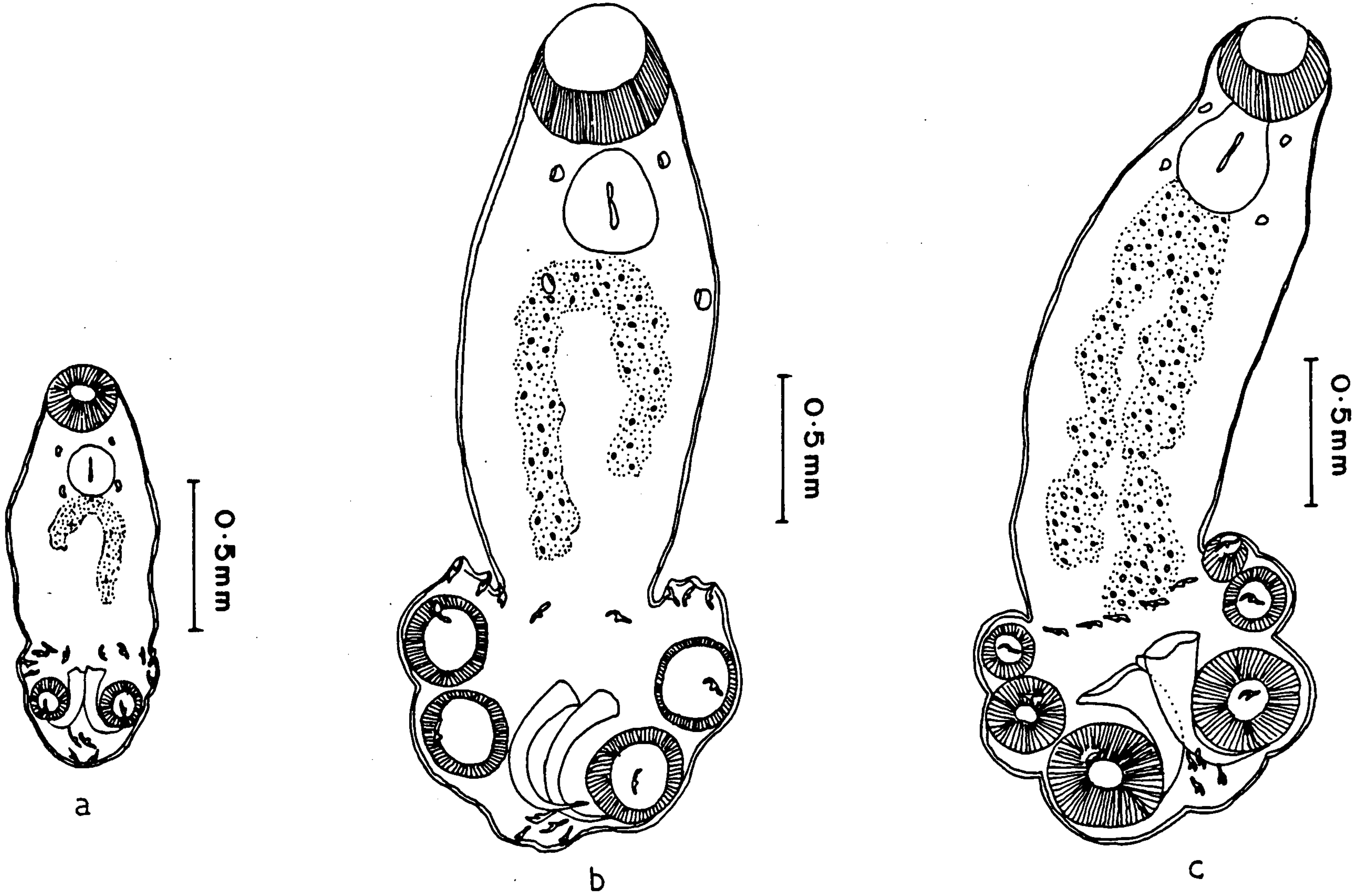
depressions connected to each other randomly. The valleys are devoid of similar arrangement (Fig.45). The oral sucker surface presents a scaly appearance and aggregation of irregular papillate thickenings along the sucker rim gives the latter an unsmooth appearance (Fig.46). In the opisthaptoral disc the pair of hamuli and microhooks are given out from the inner or ventral facet of the disc margin and their bases are covered by tegumental sheaths (Fig.47).

Post-gyrodactyloid-Ia (Figs.48-56).

This being the second stage, has a minute body, 0.30-0.44 mm in length, 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 former stage, its fore-limit from the forebody is perceptible because of the distribution of the microhooks. It is endowed with 2 clamps and a pair of well defined, though small in size, hamuli in addition to 16 microhooks. The hamuli have acquired a shape somewhat similar to that in the adult fluke. Of the microhooks 5 pairs are just anterior to the hamuli and the clamps, 2 pairs are in the region posterior to the hamuli, while one microhook is present in the middle of each clamp. 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

Fig.48 : *P. indicum*: Post-gyrodactyloid -I larva - a,b, and c stages; diagrammatic representation based on whole mounts



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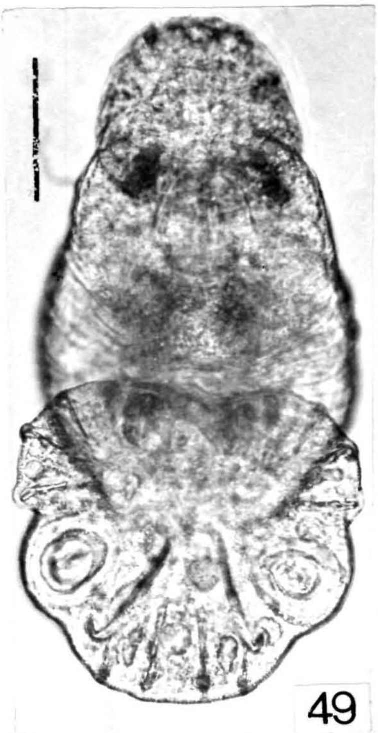
Figs.49-51 : *P. indicum* : Post-gyrodactyloid-Ia larva, photomicrographs

49 : Larva in whole mount, showing 2 distinct clamps on the opisthaptor (scale bar 0.05 mm)

50 : Anterior region in magnified view, showing 2 pairs of eyespots (scale bar 0.025 mm)

51 : Opisthaptoral disc, showing its full complement of clamps, hamuli and microhooks (scale bar 0.025 mm)



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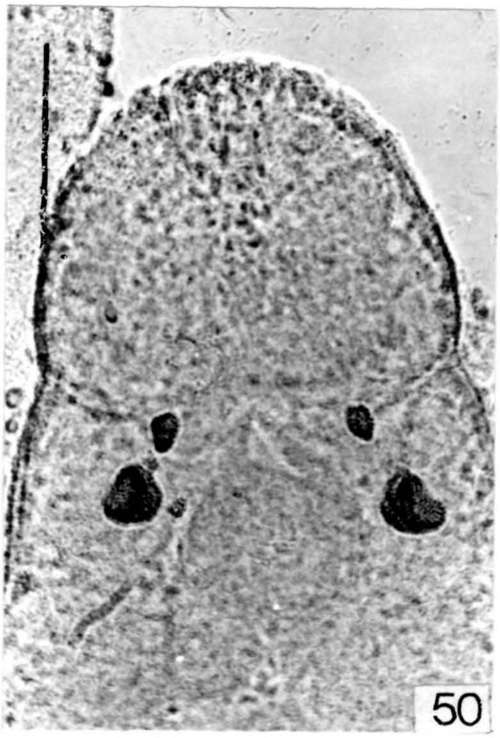

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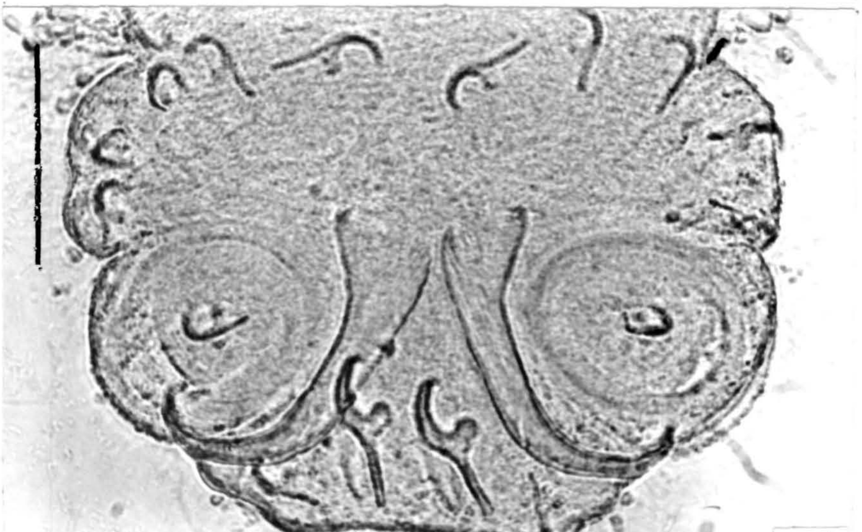

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and extend posteriad further up to the level a little behind the equatorial region of the body and close to the opisthaptor. Two pairs of eyespots, one on each side of the pharynx, are prominent.

The surface fine topography of the post-gyrodactyloid Ia stage shares similar characteristics with the gyrodactyloid-I stage, with the difference that the number of free microhooks is reduced from 16 to 14, 2 microhooks having got incorporated within the boundary of each clamp (Fig.52). The body shows the usual intricate pattern of transverse folds and ridges and is covered by the tegument displaying a fine mesh-like appearance or honeycomb texture (Fig.53). The oral sucker rim is ornamented with papillae which are more conspicuous and defined than in the preceding stage (Fig.54). The opisthaptoral clamps have a conspicuous rim protruding from the opisthaptoral general surface and depicting the same surface texture as that of the general body. The margins of the opisthaptoral disc are smooth and devoid of any spines or cilia (Figs.55,56). The bases of the microhooks and hamuli are covered by tegumental sheaths. The latter gradually loses its honeycomb pattern distally where a distinct fold marks the tegumental boundary and a naked spine emerges free of the sheath.

Figs.52-56 : *P. indicum* : Post-gyrodactyloid -Ia larva, scanning electron micrographs

52 : Whole larva in ventral view (scale bar 10 µm)

53 : Tegument as seen near the lateral aspects of the body (scale bar 1 µm)

54 : Oral opening showing papillated oral rim (scale bar 10 µm)

55 : Tegumental texture of the opisthaptoral disc (scale bar 10 µm)

56 : A single opisthaptoral sucker, showing its tegumental texture (scale bar 10µm)

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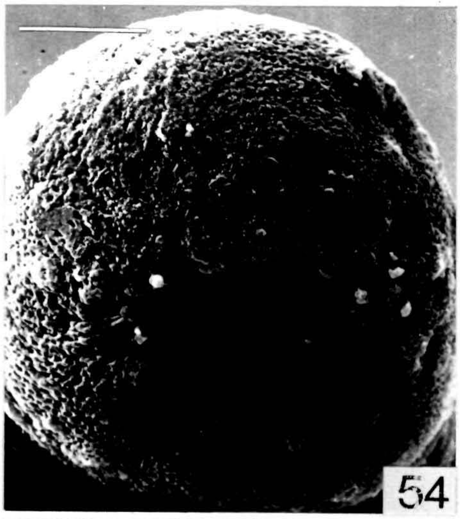

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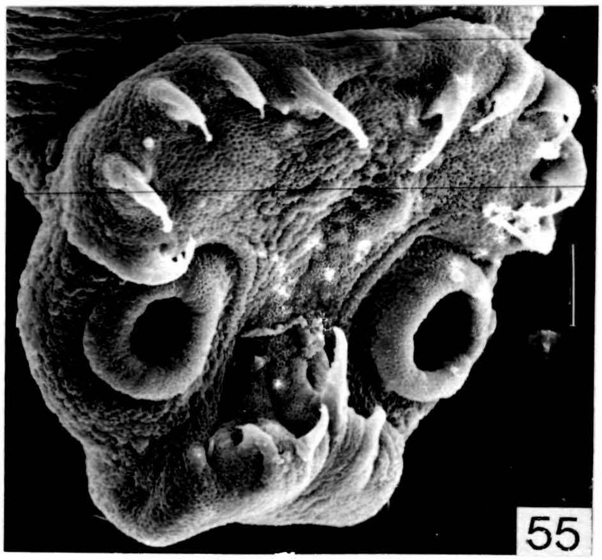

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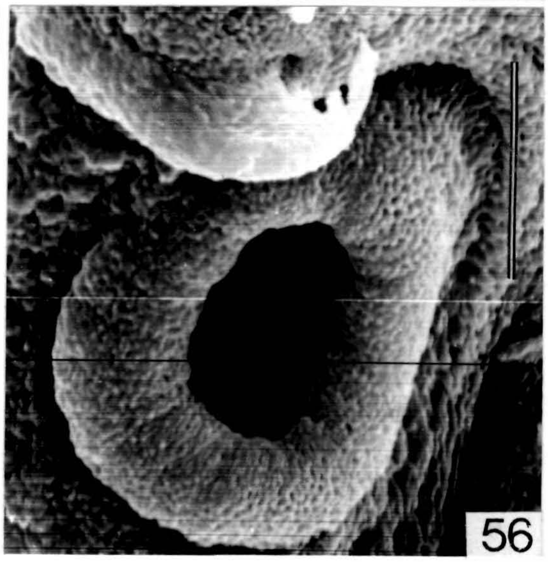

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Post-gyrodactyloid-Ib (Figs.48,57-65).

This is the third stage of development recovered during the present study. Its body is moderately large and cylindrical, 0.64-0.68 mm in length. The anterior terminal end carries the prohaptor in the form of a conspicuous prominent oral sucker. The posterior region is provided with a distinct discoidal, lobed opisthaptor having 4 prominent clamps, 2 hamuli and 16 microhooks; of the microhooks, 2 pairs are post hamuli, one microhook is associated with each clamp and 4 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. The 2 pairs of eyespots are present in this stage too (Figs.57-59).

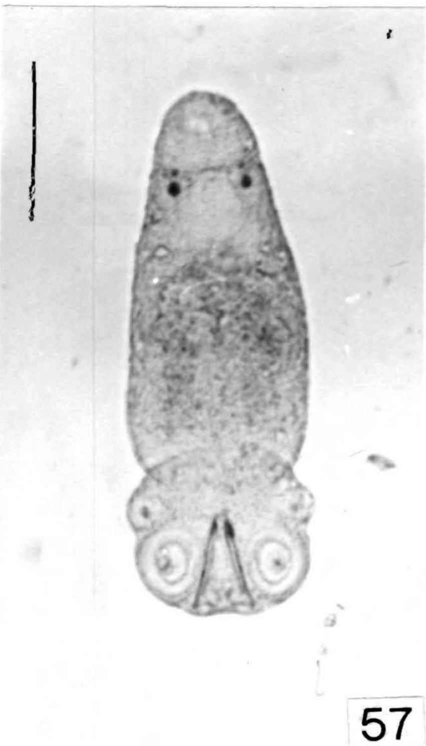
The post-gyrodactyloid-Ib stage also shares the same surface fine characteristics of the post-gyrodactyloid-Ia, but in this stage the number of clamps has increased to 4 and the free microhooks are 12 in number (Fig.60). The general body surface has somewhat mesh-like and porous texture having a spongy appearance (Fig.61). The prohaptor appears fluffy and encircles the oral opening. Its surface texture is also spongy and porous (Fig.62). The opisthaptor tegument displays the same structural features as the general body surface and the hamuli are seen projecting out of the sheath with similar surface texture

Figs.57-59 : *P. indicum*: Post-gyrodactyloid -Ib larva, photomicrographs

57 : Whole larva in ventral view (scale bar 1 mm)

58 : Anterior region with a distinct oral sucker and 2 pairs of eyespots (scale bar 0.025 mm)

59 : Posterior region with four clamps (two prominent and two developing), a pair of hamuli and microhooks (scale bar 0.025 mm)



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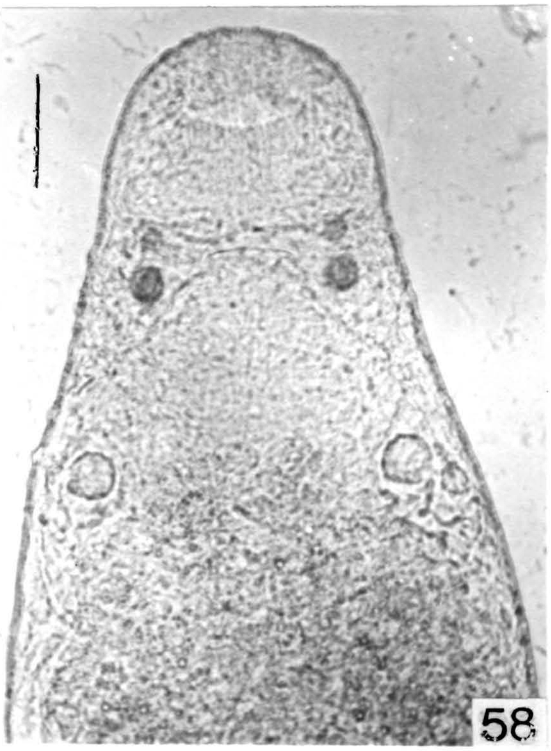

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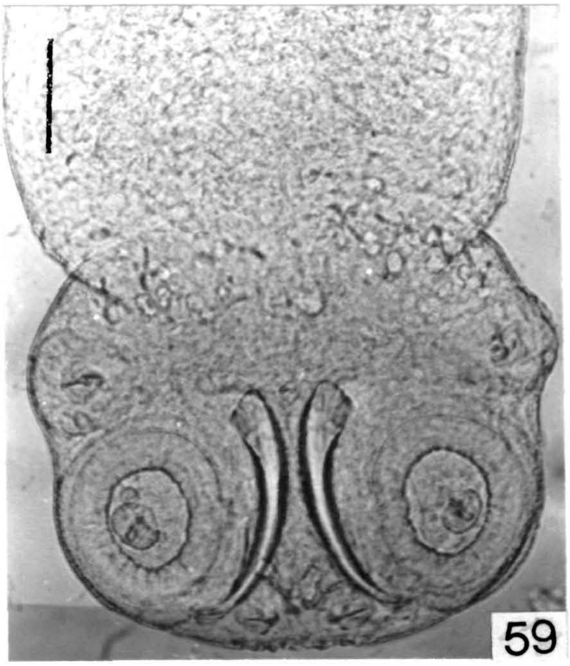

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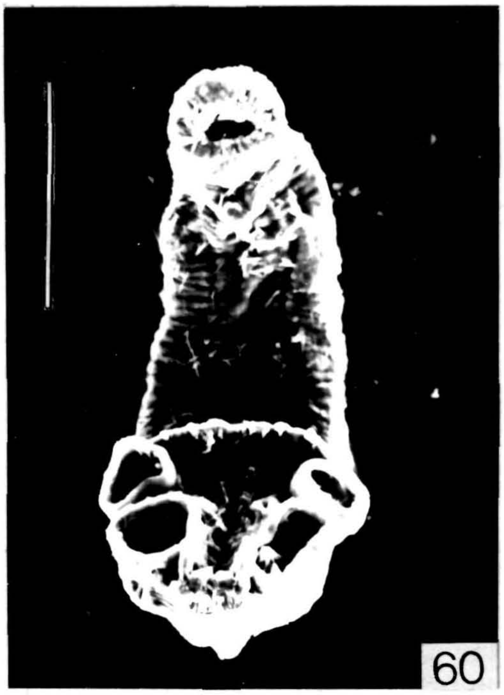
Figs.60-63 : *P. indicum* : Post-gyrodactyloid -Ib larva, scanning electron micrographs

60 : Entire larva in ventral view (scale bar 100 µm)

61 : Body surface tegument, revealing a honeycomb-like texture (scale bar 1 µm)

62 : Oral sucker, showing porous inner and outer tegumental surface (scale bar 10 µm)

63 : Opisthaptor (scale bar 10 µm)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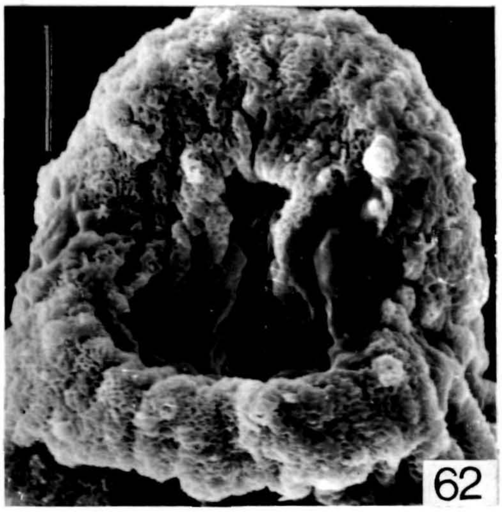
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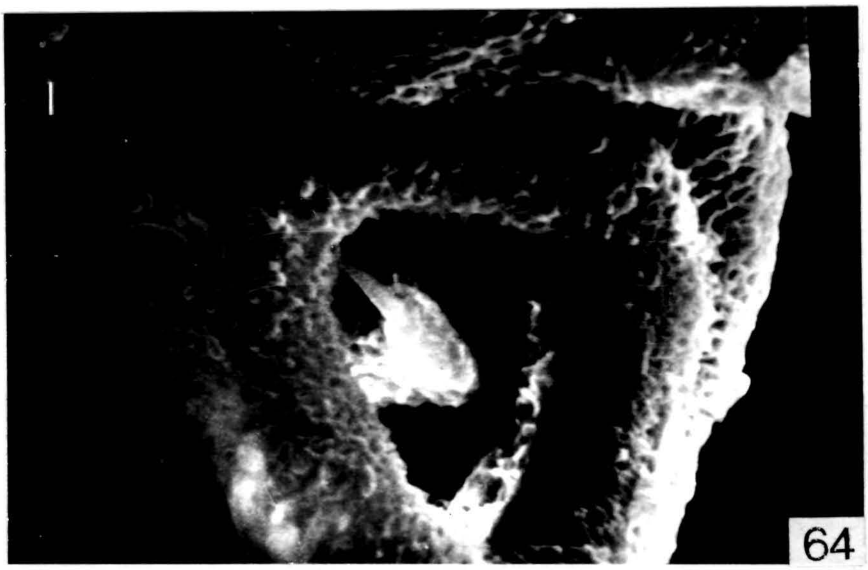
Figs.64-65 : *P. indicum* : Post-gyrodactyloid-Ib larva, scanning electron micrographs

64 : A single opisthaptoral sucker in closer view, with a microhook in the centre; porous clamp rim are distinct (scale bar 1 µm)

65 : Hamuli, showing the tegument extending to the base of spine (scale bar 10 µm)



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65

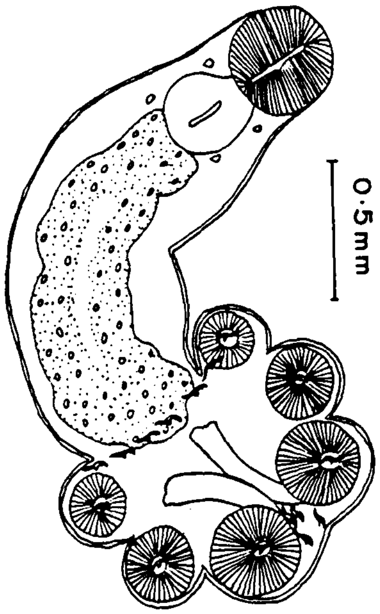
(Figs.63,65). The opisthaptoral clamps are nucleated from their respective microhook; the microhooks can be seen protruding out of the muscular tissue in the centre of the developed clamp (Fig.64).

Post-gyrodactyloid-Ic (Figs.48,66-77)

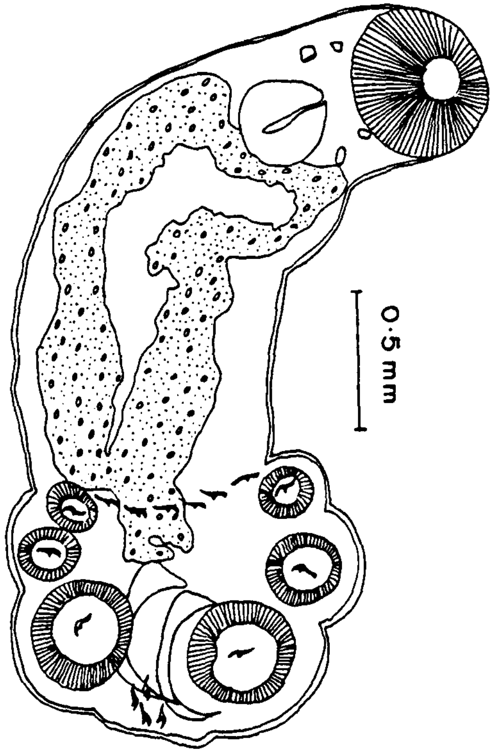
This stage is distinguishable by the presence of 6 clamps in the opisthaptor. By this stage the larvae acquire an elongated and more compact body, 0.64-0.99 mm in length, with an adult-like opisthaptor. With the incorporation of one microhook in each of the 6 clamps of the opisthaptor, the number of free microhooks is reduced to 10, of which 3 pairs are located anterior to the hamuli, and 2 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 (Fig.66). 2 pairs of eyespots are still retained in this stage. However, there is yet no trace of genital primordia (Figs.67-68).

The post-gyrodactyloid-Ic larva shows better developed surface fine characteristics as compared with the preceding younger larvae (Fig. 69). The body appears more stout with well developed oral sucker and fully armed opisthaptor with 6 clamps. The tegument of the dorsoventrally flattened body appears smooth

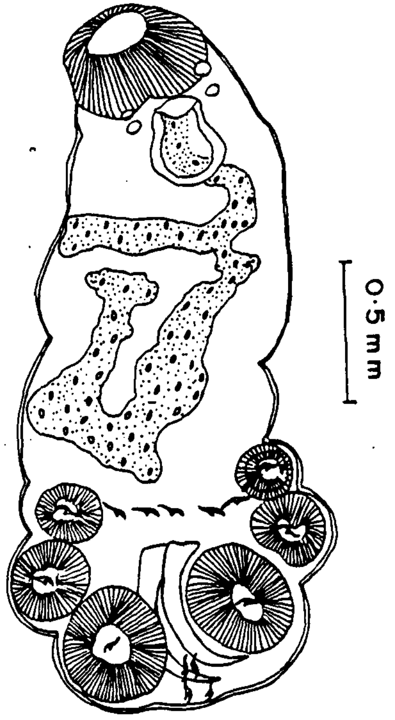
Fig.66 : *P. indicum*: Diagrammatic representation of variation in diverticulation and posterior extent of intestinal caeca in post-gyrodactyloid -Ic stages



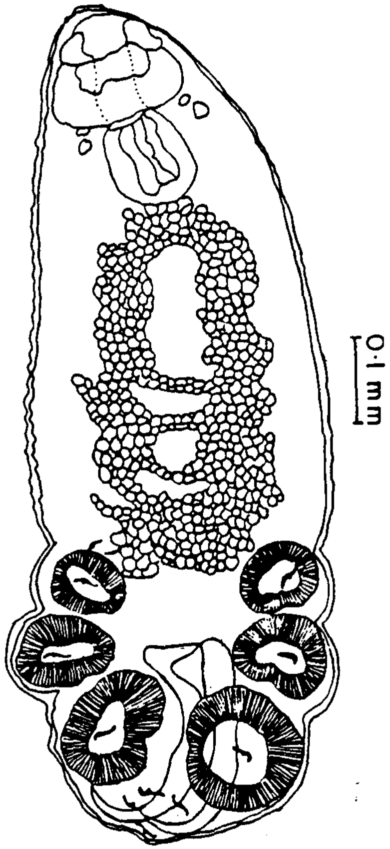
Ic(i)



Ic(ii)



Ic(iii)

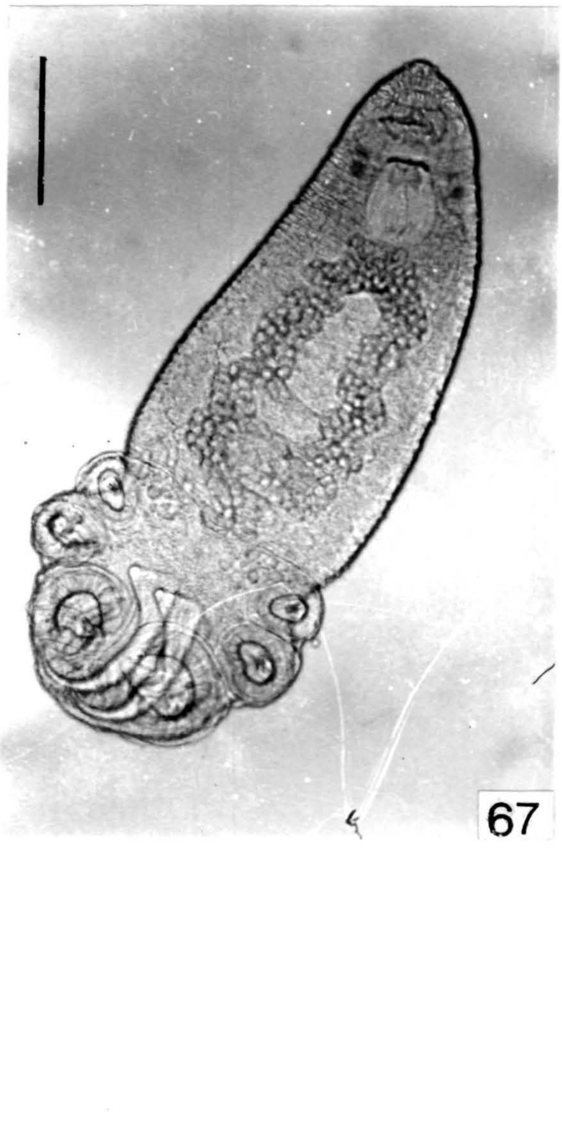


Ic(iv)

Figs.67,68 : *P. indicum* : Post-gyrodactyloid -Ic larva, photomicrographs

67 : Whole larva (scale bar 0.05 mm)

68 : Anterior region, showing oral sucker, 2 pairs of eyespots and pharynx (scale bar 0.025 mm)



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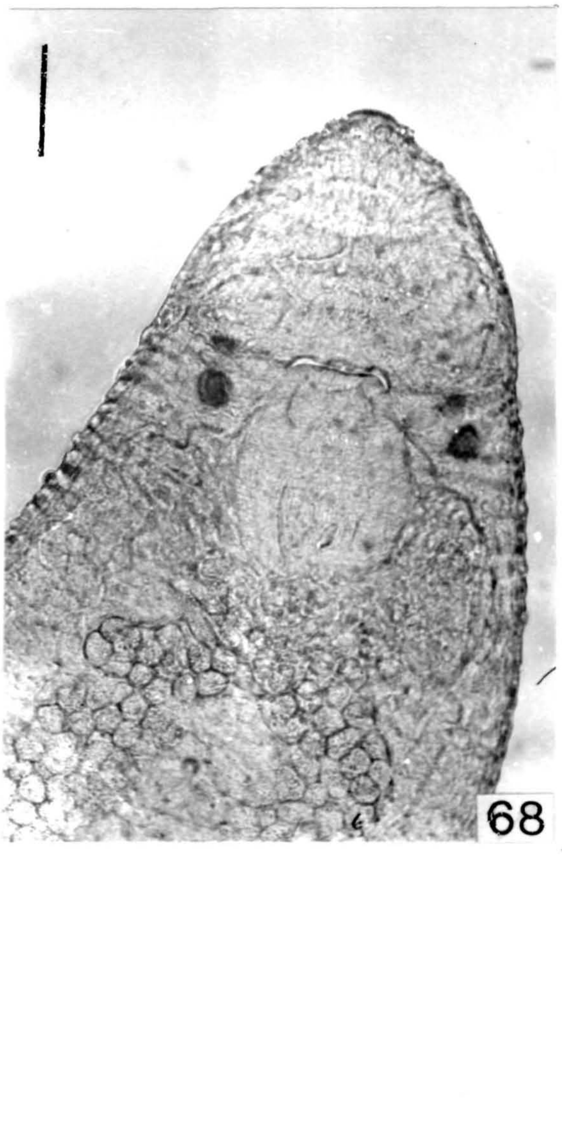

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Figs.69-74 : *P. indicum* : Post-gyrodactyloid -Ic larva, scanning electron micrographs

69 : Entire larva, in ventral view, showing distinct opisthaptor (scale bar 100 μm)

70 : General body tegument (scale bar 10 μm)

71 : Enlarged view of the same, showing honeycomb-like network (scale bar 1 μm)

72 : Papillae - like structures scattered in between the network of ridges; a single-ciliated body is evident (scale bar 1mμ)

73 : A closer view of the papillated region (scale bar 1 μm)

74 : Tegument of the lateral region of the body, showing lamellar arrangement and rounded sensory patches on the surface tegument (scale bar 10 μm)


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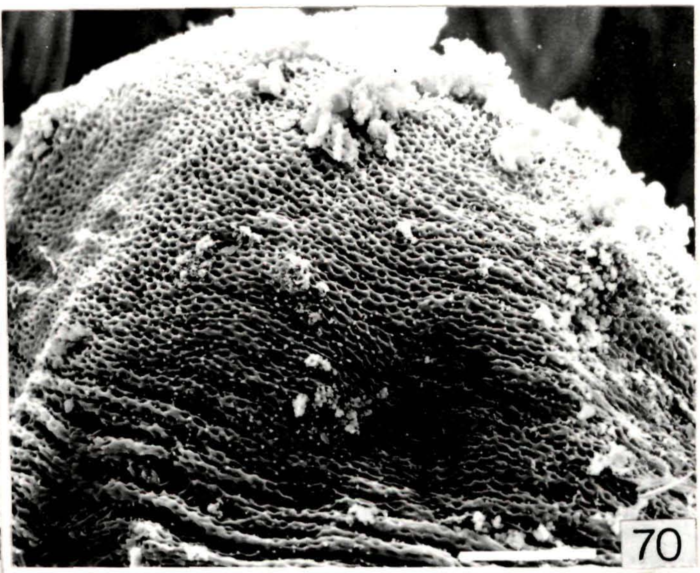
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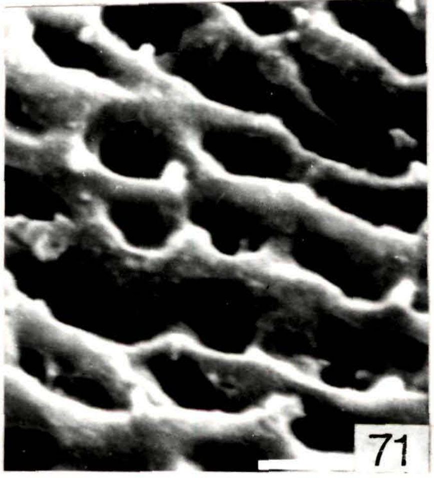
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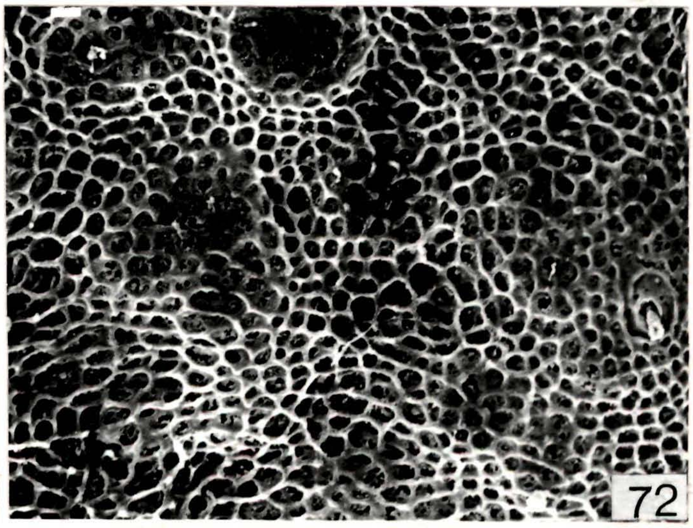
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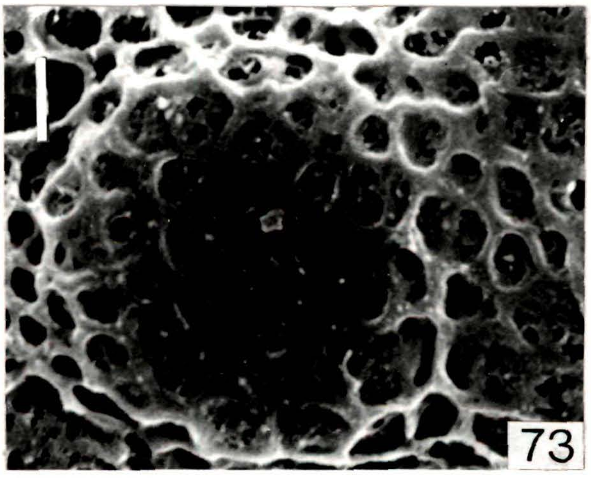
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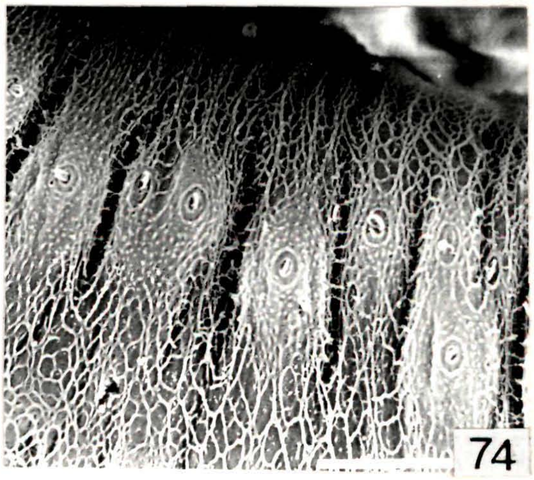
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and devoid of any spines, cilia or scales, though transverse folds with ridges and valleys clothe the entire body surface. A closer view of the ventral surface tegument reveals a honeycomb-like texture of interlacing network of ridges (Figs.69,70). Tegumentary vesicles or microvilli are seen located at the junctions of the ridge pattern forming the honeycomb (Fig.71). Also interspersed in the interlacing network are button-like papillae of different shapes and sizes distributed unevenly without specific orientations, some singly while others in clusters (Figs.72,73). The surface of these papillae appears porous with sparse distribution of tegumental vesicles. The lateral tegumental surface however displays a conspicuously different ornamentation by striae of anastomosing lamellae which are thin or sparse towards the extreme lateral margin, but as they proceed inwards, several of these transverse lamellae cluster at several points to form concentric tegumental ridges forming round patches (Fig.74). Numerous tuberculate vesicles ornament these lamellate structures. At the centre of each of such structures is an oral sensory body with a cilium. At higher magnification each of these structures is revealed to possess an outer thick rim enclosing within it an oral thick-rimmed pit or depression from which the cilium is given out (Fig.75).

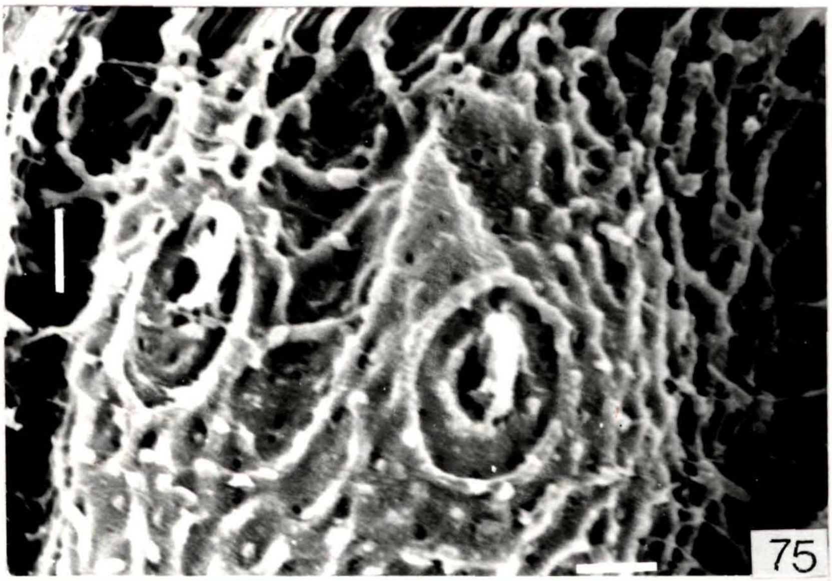
The prohaptor is subterminal, encircling the oral opening. The circumoral surface is uneven and studded with domed papillae at regular intervals. Some of these papillae have

Figs.75-77 : *P. indicum* : Post-gyrodactyloid -Ic larva, scanning electron micrographs

75 : Rounded sensory structures in a magnified view, each revealing an isolated cilium (scale bar 1 µm)

76 : Oral sucker rim studded with papillae, some with pitted apices (arrows) (scale bar 10 µm)

77 : A pair of hamuli seen protruding out of the tegumental sheath (scale bar 1 µm)



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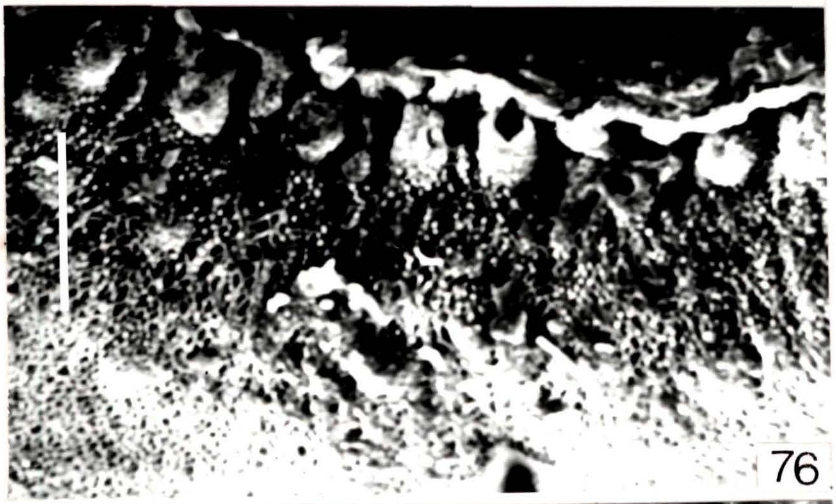

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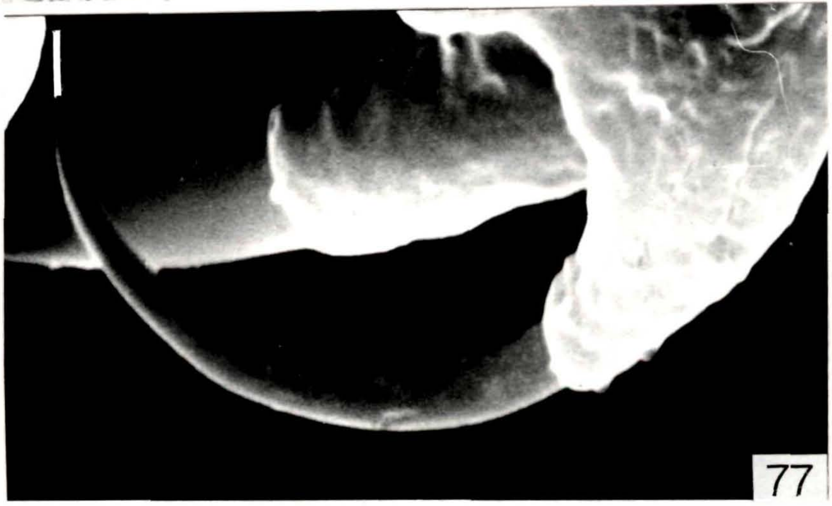

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pitted apices and appear as tegumental protrusions with a tuberculated surface due to the presence of numerous tegumental vesicles (Fig.76). The opisthaptoral clamps have well developed and conspicuous outer rim, with a single microhook within each, the texture of its surface being similar to that of the preceding larvae; the hamuli are larger in size, curved and are given out from the tegumental sheath of the opisthaptor extending up to the base (Fig.77).

While there is a gradual increase in the size of several structures, viz., oral sucker, intestinal caeca, opisthaptoral disc, clamps 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 (Fig.78).

The varying shapes of the hamuli of larval and adult parasites, which may range from smooth based to those with slight or deep incision, are shown in Figs.78 and 79. The hamuli primordia of the gyrodactyloid-I stage and hamuli of post-gyrodactyloid-I stages are with smooth bases and devoid of any incision between the guard and handle. However, in mature adults hamuli are with smooth base or with slight or deep incision at the base whereby the guard and handle could be differentiated (Fig.79). It was evident from the observations that even in the

Fig.78 : *Polystoma indicum* : Microhooks (which nucleate the clamps) and hamuli of gyrodactyloid -I and post-gyrodactyloid-I larvae, and adult fluke

(i) : Gyrodactyloid -I
(ii) : Post-gyrodactyloid - Ia
(iii) : Post-gyrodactyloid - Ib
(iv) : Post-gyrodactyloid - Ic
(v) : Adult

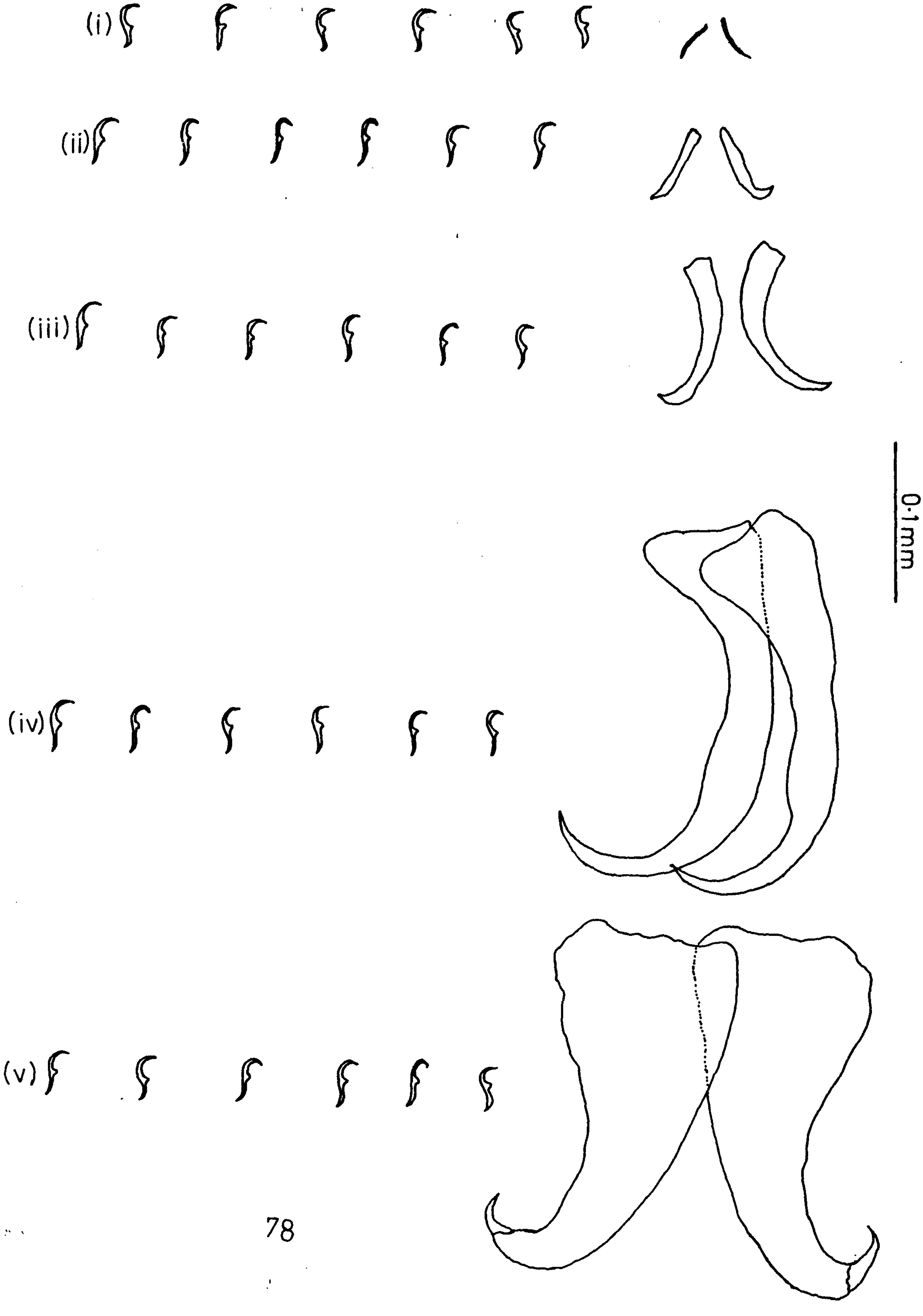
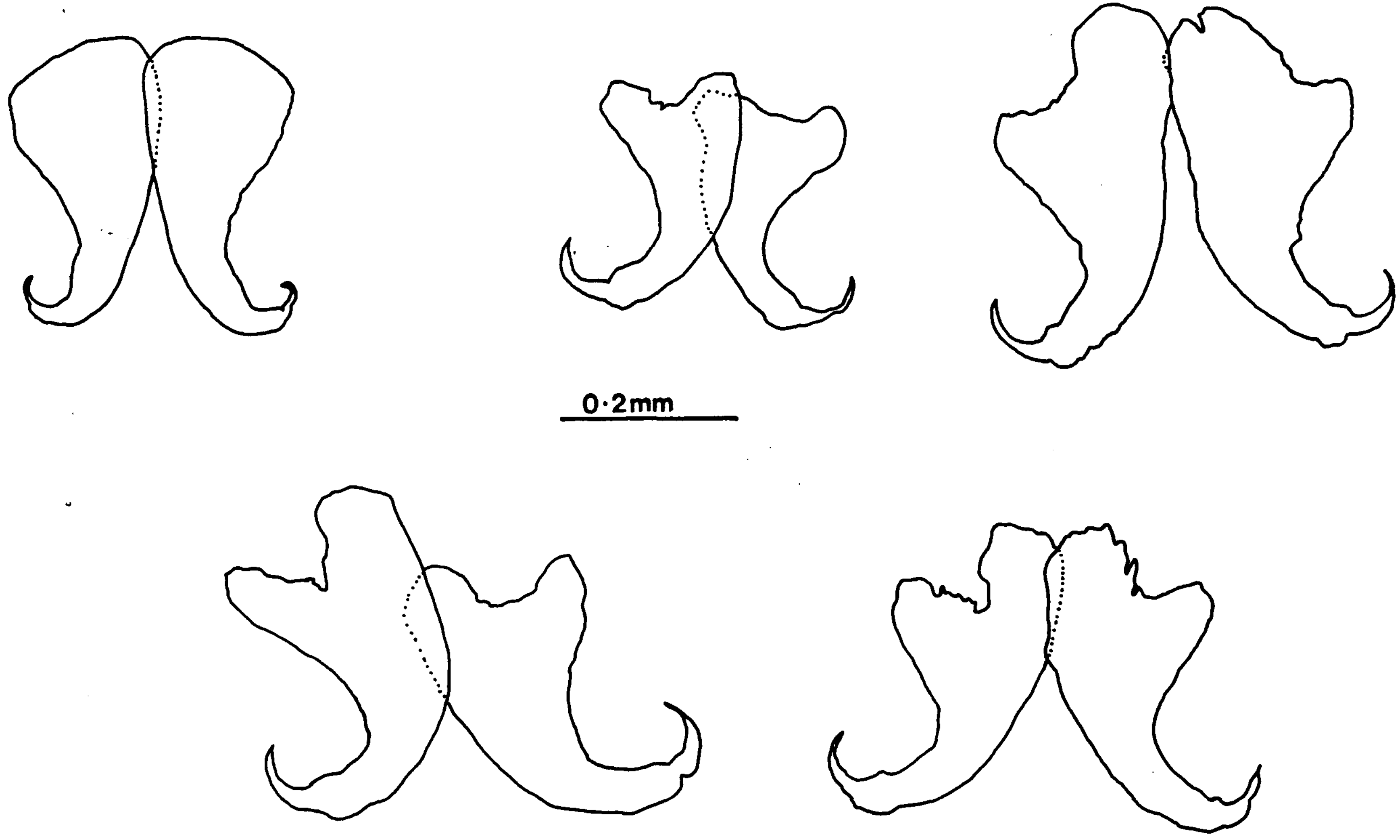

(i)
(ii)
(iii)
(iv)
(v)
0·1 mm

Fig.79 : *Polystoma indicum* : Variation in incision in hamuli, showing guard and handle



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same specimen there are variations in the relative length and width of both guard and handle and also in the depth of the incision between the two hamuli.

3. PREVALENCE AND DISTRIBUTION

The *P. indicum* infection was found to occur in rhacophorid anurans (Table 4) and confined to Cherrapunji, Mawsynram and Shillong areas. Of the three rhacophorid species, only *R. nigropalmatus* and *R. reinwardtii* showed a higher prevalence of infection as compared to *Polypedates leucomystax*; adult frogs of the last mentioned species did not harbour any adult flukes of *P. indicum*. The intensity of infection on the whole was quite low. The overall prevalence of *P.indicum* in *R. nigropalmatus* of Cherrapunji and Mawsynram was observed to be 10.89% and 25.80%, with a mean intensity of 0.1 and 0.2, respectively. The intensity of infection ranged from a single fluke up to 4 flukes in a single individual host. Similarly, the prevalence of *P. indicum* from *R. reinwardtii* of Cherrapunji, Mawsynram and Shillong was observed to be 17.18%, 16.07% and 0.125% with a mean intensity of 0.171, 0.535 and 0.2, respectively, the range of intensity being the same as in the other host species.

The prevalence data of gyrodactyloid-I and post -gyrodactyloid stages of *P. indicum* found in the 3 rhacophorid species is presented in Table 5. These larval stages were found

TABLE 4

Host-wise prevalence of adult *Polystoma indicum* in rhacophorid anurans

Host species	Locality	No. examined (No. infected)	% prevalence	Adult flukes recovered (mean intensity *)
Rhacophorus nigropalmatus	Cherrapunji	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)
	Shillong	8(1)	0.125	1(0.2)
Polypedates leucomystax (=*R. leucomystax*, Chanda, 1992)	Cherrapunji	30(-)	-	-
	Mawsynram	3(-)	-	-

- = no infection of monogenea

* = mean intensity per host examined

TABLE 5

Prevalence of Gyrodactyloid-I and Post-Gyrodactyloid-I stages of *Polystoma indicum* in tadpoles of rhacophorid anurans

Host species	No.examined (No.infected)	% Prevalence	Gyrodactyloid-I and Post-gyrodactyloid-I stages	
			No.recovered (mean intensity *)	Location in host
1. *Rhacophorus nigropalmatus*				
i) external-gill stage	60(-)	-	-	External gills
ii) internal-gill	6085(371)	6.09	1527(0.25)	Branchial chamber, internal gills, (2 specimens in intestine)
iii) lung stage with 4 limbs and rudiment of tail	436(131)	30.04	222(0.51)	Urinary bladder
2. *R. reinwardtii*				
i) internal-gill stage with 2 limbs and tail	2768(115)	4.15	263(0.095)	Branchial chamber, internal gills
ii) lung stage with 4 limbs and rudiment of tail	122(15)	12.29	131(1.07)	Urinary bladder
3. *P. leucomystax*				
i) external-gill stage	42(-)	-	-	-
ii) internal-gill stage with 2 limbs and tail	1706(58)	3.39	75(0.04)	Branchial chamber, internal gills
iii) lung stage with 4 limbs and rudiment of tail	75(6)	8.0	43(0.57)	Urinary bladder

- = no infection

* = mean intensity per host examined

to occur in the tadpoles in various stages of metamorphosis representing external-gill stages with no limbs, internal-gill stage with 2 forelimbs and tail, and lung stage with 4 limbs and rudiments 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 specimen each was recovered from the intestine as well. In tadpoles in the late stage of metamorphosis (with lungs and 4 limbs acquired and rudimentary tail), 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 stages during the present study. In *R. nigropalmatus*, while the overall prevalence in internal gills was 6.1% with a mean intensity of 0.25, that in the lung stage was 30.04% with a mean intensity of 0.51. The prevalence of infection in the internal gill stage of *R. reinwardtii* was 4.15% with a mean intensity of 0.095. In the lung stage it was much higher being 12.29% with a mean intensity of 1.07. In *P. leucomystax* the percentage of infection was 3.39 and the mean intensity, 0.04 in the internal-gill stage, and 8 with a mean intensity of 0.57 in the lung stage. The intensity of infection in a single host ranged between 1-15 larvae in the internal-gill and 20.25 larvae in the lung stages. As in the other 2 host species, no infection was found in the external-gill stages and no larvae were recovered from the intestine of the tadpoles of *P. leucomystax*.

REMARKS AND DISCUSSION

(i) Originally *Polystoma indicum* was recovered from *Rhacophorus nigropalmatus*. *R. reinwardtii* is reported herein as a new host record for the species. Some deviations from the original description have been observed. The original authors did not report the presence of microhooks. However, a full complement of 16 microhooks is definitely present in the adult fluke. Microhooks may get easily shed off during handling and many a time varying number of microhooks may be observed in different specimens. In the present study only 3 specimens were found with one intra-uterine egg each, and one specimen with a number of eggs in the uterus, although earlier authors have reported the presence of many eggs. Probably, during the short period of active egg production and deposition, many eggs are not retained in the uterus (Tinsley,1983).

Though no adult flukes were recovered from *P. leucomystax* in the present study, its tadpoles were found to harbour gyrodactyloid-I stages. Other frog species examined from the same or nearby localities did not reveal any monogenean infection. It seems therefore, that only the rhacophorid frogs serve as hosts to *P. indicum* in this region. A high degree of host specificity and restricted geographical distribution is known to be exhibited by species of *Polystoma* (see Combes,1976; Kok and van Wyk,1986; Kok and Seaman,1987). *P. indicum* appears to be another representative of the genus in the Oriental region besides *P. hakgalense* Crusz and Ching,1975 which is reported from *Rhacophorus cruciger eques* in Ceylon (=Sri Lanka). *P. rhacophori* Yamaguti, 1936 from *Rhacophorus schlegeli* var. *arborea* and *Hyla arborea japonica* representing the Palearctic region, bears a close morphological resemblance to *P. indicum.*

While usually a single adult fluke was recovered from one host, up to 4 flukes were also found harboured by the same host. The flukes when occurring more in number had a much smaller body size compared to the flukes that occurred singly. Smaller body size related to the worm burden of 4 may be taken as a crowding effect. A similar effect was reported by Combes (1972) for mature *P. integerrimum* in *Rana temporaria*. Likewise, Jackson and Tinsley (1988) also reported a crowding effect at the lower burden of 2 to 4 *Protopolystoma xenopodis* bladder parasites which were of a size comparable with that of neotenic forms but occurring in a relatively large host. However, in *P. australis,* Kok and du Preez (1989) did not find any crowding effect influencing the development and even reproduction in neotenic parasites.

(ii) The surface fine topography of *P. indicum* adult fluke reveals an intricate pattern of transverse folds and interwining ridges forming a conspicuous honeycomb-like pattern of hexagons. The general tegument, though appears devoid of any spines, seems to have a coarsely or densely beaded texture, which is primarily attributed to the numerous tegumental vesicles or microvilli that are closely associated with the rigid honeycomb. The tegumental topography of *P. indicum* closely resembles that of *Polystoma integerrimum* which also possesses the honeycomb-like pattern of tegumental ridges (Williams and Mckenzie,1995). In most monogeneans the tegument is reported to be elevated into microvilli often in restricted areas (Halton,1979; Ramasamy and Hanna,1986b,1989; Ramasamy et al.,1986). In another polyopisthocotylean monogenean, *Vallisia indica*, the microvillous structures with branchings and interconnections forming an irregular network are found covering only the forebody (Ramasamy et al.,1987). However, in a few monogeneans tegumental microvilli are reported to be completely absent (Ramasamy and Hanna,1985,1986a). The pattern of tegumental ridges as revealed in surface microtopography of many digenetic trematodes probably increase the organism's interphase with the host (Cifrian and Garcia-Corrales,1988). Further, the tegumental tiny vesicles or microvilli along with the associated glycocalyx strand are believed to have a role in stabilizing surface films or protective or adhesive layers (Lyons,1973a; Williams, 1975, 1980, 1981, 1982, 1986). The presence of minute pores in between the honeyccomb pattern of the tegument may be considered as the openings of the parenchymal or subtegumental glands. Similar structures are also observed in *P. integerrimum* by Williams and Mckenzie (1995).

In *P. indicum* there is dense aggregation of domed papillae on the tegument of the circumoral region. These structures have a resemblance to those in many digenea and are suggestive of a sensory function involved in contact reception during food finding and feeding (Silk et al., 1970; Miller et al., 1972; Morris, 1973; Bennet, 1975b; Nadakavukaren and Nollen, 1975; Kuntz et al., 1976; Sakamoto and Ishii, 1977; Bakke,1978; Bakke and Lien,1978; Font and Wittrock, 1980). The papillae having pitted apices may have a chemoreceptive function (Bennet,1975a).

The tegumental surface of gyrodactyloid-I and post-gyrodactyloid-I stages in general is somewhat similar to the adult surface in having the honeycomb texture and appears spongy. The only difference is in the presence (in the post-gyrodactyloid-Ic stage) of the striae of anastomosing lamellae arranged on the lateral margins enclosing within it an oral sensory body with a cilium inside, while in some areas there are clusters of button-like papillae. Presence of cilia in some and absence in other stages is indicative of the parasite development and establishment. However, loss of cilia is attributed to bending movements of the body at the time of searching for the host and ciliary cells are lost immediately on contact with the host (Kearn, 1967; Llewellyn, 1972). Dorsal surface being smooth and devoid of any sensory structures can play no functional role but it might provide minimum friction against passing substances excreted out of the host body through the cloaca.

(iii) The present study on the nervous system of *Polystoma indicum* exhibits a similar basic pattern as that *described for other species of* Polystomatidae *namely, Polystomoides malayi* by Rohde (1972) and *Polystoma integerrimum* by Rahemo and Gorgees (1987). Presence of five anterior pairs of nerves in *P. indicum,* which participate in the formation of the circumoral complex bears resemblance with both the species. However, in *P. indicum* two conspicuous circumoral nerve rings were observed, the anterior one formed by the joining of the most medial and medial nerves, and the posterior formed by the other nerves. Neither in *P. malayi* nor in *P. integerrimum* has a circumoral complex been described comprised of two nerve rings. However, as depicted in the photomicrographs, the circumoral region in *P. integerrimum* presents a complex picture. While the most medial nerve and medial nerve are described as contributing to the formation of the anterior part of the circumoral ring, the lateral nerve and most lateral nerve form its posterior part (Rahemo and Gorgees, 1987). These nerve components do correspond to those forming the anterior and posterior circumoral rings, respectively, in *P. indicum.* In *P. malayi* the most medial nerves are ganglionated and having three parallel branches; these nerves along with the medial nerves form the anterior part of the circumoral ring. The lateral nerves having several branches, and the most lateral nerves which are also branched also contribute to the formation of the circumoral ring. In *P. indicum,* of the anterior nerves, the most medial nerves do not show any ganglionic thickenings along their course, the lateral nerves have two branches each and the most lateral nerves are unbranched. In contrast to these species, in *P. indicum* there are two pairs of pharyngeal nerves. Similar to *P.malayi,* in *P. indicum* the haptoral commissure is quite prominent; however, in the latter only one pair of hamuli nerves, not two, could be observed. In *P. integerrimum* also, there are only two hamuli nerves. In *P. indicum* many nerve cells were observed associated with the ganglia and the main nerves, more abundantly with the haptoral suckers. Rahemo and Gorgess (1987) also observed the occurrence of neurons in *P. malayi,* but found only a few unipolar cells in the vicinity of the haptoral suckers. These authors suggested these cells to be neurosecretory in nature. Reports and information regarding the occurrence of neurosecretory components in platyhelminth parasites have accumulated in the past few years (Reuter and Gustafsson, 1989; Gustafsson, 1990) and very recently (Eriksson,1995). Further investigation seems desirable to ascertain the nature of nerve cells in *P. indicum.*

(iv) Conforming to the general shape of polyopisthocotylean eggs (Baer and Euzet,1961; Kearn,1986), in *P. indicum* the eggs are fusiform and large sized. The shape and size of the eggs of monogenea are revealed to be influenced by different environmental pressures. While the shape of the egg influences the gaseous exchange and transport of other materials through the egg shell, the shape of the egg also influences 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 sclerotin-like protein nature of the egg shell have been reported in several monogenea such as *Gastrocotyle trachuri* (see Freeman and Llewellyn, 1958), *Entobdella soleae* and *Diclidophora luscae* (see Llewellyn, 1965), and *Oögyrodactylus farlowellae* (see Harris, 1983). Smyth and 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 coalasce to form the shell. According to Râmalingam (1970, 1971, 1973b), phenolase exists as an inactive precursor (prophenolase) in the vitellaria of monogeneans belonging to the genera *Pricea* and *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 like in many other monogenea (Llewellyn,1957) and it seems that hatching could occur through the opercular opening by splitting open 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 by longitudinal splitting of the egg shell (Tinsley, 1978a).

In the present study the number of eggs observed in the uterus of the adult worm varied from 1 to 12. The egg-laying period of *P. indicum* under laboratory conditions lasted for 48 h during which a total of 189 eggs could be harvested with 7 eggs still retained in the uterus when it died after 48 h of its detachment from the host. Tinsley (1983) generalized that most monogenea deposit fewer than 100 eggs per fluke per 24 h. However, Combes (1972) recovered 1000-2500 eggs in one day by one parasite of *P. integerrimum* parasitizing an isolated frog. Other polystomatids, viz., *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopodis*, are reported to produce much fewer eggs, their annual output not exceeding 250 eggs (Tinsley, 1983), though other estimates indicate 10-20 eggs/parasite/24 h (Maeder, 1973; Tinsley and Owen,1975). In the present observations the eggs in utero or after release by the parasite 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 and Earle, 1983).

During the present investigation no oncomiracidium of *P. indicum* could ever be encountered, although it is supposed to be the earliest stage of development in the biology 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 4 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 and 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 vaginae, uterus and genito-intestinal canal (Prudhoe and 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 2 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 a high loss of larval parasites at the time of migration must have resulted in the occurrence of less number in the urinary bladder. No tadpole mortality has been observed as caused by parasite burden in case of *P. indicum*, 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 of 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, 1981b).

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 larva attaching to a young tadpole having external gills is well established in *P. integerrimum*, the common European species (Prudhoe and 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). It seems, therefore, that larval stages of different polystome species also may differ in their response to the stimuli associated with the metamorphic changes in the host. Within the anuran polystomatid parasites the intimate adaptation of the parasites' life cycle to the ecology of the hosts is well known (Tinsley, 1978; Murith, 1981b, 1982; Tinsley, 1983). Polystomid parasites of anurans with more terrestrial life may reproduce by either a neotenic cycle or by a vesicular cycle in urinary bladder (Murith,1981b). In *Metapolystoma cachani* parasitizing *Ptychadena longirostris* the life cycle involves both a vesicular cycle in the adult amphibian and a 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 host (Murith et al.,1977). Similar appears to be the case in *P. africanum* which can multiply as egg-laying neotenics on the tadpoles (Combes et al., 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 et al., 1973; Combes et al., 1976; Dupouy and Combes, 1977; Fournier and Combes, 1979). Usually in aquatic hosts the parasite reproduction is reported to be continuous but punctuated in parasites of terrestrial host, ultimately leading to ovoviviparity in certain polystomids (Tinsley, 1983). The rhacophorid hosts, viz., *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).

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 South African polystomes (Murith, 1981a). 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, variations with regards to the hamuli shape was found to be wide ranging; while the hamuli primordia and the developing hamuli besides having a much smaller size than those of the native form lacked incised bases, the juvenile and the adults exhibited varying depths of incision at the base within the same fluke and also among different specimen of the same species. Again, the shape and size of hamuli have been considered to be of taxanomic value and used as differential characters for species of many polystomes (Kok and van Wyk, 1986). In view of the variation observed in this regard in the present study the mere shape of the hamulus base and the size should not be considered so authentic differential characters in respect of polystome taxonomy.

In the present study the occurrence of *P. indicum* confined to only 3 species of rhacophorid frogs and also restricted to certain localities of the region supplements the commonly postulated hypothesis that the monogenea, particularly polystomes, exhibit high host-specificity and restricted geographical distribution (Combes,1976; Combes and Channing, 1979; Kok and van Wyk, 1986; Kok and Seaman, 1987). Other frog species, both adults and tadpoles, occurring in the same locality as of rhacophorid frogs did not reveal any monogenean infection. The reasons for predilection towards one host species and rejection of the others as potential hosts need to be sorted and investigated. Perhaps, physiological and biological characteristics of the host may have a determining role in establishing the host-specificity of the parasite, as well documented in respect of many parasitic species (Smyth and Halton, 1983).

All through the study a low prevalence and a low intensity of infection was observed. While this may be related to the high mortality rates of the infective stage before it is able to find an appropriate host, it might also be accounted to mechanical loss from the host's branchial chamber or their predation (Murith, 1981b). A low intensity seems to be the usual feature in respect of amphibian hosts as indicated by most reports. While usually a single parasite is harboured by the infected host, the maximum number recorded does not exceed 6 or 7 per infected host (Kok and van Wyk, 1986; Harris and Tinsley, 1987; Vojtková, 1989; du Preez and Kok, 1992). Kok and Seaman (1987) reported the occurrence of as many as 15 flukes of *P. umthakathi* in *Natalobatrachus bonebergi*. A low intensity of infection also relates to the usually encountered low prevalence of polystomid parasites.

Parapolystoma bulliense (Johnston, 1912) Ozaki, 1935

Parapolystoma bulliense (Johnston, 1912) Ozaki, 1935

Johnston (1912) described a monogenean species, *Polystomum bulliense* from the urinary bladder of the Australian frogs, *Hyla phyllochroa* and *H. lesueurii*. Ozaki (1935) erected a new genus *Parapolystoma* for this species, on the basis of the uterus extending posteriorly between ovary and posterior end of the body unlike the genus *Polystoma* in which the uterus lies anterior to the ovary. Ozaki (1935) also transferred *Polystoma alluaudi* Beauchamp, 1913 to the newly created genus *Parapolystoma*. However, Yamaguti (1963) erected the genus *Beauchampia* for *P. alluaudi* but Euzet, Combes and Knoepffler (1966) considered this species to be congeneric with *Eupolystoma rajae* Kaw, 1950; Prudhoe and Bray (1982) regarded *Beauchampia* as a synonym of *Eupolystoma*. Williams (1960) provided supplementary information on the morphology of *P. bulliense* (Johnston,1912) Ozaki, 1935.

The genus *Parapolystoma* is hitherto represented in anuran hosts in the Asian South-East, *P. bulliense*, the type species having been reported from Australia and Papua New Guinea and *P. crooki* Vande Vusse, 1976 from the Philippines. During an exploratory survey of the monogenea of Amphibia in Meghalaya state of North-East India, some raniid frogs were found to harbour a polystomatid species, the morphological features of which tally with *P. bulliense*.

Parapolystoma bulliense (Johnston,1912) Ozaki,1935, being the first report of the species from the Indian subcontinent, is redescribed herein.

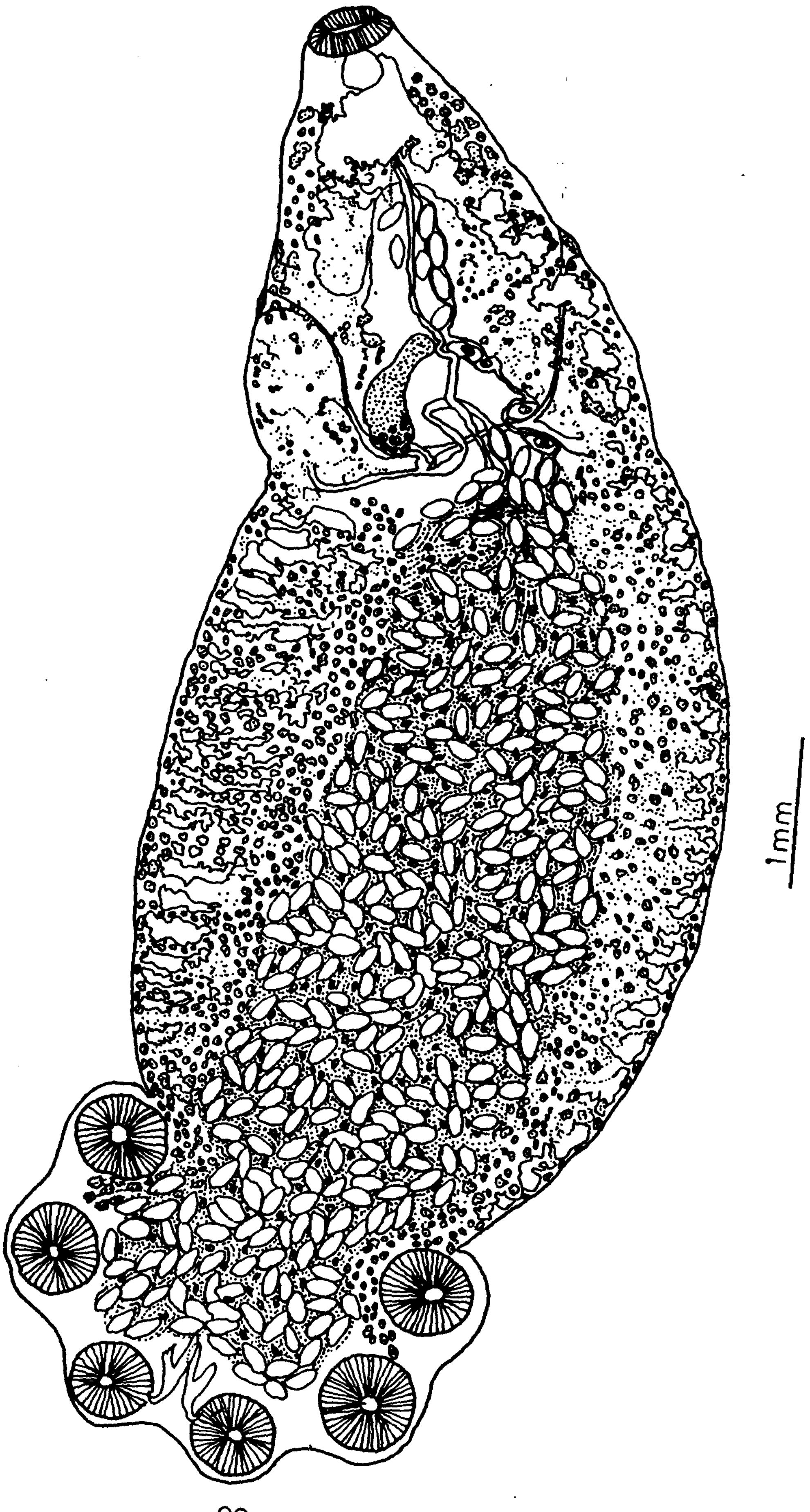
Order - Polyopisthocotylea Odhner, 1912
Family - Polystomatidae Carus, 1863 emended Gamble,1896
Genus - *Parapolystoma Ozaki*, 1935
Parapolystoma bulliense (Johnston,1912) Ozaki,1935

1. ADULT WORM

(i) Morphology and Surface Topography (Figs.80 - 88)

Description (based on 10 mature specimens, 5 of which contained eggs in the uterus).Body foliate, elongate, somewhat tapering towards anterior region. Prohaptor a suberminal oral sucker encircling the mouth. Pharynx prominent, muscular, leading to bifurcate intestine. Intestinal caeca indented at irregular

Fig.80 : *Parapolystoma bulliense* : Whole mount, ventral view



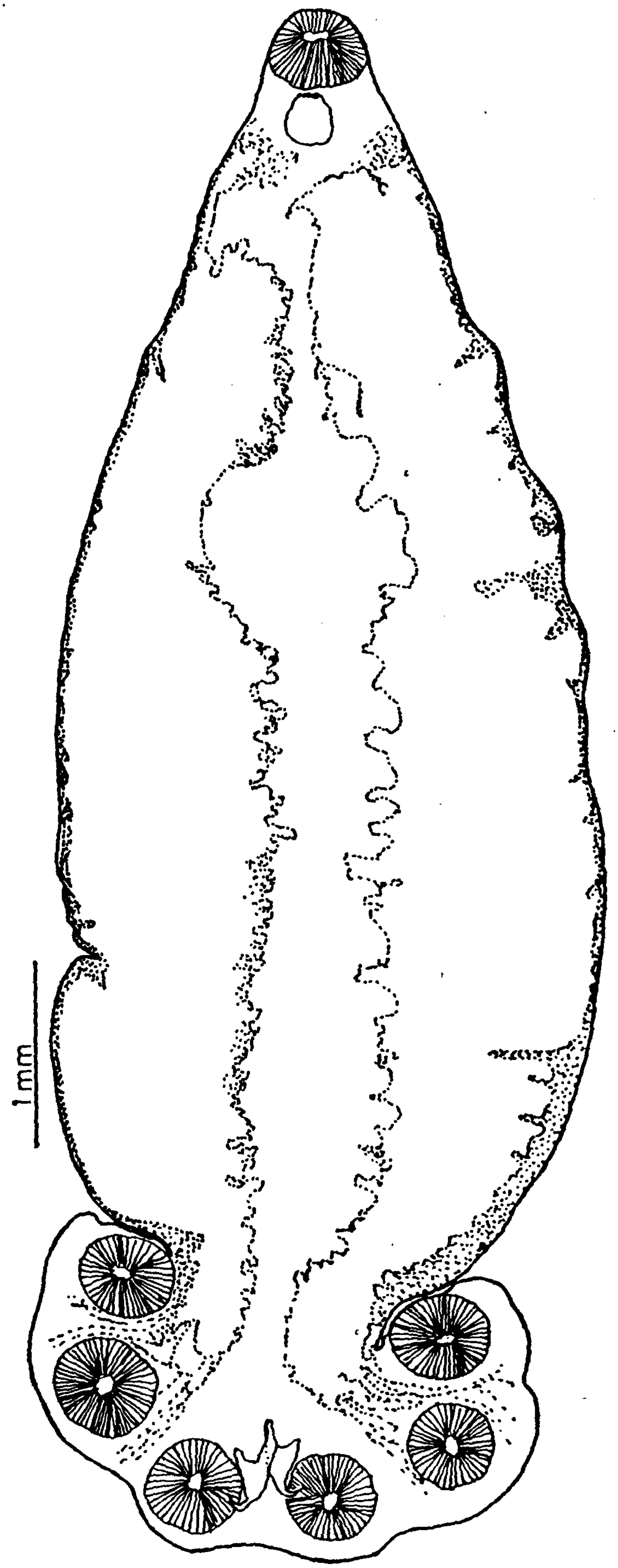
80

Fig.81 : *Parapolystoma bulliense* : Whole mount

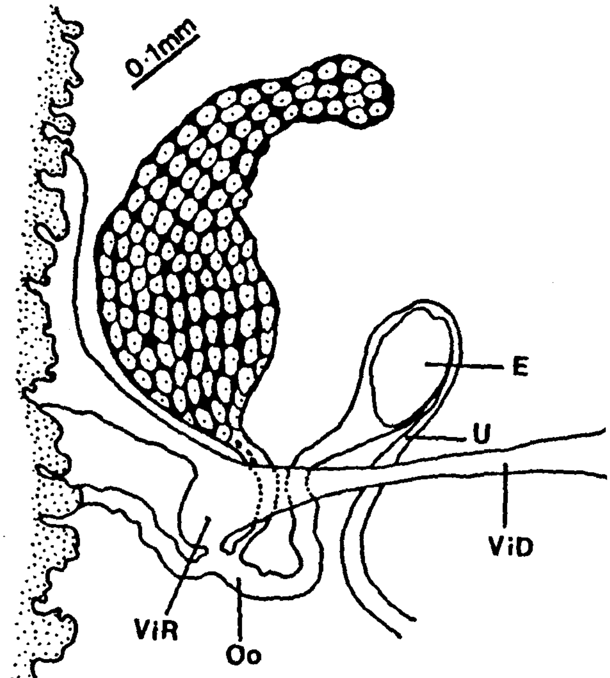
(i) : Showing extent of vitellaria (represented by dotted limits)

(ii) : Genital complex

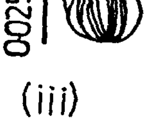
(iii) : Crown of genital spines



(i)



(ii)



(iii)

Figs.82-85 : *Parapolystoma bulliense* : photomicrographs

82 : Anterior two-thirds of juvenile fluke

83 : Opisthaptor with a pair of hamuli in a juvenile fluke

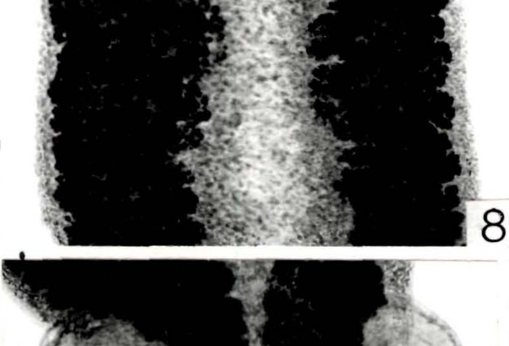
84 : Anterior end showing well developed genital complex of adult fluke

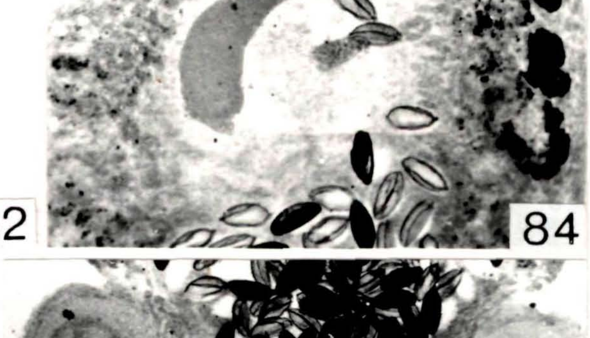
85 : Opisthaptor of adult fluke

(scale bar 0.1 mm)


82


84


83


85

intervals on lateral aspects, sending out prominent diverticula from their inner facet which in turn give off secondary branches, interspersed with smaller unbranched diverticula, extending into opisthaptor and may touch but apparently do not fuse, diverticulation almost absent in region of caecal union. Opisthaptor small compared to body dimensions, usually less than half of body length; with 6 prominent clamps or suckers, a pair of hamuli, 16 microhooks; of microhooks, 1 in each clamp, 2 pairs below hamuli, 3 pairs in anterior region of opisthaptoral disc; hamuli with incised and biforked base and sharply pointed recurved tip in most specimens, in others (both juvenile and adults) without incision and biforked condition. Testis a compact mass in juvenile stage, gradually becoming follicular, forming a reticulate network, commencing from behind intestinal bifurcation, ventral net-like extensions from edge to edge in middle third of body with narrow anterior extensions behind intestinal caeca and broad posterior extensions tapering towards lateral margins; vas deferens arising from mid dorsal surface of testis, extending anteriad at penis bulb at level of intestinal bifurcation; penis bulb with genital crown of 11 hooklets. Ovary retort shaped, on left or right of median line in anterior third of body; faintly stainable Mehlis' gland complex opposite to base of ovary; uterus intercaecal, descending arm with broad transverse loops reaching opisthaptor, ascending arm straight and narrow reaching anterior edge of genital complex, containing numerous eggs. 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, vaginal ducts passing posteriad and joining vitelline ducts. Vitellaria follicular, extensive, commencing from pharyngeal level, extending lateral and dorsal to caeca, posteriorly to opisthaptoral zone, intruding in spaces between suckers; common vitelline reservoir ventro-posterior to ovary. Eggs numerous, large, oval, slightly flattened on one side, operculate at narrow end, with terminal antopercular depression, mature eggs in uterus fully embryonated containing a well developed oncomiracidium.

The morphometric measurements of the adult are presented in Table 6.

Surface Fine Topography

The tegument in the adult fluke has fine transverse folds and craters throughout the pre-opisthaptoral part of the body and is smooth, completely devoid of any spines or scales (Fig.86). At higher resolution the general tegument exhibits a pattern of interlacing network of ridges forming honeycomb-like hexagons (Fig.87); both the ridge walls and depression floors have fine tuberculate tegumental vesicles (Figs.89-91). While the mesh pattern appears denser on the ridged parts of the folds, it is quite stretched in the craters (Figs.86,87). On the ventral surface of the body domed button-like papillae are also found scattered in between the tegumental network of ridges (Fig.89). The tegument of the antero-dorsal end of the fluke,

TABLE 6

Comparison and morphometric measurements of species of *Parapolystoma*

Characters		*P. bulliense*(Johnston,1912) Ozaki,1935				*P. crooki* Vande Vusse, 1976	
		Present Observations		Original Observations			
		Range(mm)	Mean($\bar{x}$)	Range(mm)	Mean($\bar{x}$)	Range(mm)	Mean($\bar{x}$)
Body:	Length	7.344 - 11.903	9.387	3	-	1.7 - 7.5	3.5
	Breadth	2.493 - 3.396	2.9	1	-	0.5 - 2.2	1.1
Oral sucker	Length	0.229 - 0.413	0.373	-	-	$0.177^{(*)}$	$0.113^{(*)}$
	Breadth	0.382 - 0.535	0.45	-	-		
Opisthaptor	Length	1.453 - 2.142	1.973	-	-	0.6 - 1.485	0.953
	Breadth	2.264 - 3.044	2.772	-	-	0.679-1.943	1.127
Haptor length : Body length		1:5.054-1:5.556	1:4.757	-	-	1:2.833-1:5.05	1:3.672
Haptor Breadth : Body Breadth		1:1.101-1:1.115	1:1.046	-	-	1:0.736-1:1.132	1:0.976
Hamulus length		0.428 - 0.703	0.55	smaller than *P. crooki*		0.329 - 0.512	0.401

cont.

Microhooks	Length	0.019 - 0.034	0.025	–	–	0.019	–
	Breadth	0.003 –	–	–	–	–	–
Opisthaptoral clamp	Length	0.413 - 0.651	0.556	–		0.207 - 0.559$^{(*)}$	0.265$^{(*)}$
	Breadth	0.41 - 0.593	0.531	–			
Pharynx	Length	0.184 - 0.275	0.244	–	–	–	–
	Breadth	0.153 - 0.306	0.243	–	–	–	–
Ovary	Length	0.780 - 1.04	0.966	–	–	0.130 - 0.422	0.260
	Breadth	0.214 - 0.291	0.269	–	–	0.05 - 0.206	0.104
Genital spine	Length	0.023 - 0.03	0.025	–	–	0.012 - 0.017	–
	Number	11		8 - 13	–	9 - 13	–
Distal of vagina from anterior end		1.759 - 1.913	1.814	–	–	–	–
Embryonated eggs in utero	Length	0.22 –	–	–	–	0.06	–
	Breadth	0.13 - 0.15	0.14	–	–	0.038	–
	Length	0.22	–	–	–	–	–

cont.

Unembryonated eggs in utero Breadth	0.10 - 0.12	0.11	-	-	-	-
Host	*Rana malabarica*		*Hyla phyllochroa* *, *Hyla lesueurii* *, *Litoria arziana* **, *Litoria becki* **, *Litoria lesueurii* ***, *Litoria phyllochroa* ***, *Nyctimystes kubori* **, *Nyctimystes papua* **,		*Rana magna*	
Locality	Meghalaya		Oriental and Australasian * Papua, New Guinea ** Australia ***		Philippines	

(*) = Diameter

* ¦
** ¦ = Hosts with their corresponding locality.
*** ¦

Figs.86-91 : *Parapolystoma bulliense* : scanning electron micrographs of the adult fluke

86 : Adult fluke (scale bar 100 µm)

87,88 : Tegument of midbody region (scale bar 1µm)

89 : A closer view of the same, at a high resolution (scale bar 1 µm)

90 : Tegument of the antero-dorsal region near the oral sucker. Pitted areas (arrows) with thich rims are scattered on the honeycomb mesh (scale bar 10 µm)

91 : A tegumental pit at a higher resolution (scale bar 1µm)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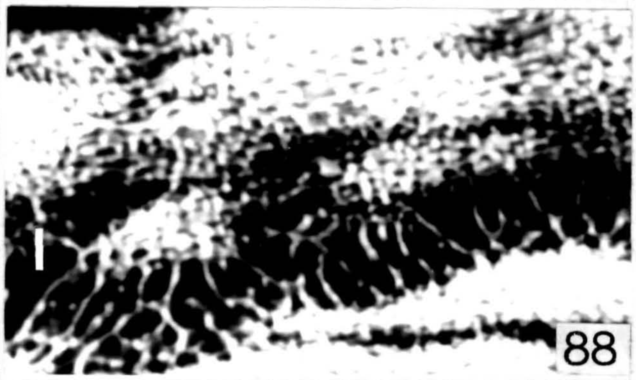
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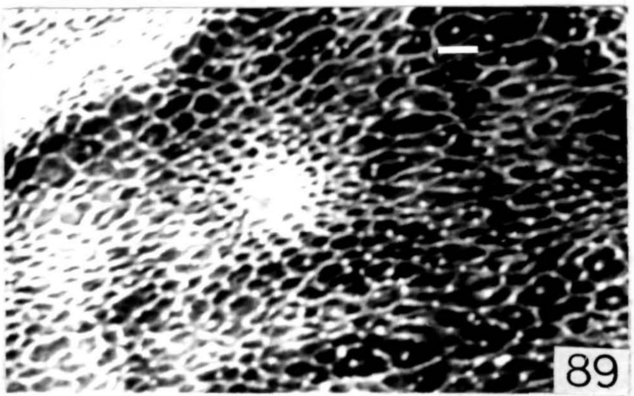
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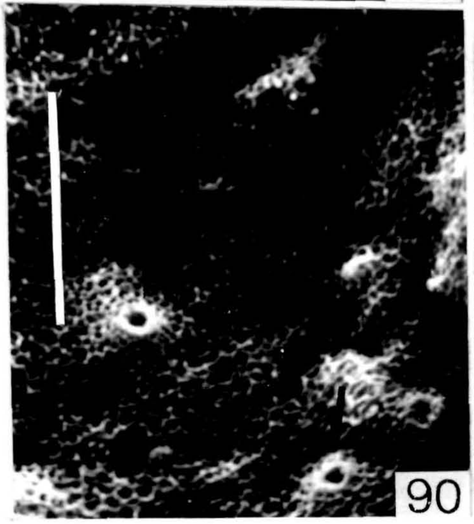
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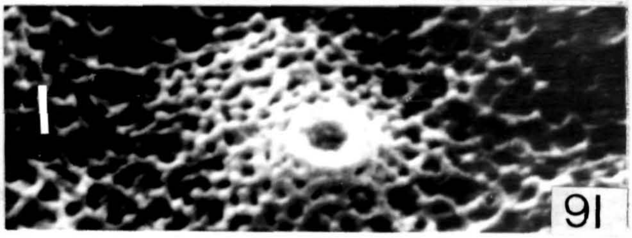
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adjacent to the prohaptoral oral sucker has randomly scattered pits each of which is surrounded by a rosette of tegumental vesicles which form a thick rim (Figs.90,91). The elevations of the vaginal openings on anterolateral aspects of the body are conspicuous; the tegument covering these bulges also has the basic network pattern (Fig.92). The tegument of the opisthaptoral region also exhibits a folded texture. The rim of the opisthaptoral clamps shows radial foldings forming regular ridges and valleys (Figs.93-95). A similar network as that of the tegument of the main body exists on the rim of the clamps (Fig.96).

Host : - *Rana malabarica*
Location : - Urinary bladder, ureter, cloacal chamber
Locality : - Mawsynram, Tura (Meghalaya,India)

2. DEVELOPMENTAL STAGES

Only one larval stage, i.e., oncomiracidium and juvenile were recognizable during the development of the fluke from embryonated egg to mature adult (Figs. 98 - 102).

(1) Culture of the eggs from live worms in vitro

The adult gravid flukes were found to contain eggs numbering 271 to 476. Eggs dissected out from these flukes showed varying degree of embryonation and some increase in their

Figs.92-96 : *Parapolystoma bulliense* : scanning electron micrographs of the adult fluke

92 : Lateral margin of the body, showing vaginal protrusion (scale bar 10 µm)

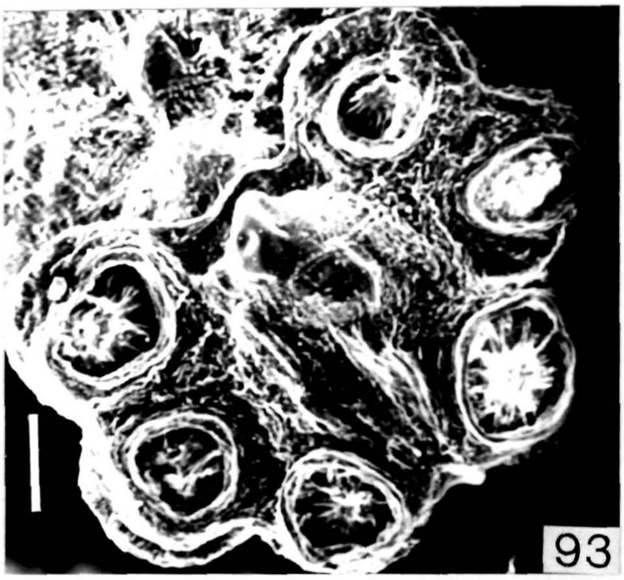
93 : Opisthaptoral disc, showing 6 well-developed clamps (scale bar 100 µm)

94 : A single opisthaptoral clamp in a magnified view (scale bar 10 µm)

95 : A portion of the rim of the opisthaptroal clamp (scale bar 10 µm)

96 : Same in a further magnified view (scale bar 10 µm)

92



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dimensions with development. The flukes when kept in tap water under laboratory conditions laid between 80 and 235 eggs per day for three and half days, whereafter they did not survive.

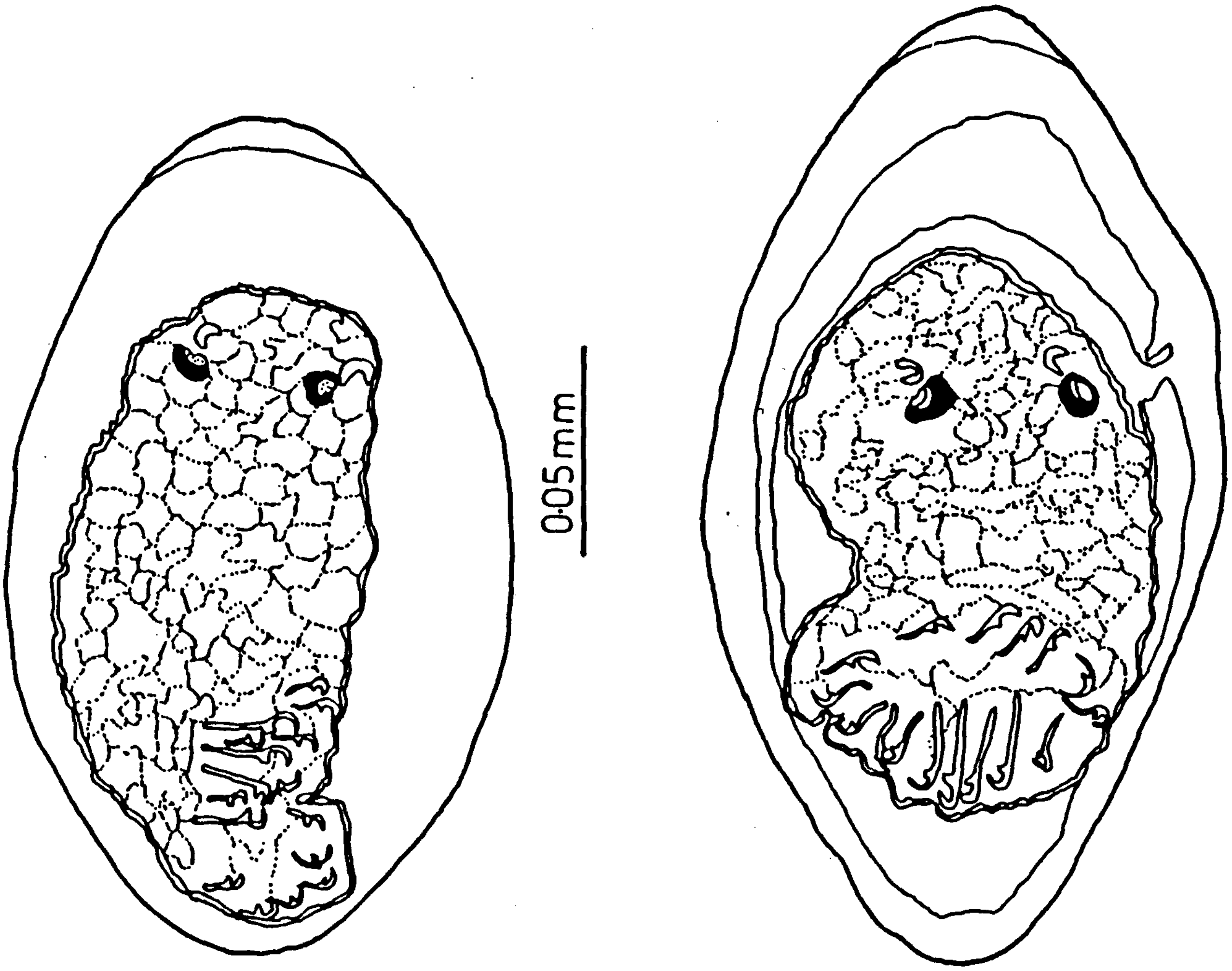
Freshly laid unembryonated eggs (Fig.98), maintained in Ringer's solution at the ambient room temperature (17-21°C) took about 33 days, and in tap water 21 to 26 days, to develop an oncomiracidium within (Fig.99). However, at 30°C in tap water they took about 10 to 15 days for the fully developed mobile oncomiracidium (Fig.100) to come out. In contrast, the embryonated eggs from utero when placed in tap water, began hatching within 3 to 4 h which continued for 24 to 48 h. The oncomiracidium emerges out through the opercular opening with its opisthaptor first, swims rapidly in a darting, searching manner. While swimming the body elongates front and backward with eyes always upwards and later attaches to the bottom of the culture petridish.

(2) Morphology And Surface Topography

(i) Egg (Figs.97-99,103-105)

The eggs of *Parapolystoma bulliense* are large, oval, operculate and deep brown in appearance. Both embryonated and unembryonated eggs are contained in the uterus. The egg shell surface is smooth and the egg shell gives a positive reaction with catechol staining, indicating the presence of phenolase, and thereby a sclerotin-like protein nature.

Fig.97 : *Parapolystoma bulliense* : Diagrammatic representation of embryonated eggs containing fully developed oncomiracidium



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Figs.98-102 : *Parapolystoma bulliese* : photomicrographs

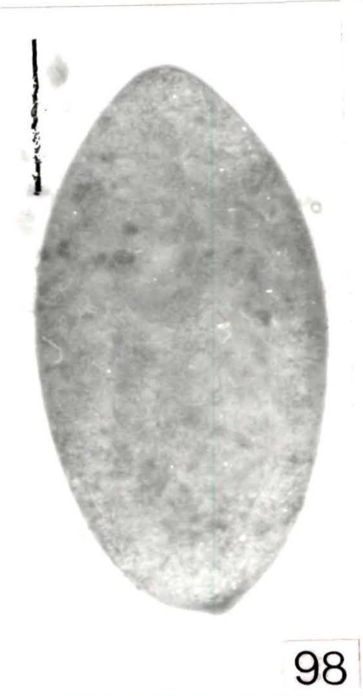
98 : An unembryonated laid by the fluke kept in tap water (scale bar 0.025 mm)

99 : Embryonated egg, enclosing a fully developed oncomiracidium (scale bar 0.025 mm)

100 : Oncomiracidium hatched out fom egg (scale bar 0.025 mm)

101 : Juvenile fluke (scale bar 1 mm)

102 : A mature adult flukle, ventral view (scale bar 1 mm)



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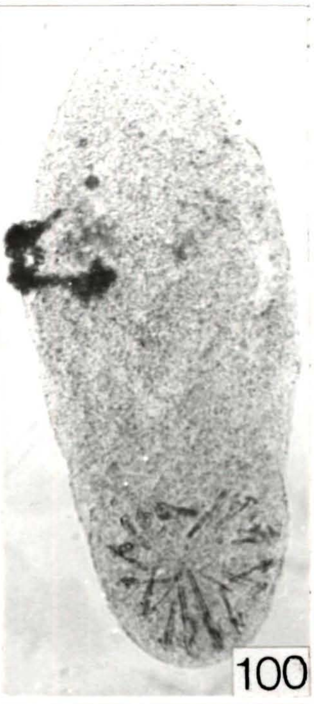

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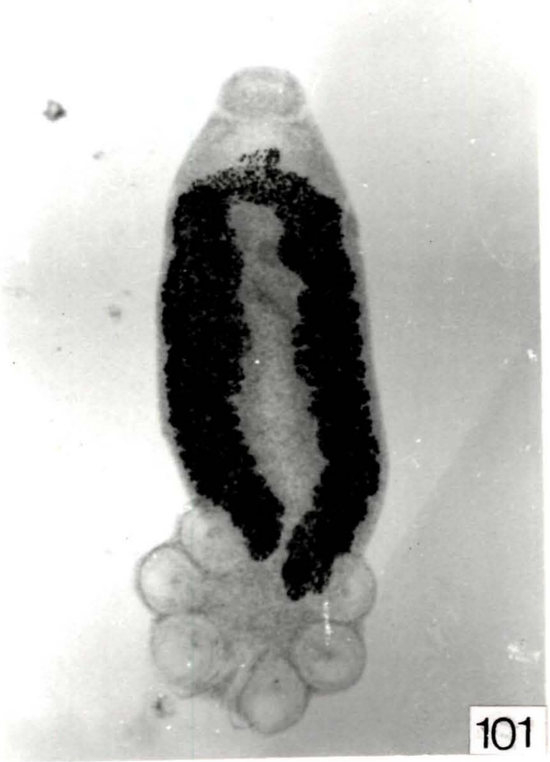

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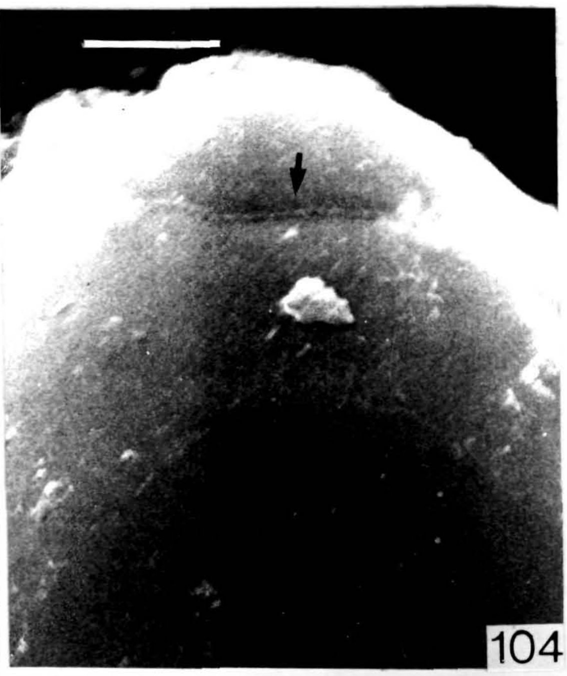
Figs.103-105 : *Parapolystoma bulliense* : Egg, scanning electron micrographs

103 : One fully developed egg (scale bar 10 µm)

104 : Operculum (arrows) of fully developed egg (scale bar 10 µm)

105 : A magnified view of the surface (scale bar 10 µm)

103



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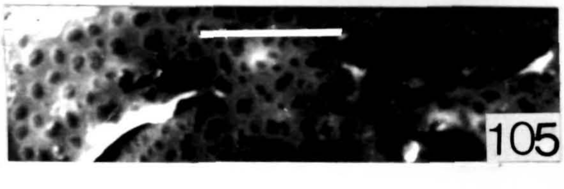

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Scanning electron microscopic studies revealed that the eggs have apparently smooth surface with bluntly rounded posterior end and a slightly tapering anterior end at which an operculum is present (Figs.103,104). At higher resolutions, however, the egg shell is revealed to have a blebbed contour, the blebs being irregular with varying shapes and dimensions (Fig.105).

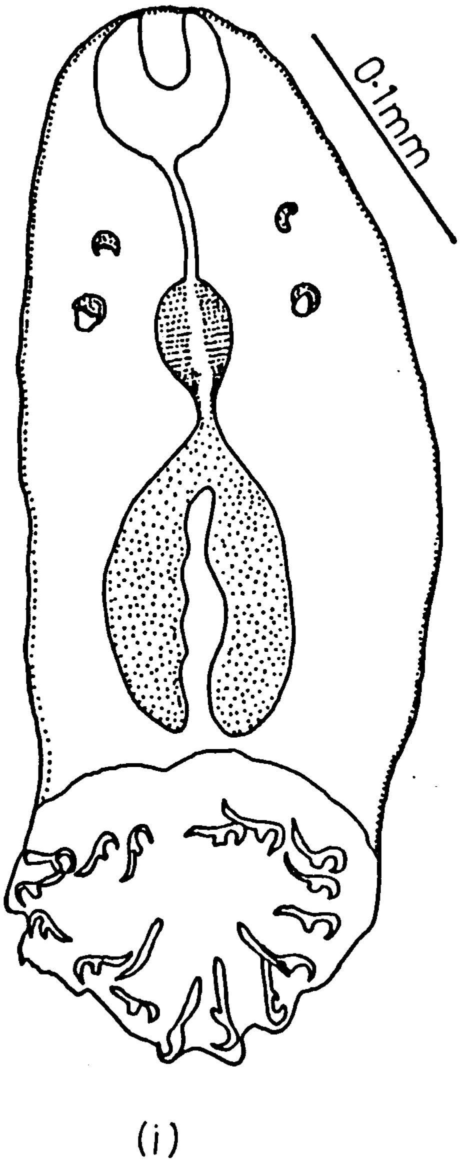
(ii) **Oncomiracidium** (Figs.106-110)

This is the earliest stage recovered from the mature eggs of the adult fluke. It possesses a minute body, highly mobile, and somewhat cylindrical with a terminal oral opening in the form of a prohaptor surrounding the mouth. The posterior quarter of the body is made up by the opisthaptoral disc. The opisthaptor has wavy margins but its anterior boundary is not constricted off from the main body. The haptoral disc contains 16 microhooks and a pair of hamuli primordia. The latter are simple structures without any incision or bifurcation at their base. Regarding the microhooks arrangements, 3 pairs are anterior to the hamuli primordia, 2 pairs occupy the region latero-posterior and in between the latter and 6 microhooks are lodged in the positions where future 6 clamps would develop. The prohaptor leads to the pharynx and behind it is the bifurcated intestine. The intestinal caeca are simple, thin walled and unequal, extending up to the middle third of the body. 2 pairs of conspicuous pigmented eyespots are observed on either side of the pharynx.

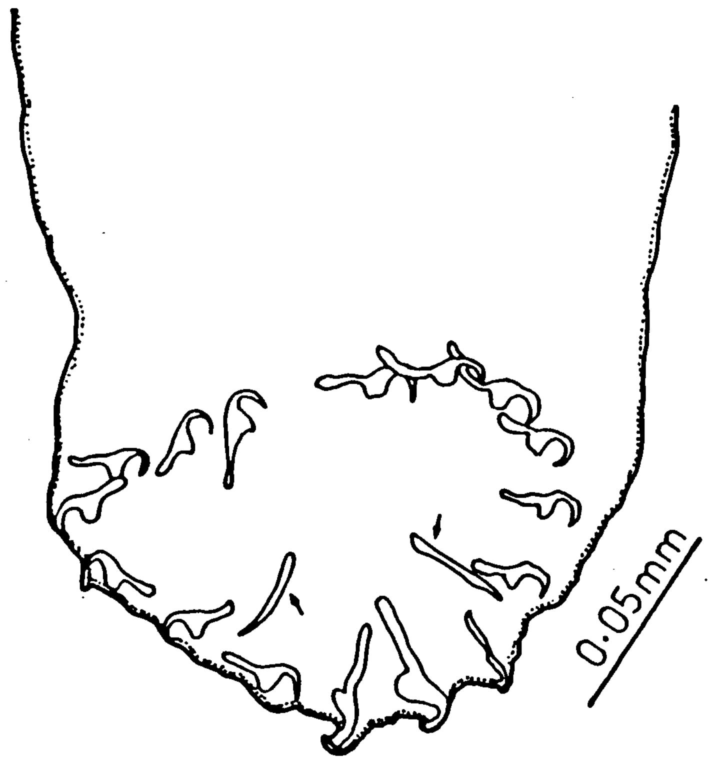
Fig.106 : *P. bulliense* : Oncomiracidium

(i) : Whole mount, ventral view (scale bar 0.1 mm)

(ii) : Opisthaptoral disc, magnified, showing microhooks and hamuli primordia (arrows) (scale bar 0.05 mm)



(i)



(ii)

Morphometric measurements of the body and other structures of the oncomiracidium are presented in Table 7.

Scanning electron microscopy of the oncomiracidium revealed that its body surface is thrown into irregular transverse folds. The whole body of the larva has a porous texture and appears spongy, ornamented by numerous singly arranged cilia with blunt tips and sparsely distributed randomly throughout the surface and interspersed with a number of prominent tegumental folds presenting an uneven and rough contour to the surface (Figs.107,108). The ciliary plate cells, however, are not traceable anywhere. The oral opening is also surrounded by numerous short cilia coming out singly from the sucker tegument (Fig.109). The opisthaptoral disc is endowed with the microhook armature given out from the tegumental tissue, with sharp anterior tips projecting outwards (Fig.110).

(iii) **Pre-adult** (=juvenile) (Figs.111,112)

This stage is identifiable by the presence of 6 clamps on the opisthaptor. In this stage the juvenile fluke acquires an elongated, dorsoventrally flattened and more compact body and opisthaptor like the adult fluke. With the development of 6 clamps incorporating 1 microhook in each the number of free microhooks gets reduced to 10. Eye spots are not traceable in this stage. The caecal bifurcation begins immediately behind the pharynx and the two caeca become thick walled and well

TABLE 7

Morphometric measurements of oncomiracidium of *P. bulliense*

Characters		Range(mm)	Mean ($\bar{x}$)
Body size	Length	0.214 - 0.306	0.263
	Breadth	0.076 - 0.137	0.113
Opisthaptor	Length	0.061 - 0.091	0.078
	Breadth	0.076 - 0.122	0.099
Hamulus primordium	Length	0.022 - 0.030	0.023
Microhook	Length	0.019 - 0.034	0.025
	Breadth	0.003 -	-

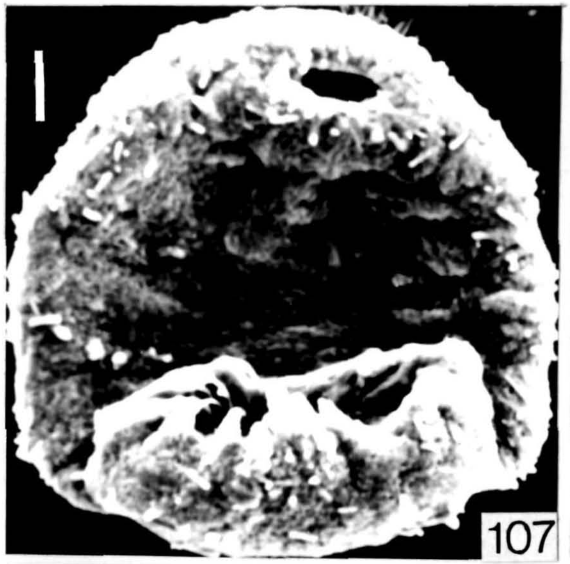
Figs.107-110 : *Parapolystoma bulliense* : Oncomiracidium, scanning electron micrographs

107 : Oncomiracidium in ventral view (scale bar 10 µm)

108 : General tegument of the body (scale bar 10 µm)

109 : Anterior region magnified, showing oral opening (scale bar 10 µm)

110 : Opisthaptoral disc displaying microhooks (scale bar 10 µm)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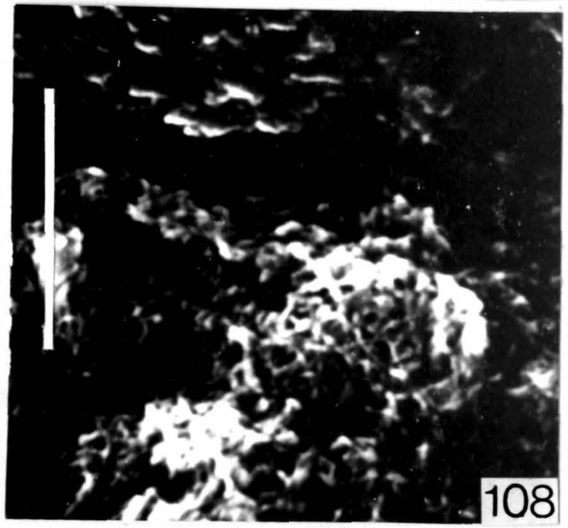
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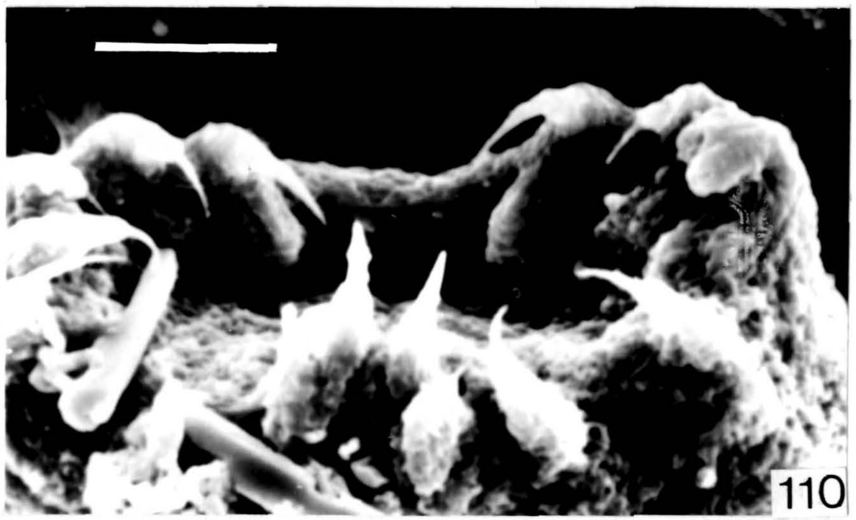
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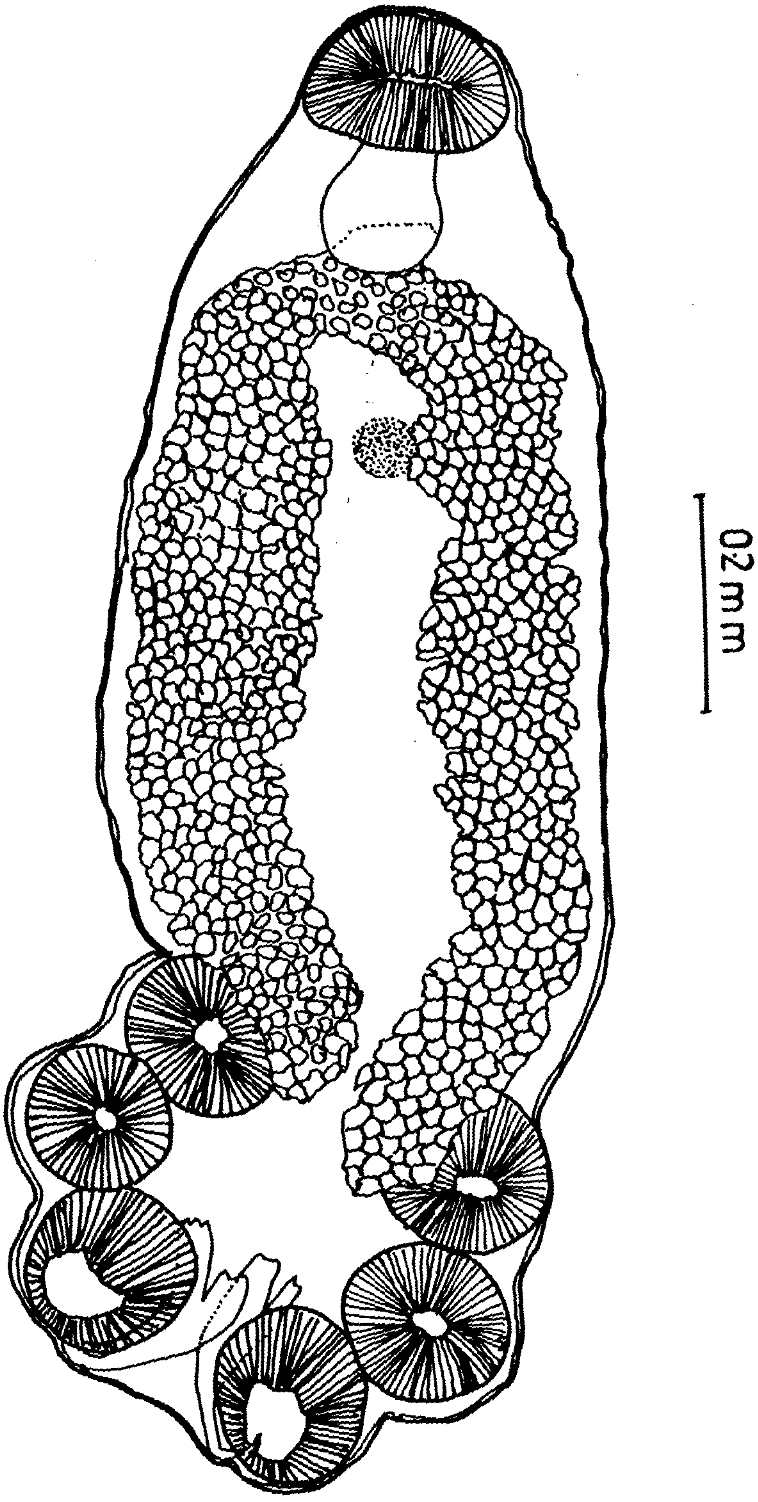
Fig.111 : *P. bulliense* : Juvenile fluke in ventral view; diagrammatic representation



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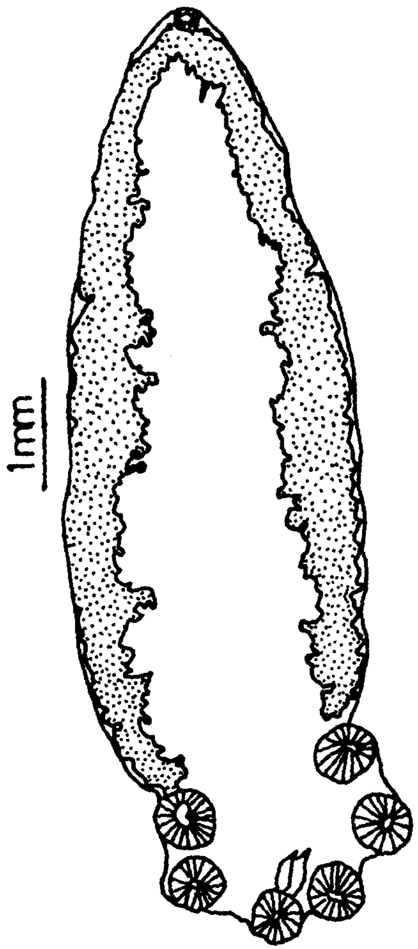
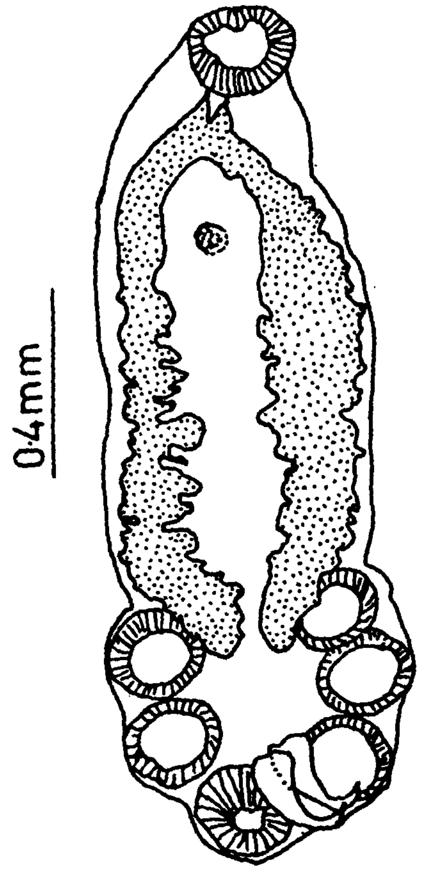
developed, extending posteriad up to or a little before the opisthaptoral disc. The outer margins of the intestinal caeca show irregular indentations. The caeca do not join each other posteriorly. However, even among the individuals recovered from the same host the intestinal caeca present variations. In some, the caeca are with slightly indented outer margins lacking prominent diverticula, while in others both their inner and outer margins have diverticulations and they extend up to the level of the second pair of clamps. In some others the outer margins are somewhat smooth but inner margins are thrown into extensive diverticulation; diverticula are of different shapes and sizes, and those of the two sides may fuse medially to form bridge-like structures giving an anastomosing pattern (Fig.112).

There is gradual increase in the shape and size of several structures, particularly the oral sucker, pharynx, intestinal caeca, opisthaptoral clamps and hamuli along with the increase in the body size of the juvenile. However, there is no remarkable change in the dimensions of the microhooks from larval to adult stage. The juvenile form resembles the adult in all general features except that it lacks a conspicuous reproductive system. The shape, size and incision of hamuli varies among different individual flukes. Variation occurs in the relative length and depth of the incision; while in some flukes the hamuli are with even, undulating bases, in others they may exhibit

Fig.112 : *P. bulliense* : Diagrammatic representation of the variations in the caecal diverticulation in the juvenile (i,ii) and adult (iii - v) flukes



(i)



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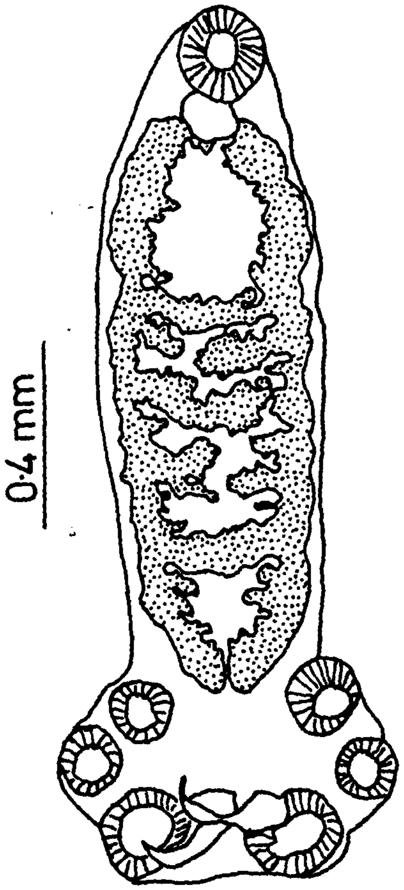

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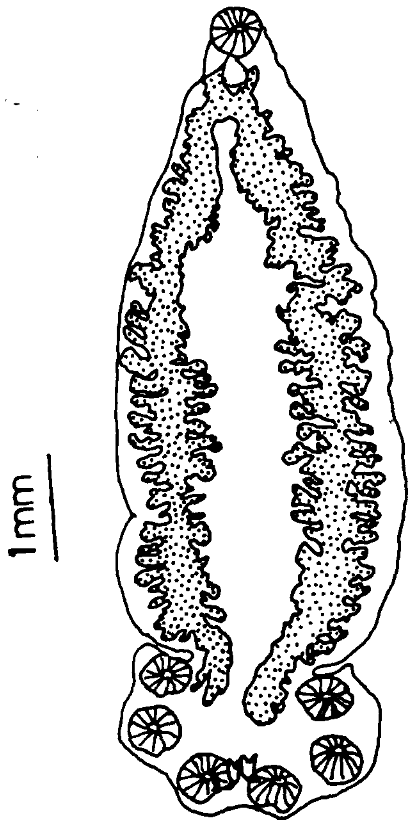

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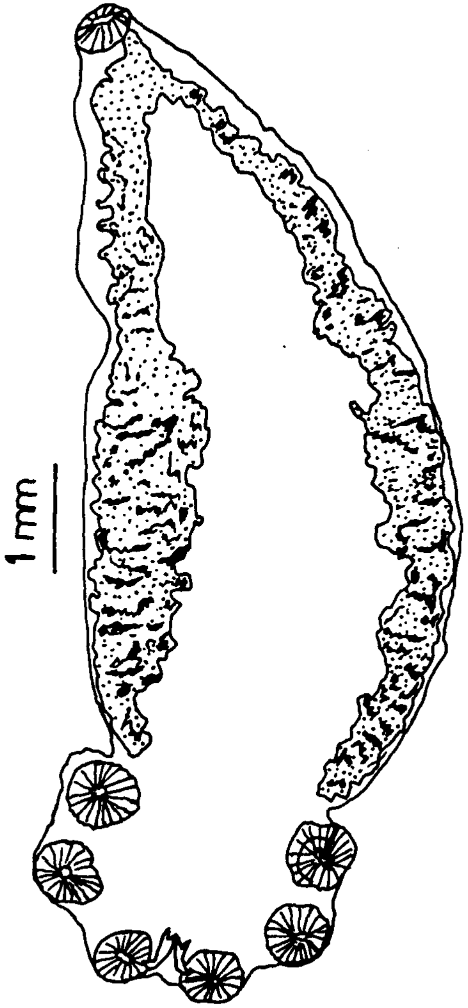
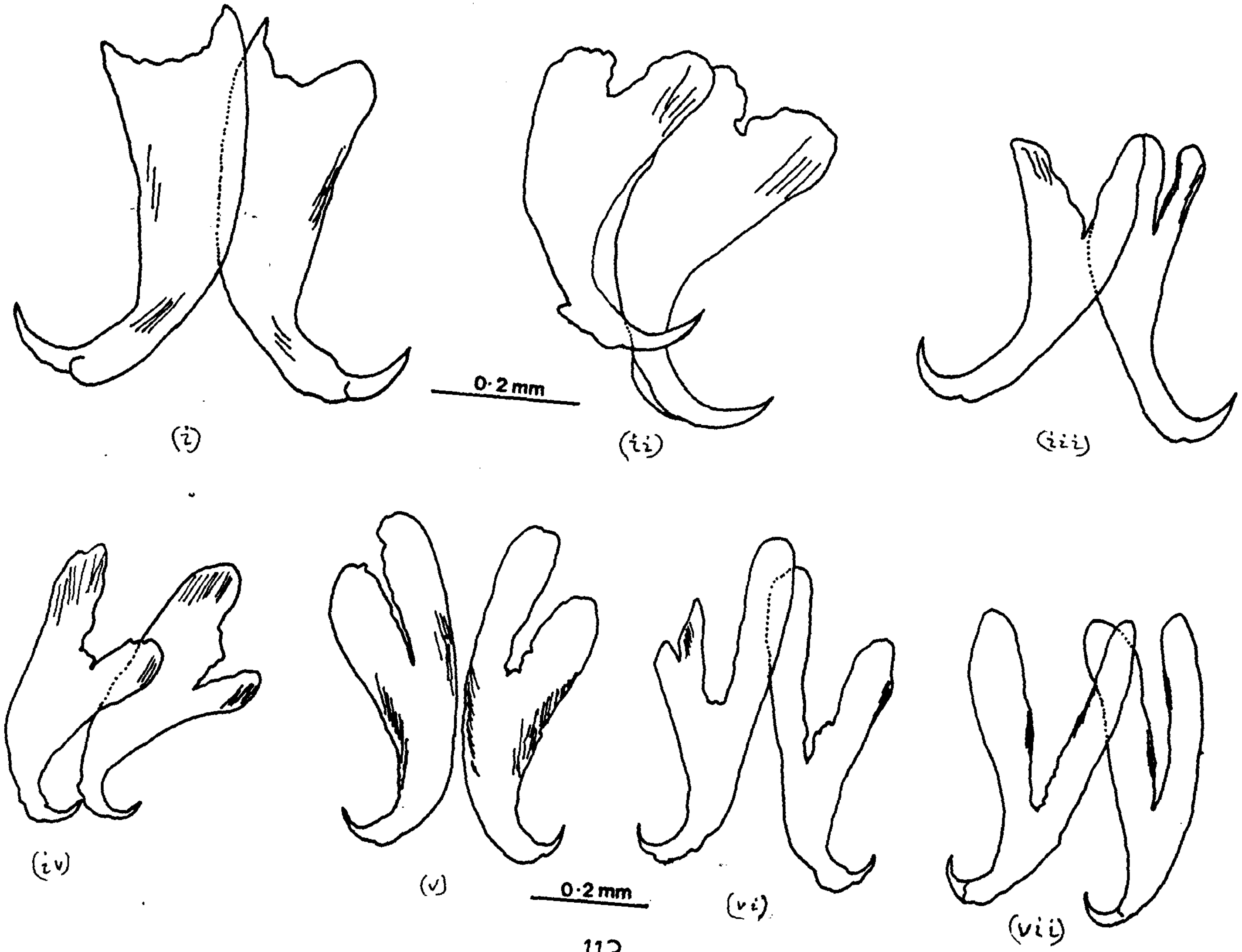


(v)

Fig.113 : *P. bulliense* : Diagrammatic representation of the variations in the shape and size of the hamuli in juvenile (i - iii) and adult (iv - vii) flukes



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slightly indented to deeply indented bases, the incision forming conspicuous guard and handle (Fig.113).

3. PREVALENCE AND DISTRIBUTION

The *P. bulliense* infection was found to occur only in *Rana malabarica* (Table 8) and restricted to Mawsynram and Tura. The adult frogs were found to harbour both the adult and juvenile flukes. The prevalence of *P. bulliense* in *R. malabarica* of Mawsynram and Tura was observed to be 50% and 15.96%, respectively; however, the host sample size from Mawsynram was too small to lead to any significant results. The overall intensity of infection was quite low; it ranged from a single fluke, up to 6 in a single infected host, the mean intensity of infection being 0.5 and 0.19 in the two localities. Three adults and 2 juveniles were recovered from the same host in two instances; otherwise usually a single fluke was recovered per infected host. The prevalence of larval stages in tadpole hosts was found to be nil.

REMARKS AND DISCUSSION

(i) On the basis of having the male gonad in the form of a reticular or follicular mass, the form under the present study belongs to the family Polystomatidae Gamble, 1896; the character of the uterus, that is extending posteriorly well beyond the ovary, places it under the genus *Parapolystoma*. The

TABLE 8

Host-wise prevalence of adult *Parapolystoma bulliense* in *Rana malabarica*

Host species	Locality	No. Examined (No. infected)	% of occurrence		Adult flukes recovered *(mean intensity)
Rana malabarica	Mawsynram	4(2)	50^{**}	12.1	2(0.5)
	Barapani	50(-)	-		-
	Tura	119(19)	15.96		23(0.193)

- = no infection

* = mean intensity per host examined

** = sample size too small

morphological details of the present form tally with those of *P. bulliense* (Johnston, 1912) Ozaki, 1935 and set it apart from *P. crooki* Vande Vusse, 1976. However, there are minor variations from the original description of *P. bulliense* with regard to the size of the body and its organs. The only major deviation observed in the specimens studied herein relates to the nature of the intestinal caeca; the latter are described to be without diverticula in *P. bulliense*. However, the present observations strongly suggest that caeca could exhibit varying degree of diverticulation in different individuals. Therefore, it appears that caecal diverticulation, being a variable character, cannot be considered an authentic criterion for species differentiation in the present case and that it represents only an intraspecific variation. A close comparison of the present form with the other known species, *P. crooki*, also reveals a similarity of morphological characters between the adults of the two. However, the oncomiracidium stage of the present form conspisuously stands apart from that of *P. crooki* which is characterized by having 55 ciliated epidermal plates arranged in 6 rows. In the present form the oncomiracidium stage was found to have a randomly ciliated body, without any precise arrangement of cilia in epidermal plates. In view of the foregoing, the present form has been identified as *P. bulliense*. Characters of spermiogenesis and spematozoon ultrastructure have recently been exploited as newer tools in monogenean taxonomy and phylogeny (Justine, 1993).

Further study on similar aspects will help ascertain precise identification of the species.

P. bulliense has so far been reported from *Hyla phyllochroa* and *H. lesueurii*, *Litoria arziana*, *L. becki*, *L. lesueurii*, *L. phyllochroa*, *Nyctimystes kubori* and *N. papua* from Australia and Papua New Guinea. *Rana malabarica* is reported herein as a new host record for the species. While all other frog species from the same and nearby localities were examined, there was no sign of any monogenean infection in them. It is likely that only *Rana malabarica* serves as host to *P. bulliense* in this region. Restriction of *P. bulliense* to only this anuran species in the region again reiterates the high degree of host specificity and a restricted geographical distribution exhibited by polystomes (Combes, 1976; Kok and van Wyk, 1986; Kok and Seaman, 1987).

The number of flukes per adult host ranged from 1 - 6. Both adult and juvenile flukes were also recovered from the same host specimen. The juvenile having a smaller body size than the adult and primordia of reproductive system were observed only in some cases. Recovery of both adult and small-sized juvenile forms from the same host may relate to the crowding effect as reported in the case of *P. integerrimum* in *Rana temporaria* and *Protopolystoma xenopodis* (Combes,1972; Jackson and Tinsley,1988).

(ii) The present investigation on the surface fine topography of the adult *P. bulliense* flukes reveals more or less similar features as those of *P. indicum*. Like *P. indicum*, in *P. bulliense* also the surface tegument presents a non-spinous nature, with conspicuous ridges and craters covered all over by an intricate pattern of honeycomb-like mesh work of hexagons. However, this pattern is more finely and discretely displayed in *P. bulliense*. While in *P. indicum* the circumoral region is provided with a dense aggregation of domed papillae, in *P. bulliense* such an aggregation is not observable. Instead, discrete round pits with thick tuberculated rim abound in the antero-dorsal region. The location and morphological features of these pits are suggestive of a sensory function (Morris and Threadgold, 1967; Erasmus, 1967; Lyons, 1972; Page et al., 1980; Hoole and Mitchell, 1981; Otubanjo ,1987). Though the densely beaded texture of the general tegument of *P. indicum* is not evident in *P. bulliense*, tiny tegumental vesicles or microvilli are spread over in between the fine meshwork of hexagons. On the whole the surface exhibits a spongy appearance.

The infective larvae of many monogeneans have a ciliated epidermis with few exceptions (Erasmus, 1972). In *P. bulliense* the oncomiracidia possess singly and sparsely distributed cilia all over the body. No ciliary plates nor any discrete pattern of the arrangement of cilia were observed on the body surface of the oncomiracidia. The oncomiracidium of

Parapolystoma crooki, on the other hand, possesses ciliated epidermal plates, 55 in number and arragned in 6 rows (Vande Vusse, 1976). It has been reported that ciliary cells are lost during parasite establishment in *Entobdella soleae* on host scales (Kearn, 1967; Llewellyn, 1972). Loss of cilia from ciliary cells is also related to delayed hatching of oncomiracidia (du Preez and Kok, 1987). Tinsley and Earle (1983) reported that some other species of monogeneans do not shed their cilia after contact with the host.

The operculate eggs of *P. bulliense* are brown in colour when contained in the uterus but of lighter shade than those in *P. indicum*. Both embryonated and unembryonated eggs have a similar brown tinge of egg shell which is translucent. The presence of the egg shell precursors for sclerotin-like basic proteins is also indicated in both vitellaria and egg shell of *P. bulliense*. The lighter tanned tinge of the egg shell in this species may indicate a difference from *P. indicum* in the process and degree of stabilization of the egg shell protein. Inspite of sclerotinization, the egg shell remains sufficiently translucent. This translucency is perhaps significant in view of the fact that many monogenean eggs contaning larvae are stimulated to hatch under the influence of light (Macdonald and Combes, 1978). The egg shell surface in *P. bulliense* has tiny blebs all over and shows a texture quite different from that of *P. indicum*.

Numerous eggs could be observed in the uterus of the adult worm. The egg-laying period for *P. bulliense* when kept under laboratory condition lasted for 60 h during which 271 to 476 eggs could be harvested per fluke, which still retained a few eggs in the uterus when it died 60 h after its removal from the host. The rate of egg production was 80 - 235 eggs per parasite per 24 h. This rate is comparable to that reported for other monogenean flukes. The eggs released by the parasite were at different stages of development and some eggs were also embryonated with well developed oncomiracidium within them.

Retention of large number of eggs in the uterus has been reported for several polystomatids. For example, *Pseudodiplorchis* and *Neodiplorchis* may have up to 250 eggs stored in the uterus (Tinsley, 1983).. As suggested by Harris (1983) the retention of eggs in the uterus would help reduce the exposure time of eggs to water currents. In some instances time span for breeding is so short and so unpredictable that there would not be enough time for a monogenean to mobilize an inactive egg assembly and the eggs that are produced to develop, hatch and invade a host. In such instances the polystomes have adopted an alternative method to carry on their life cycle. The eggs are retained in the uterus of the adults during the hibernation period of the frog or toad, during which they develop in the uterus and hatch immediately when they enter water for breeding (Tinsley, 1978a).

In *P. bulliense* it took 10 - 15 days at 30°C under laboratory condition to develop from non-embryonated eggs to egg containing mobile oncomiracidia; at room temperature this development took 20 - 26 days. In a number of monogenean species the egg development time varied from 15 - 147 days depending on the temperature (ref.Kearn,1986). In *Protopolystoma xenopodis* the egg development time was 20 - 32 days at 26°C (Tinsley and Owen 1975). The occurrence of well developed larvae in some of the eggs in utero would perhaps afford an opportunity for reinfection of the same host individual. Similar is the case of *Pseudobenedenia shorti* and *Acanthocotyle greeni* (Hargis and Dillon, 1968; Gibson, 1976; Macdonald and Llewellyn, 1980) and polystomids like *Choricotyle australiensis* (Roubal et al., 1983).

Slight increase in the size of eggs during development was noticeable in the present study. Similar increase in size has been reported in certain polystomatids that retain eggs inside the uterus and may be related to the transport of nutrients through the egg shell for the growth of the embryo within (Tinsley, 1983). Fully embryonated eggs of *P. bulliense* hatched in the absence of any obvious environmental stimulus. Similar is tha case for other monogeneans, though such eggs can be activated experimentally by warmth, light and mechanical disturbance, thereby promoting hatching (Tinsley and Owen, 1975). Since the eggs are operculate, the muscular extension of the fully developed oncomiracidium is believed to provide the necessary force for detaching the operculum during hatching (Frankland,1955; Bychowsky,1957; Llewellyn, 1957; Bovet, 1967; Macdonald, 1974; Molnár, 1971).

During the present investigation a large number of oncomiracidia were recovered from the eggs maintained in the laboratory at 23°C (±1°C) and 30°C. No oncomiracidia were recovered from the urinary bladder or cloacal chamber of the adult fluke. Since no oncomiracidia or gyrodactyloid-I stages were recovered from the tadpoles of *R. malabarica* (Which harboured the juvenile flukes) it seems probable that an internal development cycle exists in this polystomid fluke. The characteristics of the oncomiracidial stage of *P. bulliense* (4 eyespots, a conspicuous oral sucker, small intestinal caeca, opisthaptoral disc with 16 microhooks and a pair of hamuli primordia) resemble those of the gyrodactyloid-I stage of *P. indicum* and *P. integerrimum.* No neotenic stages were ever recovered from tadpoles in the present study.

In *P. bulliense,* the shape and size of the larval microhooks remained more or less the same in oncomiracidia up to the adults, while a gradual increase in the dimensions of the hamuli was observed. The hamuli increased in length and breadth with simultaneous increase in the depth of the incision from juvenile to adult stage. It is thus evident that the microhook armature is acquired very early during the development of the parasite and is retained as such even in the adult stage. Occurrence of both juvenile and adult flukes in the urinary bladder of the host as seen in the present study may be related to an internal cycle of reproduction in the host. An internal cycle has been reported for *Polystoma grassei* and *Eupolystoma alluaudi* (ref. Dupuoy and Combes, 1973; Combes, 1977). In case of *E. alluaudi* multiplication takes place in the bladder of the adult amphibian and does not require a much aquatic environment. It infects older toads and infection is transmitted during coupling from one mate to the other. As *E. alluaudi* characterizes the more dry areas the mode of trasmission has an adaptive value towards the climate (Combes et al., 1976). In *E. alluaudi* occurrence of two types of larvae signifies two modes of reproduction - an internal cycle programmed in the development of the parasite and an external cycle involving tadpoles hosts. `Creeping larvae' which lack cilia and sensilla as free-living stage, hatch in the uterus; and `Swimming larvae', ciliated and with a complete sensory apparatus, hatch when the eggs are expelled from the bladder, and are not involved in the internal cycle. The production of any one of these larval stages may be dependent on a process of population regulation (Fournier and Combes, 1979). Immature worms of *P. crooki* of various sizes were recovered from the gills of tadpoles, indicating an oviparous mode of transmission for the parasite (Vande Vusse, 1976). *P. crooki* which infects *Rana magna*, a riverine frog, possibly has an external cycle adapting to the host's aquatic ecology (Tinsley, 1978; Murith, 1981b, 1982; Tinsley, 1983); in contrast *P. bulliense* recovered from *Rana malabarica,* a semi-terrestrial host, might show a similar feature like *E. alluaudi* having a direct internal cycle.

Similar to *P.indicum* low prevalence and low intensity of infection were also observed in the case of *P. bulliense*. This may be on account of failure of transmission, the infective stage suffering a high mortality of not being able to contact the appropriate host. Low intensity of infection appears to be a normal feature in the case of endoparasitic monogenea.

Summary

Summary

1. The anuran amphibian fauna of Meghalaya was explored for the occurrence of monogenean parasites. Various localities of the State were surveyed for the host collection. The host species collected include *Rhacophorus nigropalmatus*, *Rhacophorus reinwardtii*, *Polypedates leucomystax*, *Rana mawphlangensis*, *Rana malabarica*, *Rana limnocharis*, *Rana cyanophlyctis*, and *Amolops afghanus*, tadpoles and adults of all of which were examined for the recovery of parasites. The monogenean parasites spectrum was found to be represented by only two polystomatid species, namely *Polystoma indicum* Diengdoh et Tandon, 1991 and *Parapolystoma bulliense* (Johnston, 1912) Ozaki,1935. The present investigation deals with a detail study of the morphology, biology and prevalence of these two species.

2. *Polystoma indicum* Diengdoh et Tandon,1991, was found restricted to *R. nigropalmatus* and *R. reinwardtii* from Cherrapunji, Mawsynram and Shillong.

(i) The present study supplements the original morphological description of this species. The presence of 16 microhooks in the opisthaptor of the adult fluke is established. Scanning electron microscopic studies of the adult fluke revealed that the surface is devoid of any spines or scales but is coarsely covered with tegumental folds, ridges and craters. Minute tubercules abounding all over the surface give it a beaded texture. Tuberculate elevations or tegumental vesicles on the ridges possess pitted apices. Domed papillae, some pitted at their apices, encircle the oral sucker. The general surface of both the forebody and opisthaptoral disc has a fine tuberculate texture.

(ii) The nervous system could be visualized in toto with the localization of non-specific esterases. It exhibits a similar basic pattern as that described for other polystomatid species. The nervous system is bilaterally symmetrical. Cerebral ganglia, which are connected by a thick dorsal commissure, are present dorsal to the pharynx. From the cerebral ganglia arise five anterior pairs and four posterior pairs of nerve trunks. The anterior nerves and/or their fine branches join to form a conspicuous circumoral nerve ring complex. The posterior nerves unite in the opisthaptoral region to form two main haptoral nerves, the branches from which innervate the components of the opisthaptor. Presence of nerve cells is also revealed in association with the main nerves, more predominantly in the vicinity of the opisthaptoral suckers.

(iii) 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 189 eggs could be harvested from a single fluke. Surface fine topography of the egg surface reveals a pitted texture, uneven with small tubercules dispersed all over.

No oncomiracidium was ever recovered during the present study. Unciliated larvae with 16 microhooks, 2 pairs of eye spots and a pair of hamuli primordia representing gyrodactyloid - I stage were the 1st 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 2,4 and then 6 opisthaptoral clamps, 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 remain almost the same from the 2-clamped 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 distinct guard and handle. Variations in the relative length and depth of the incision between the two hamuli exist even within the same specimen.

Surface fine topography of these larval stages reveals the presence of transverse folds and ridges giving the surface a honeycomb-like texture. However, in post gyrodactyloid-Ic stage tegumentary vesicles or microvilli and button-like papillae are unevenly distributed and striae of anastomosing lamellae are observed to from concentric round patches with a cilium within at several places. The tegument of the prohaptor in all the stages has domed papillae, the latter having apical pits in post-gyrodactyloid -Ia and Ic stages. The tegument of the opisthaptor and that covering the clamps appears spongy.

The larval and juvenile stages were always recovered from the internal gills, branchial chambers and urinary bladder of the metamorphosing tadpoles and rarely from the intestine. The larval migration to the final destination seems to follow an internal 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 even in the branchial chambers of the older ones. Neotenic development thus does not seem to occur in *P. indicum.*

(iv) The prevalence of *P. indicum* infections was found to be quite low. The intensity of infection of adult flukes in adult hosts is lower than that of the larval stages recovered from metamorphosing tadpoles. No tadpole mortality was observed as caused by parasite burden. *P. indicum* also exhibits host specificity and restricted geographical distribution. A predilection towards *R. nigropalmatus* amongst the three rhacophorid hosts is indicated.

3. *Parapolystoma bulliense* (Johnston,1912) Ozaki, 1935, has been redescribed and forms the first report of its occurrence from India. *Rana malabarica* from Mawsynram and Tura (Meghalaya) forms a new host record for the species.

(i) The morphological details of the adult fluke tally with the original description except that the intestinal caeca, which are described to be without diverticula, exhibit varying degree of diverticulation in different individuals under the present study.

Surface fine topography of the adult fluke reveals the presence of ridges and valleys, with a honeycomb-like network. Tegumental vesicles are distributed unevenly all over the body. Pits are observed on the antero-dorsal surface.

(ii) The developmental biology of *P. bulliense* presents differences from *Polystoma indicum*. The eggs of *P. bulliense* are brown in colour, operculate and with a sclerotin-like egg shell. Both embryonated and unembryonated eggs are present in the uterus of the same fluke. In vitro the egg laying period lasted 60 h when 271 to 476 eggs could be harvested per fluke. Surface fine topography of the egg surface reveals blebs of different shapes and sizes all over the egg shell.

No oncomiracidia and/or other larval stages were ever recovered from the tadpole hosts. Fully developed oncomiracidium is present in the embryonated eggs in utero. Non-embryonated eggs, when kept in vitro conditions, developed oncomiracidia within 26 days at room temperature. The oncomiracidium, with 16 microhooks and 2 hamuli primordia on its opisthaptoral disc, has a randomly ciliated body, cilia emerging singly and not arranged in discrete pattern of ciliary plates.

Juvenile flukes were also recovered along with mature flukes parasitizing adult frogs. These resemble the adult fluke in all respects except for the absence of the reproductive components.

No neotenic larval stages were ever recovered from tadpoles of *R. malabarica*. An internal vesicular cycle of development seems to be operative in *P. bulliense*.

(iii) The prevalence and intensity of infection were found to be quite low. Restriction of *P. bulliense* to only *R. malabarica* is suggestive of a narrow host specificity of this monogenean species, too.

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