

**STUDIES ON CARYOPHYLLIDEAN CESTODE PARASITES OF
SOME CATFISHES AND HISTOPATHOLOGY OF THE HOST**

ABSTRACT

REBA BHATTACHARJEE, M. Sc.

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



To



**THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA**

SEPTEMBER, 1986

597.5204828
BHA

Acc. No. 102010
Acc. by R. Non... 25.10.88
Class by ...
Sub. Recd. by ...
Date. by ...
Prescribed by ...

SUMMARY

1. Exploration of the edible cat-fishes, *Clarias batrachus* and *Heteropneustes fossilis*, for their caryophyllidean fauna revealed that, nine different types of caryophyllids parasitize these piscine hosts of north-east India. While eight of them represent the subfamily Lytocestinae and belong to the genus *Lytocestus*, only one represents the subfamily Djombanginae and belongs to the genus *Djombangia*. Out of the eight *Lytocestus* spp., four, namely *L. indicus*, *L. birmanicus*, *L. filiformis* and *L. longicollis* are already known forms representing new locality record and the remaining four appear new to science. These have been named as *L. clariae* n.sp.; *L. attenuatus* n.sp.; *L. assamensis* n.sp. and *L. heteropneustii* n.sp. The validity of the new species has been discussed.

The spectrum of the parasitic infection was more diversified in *C. batrachus* in comparison to *H. fossilis* which also shares the same benthic habitat, since only a single species of *Lytocestus* was obtained from the latter, throughout the period of caryophyllidean faunistic survey.

2. Scanning electron microscopic studies on the surface fine topography of *L. indicus* and *D. penetrans*, the two most commonly occurring types revealed a dense and uniform covering of microtriches throughout the surface of the body, without showing any regional differentiation with regard to their morphology.

3. Histochemical studies on *L. indicus* and *D. penetrans*, the two most apparently pathogenic species, indicates towards a similarity in the distributional pattern of the different metabolites between the two species.

While in both the carbohydrates, proteins and lipids showed a generalised pattern of distribution in the tegument, parenchyma, reproductive organs as well as the scolex gland cells, differences occurred with regard to glycogen and lipid in the eggs of two species. The vitelline cell nuclei in the egg of *L. indicus* contained a higher concentration of glycogen than lipid, but those in case of *D. penetrans* had lesser concentration of glycogen and higher of lipid. Besides, another distinguishing feature between the two species is that, in the vitelline lobes of *L. indicus* usually 3 to 4 patches of glycogen masses were observed whereas in *D. penetrans* only few cells of the vitelline lobe became vacuolated and stained positively for glycogen.

DNA concentration appeared to be higher in the eggs of *D. penetrans* as compared to *L. indicus*.

4. Incidence pattern for the different caryophyllid types recovered appeared to be species specific. Even for the most frequently occurring types like *L. indicus*, *L. birmanicus*, *L. filiformis* and *D. penetrans*, seasonal fluctuation was distinct. While there occurred low incidence of infection during the summer months, the peak period of incidence obtained by the different species was during late winter or early spring months. Only a single species, *L. assamensis* n.sp. depicted its peak during autumn.

The prevalence of infection was more in the male hosts than in the female host fishes. However, though host reactions or immunity stimulated by temperature appears to be responsible for the population dynamics within the vertebrate host, the general incidence picture is more a function of temperature than any other factor.

5. Based on histopathological changes *D. penetrans* was found to be the most highly pathogenic type, producing large nodules in the intestinal wall as a result of complete penetration. Like *D. penetrans*, *L. indicus* is also the burrowing type, but its penetration was

not as deep (i.e., up to the serosa), as that of *D. penetrans*, but was restricted only up to the muscularis layer. Hence *L. indicus* is comparatively less pathogenic causing ulceration of the affected tissue. In severe cases of pathogenicity however, hyperplasia of the muscularis was observed.

Pathogenicity due to multiple infection comprising several species of caryophyllids was minimum, since only the denudation of the mucosal folds was observed. Similar host reaction was also produced due to *L. assamensis* n.sp. infection, which appeared singly and not along with multiple infection and occupied a considerable length of the intestine.

Thus extent of damage was maximum due to a single worm of *D. penetrans* and minimum due to multiple invasion of the caryophyllids.

SERIALIZED
 Acc. No. 102010
 Acc. by R. N. Singh
 Class by
 Sub. Heading by
 Date by
 Indexed by

**STUDIES ON CARYOPHYLLIDEAN CESTODE PARASITES OF
SOME CATFISHES AND HISTOPATHOLOGY OF THE HOST**

REBA BHATTACHARJEE, M. Sc.

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

To



**THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA**

SEPTEMBER, 1986

DS
597.5204828
BHA

App. No. 102010
App. by R. N. Noyce 25-10-88
Class by [initials] 29/11/89
Pub. Reading by 5/10
Date [initials]
Transcribed by O. Noyce
6/10/89

**DEDICATED
TO
MY PARENTS**



Phone :
Grams : NEHU

North-Eastern Hill University

Mayurbhanj Complex

Nongthymmai Shillong - 793014 (Meghalava)

Dr (Mrs) Veena Tandon, Reader
Department of Zoology

I certify that the thesis entitled "Studies On Caryophyllidean Cestode Parasites Of Some Catfishes And Histopathology Of The Host" submitted by Miss Reba Bhattacharjee 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 been 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: 26th Sept, '86
Place: Shillong

Veena Tandon
(Dr.(Mrs) Veena Tandon)
Supervisor

ACKNOWLEDGEMENTS

It is a great pleasure penning my acknowledgement towards all those from whom I have incurred many debts and obligations during the compilation of this manuscript.

First and foremost, I owe my heartfelt gratitude towards my reverend teacher Dr.(Mrs) V. Tandon, Reader, Department of Zoology, North-Eastern Hill University, for her invaluable guidance, moral and unstinted encouragement throughout the course of this work.

I am also grateful to Dr. K. Chatterjee, Head, Department of Zoology, North-Eastern Hill University for providing me with all the laboratory facilities for accomplishing the work.

I am highly indebted to Dr. M.K. Khare, Dean, School of Life Sciences, North-Eastern Hill University and Dr. P. Tandon, Reader, Department of Botany, for evincing a keen interest in my work.

For my friends and colleagues, it remains only to write a word of thanks, whose never failing assistance and encouragement have contributed substantially to the pleasure I have had in compiling this thesis. I am specially indebted to Mr. D. Paul, Mrs. N. Mishra, Mrs. C.A.R. Diengdoh Darlong, Mr. V. Darlong, Mr. A. Yadav, Mr. B. Roy, Mr. N. Saha and Mr. P.K. Prabhakaran for rendering their timely help and enthusiastic support towards the success of this endeavour.

I express my appreciation to Mr. B.K. Das, Mr. S. Roy Choudhury and to Mr. N.K. Paul Choudhury for assisting me in photography, diagrammes, and typing the manuscript respectively.

Words are inadequate to express my deep sense of gratitude towards my parents whose kindness above and beyond the call of duty has greatly lightened the process of compilation.

Last but not the least, thanks are also due to Mr. S. Chakravarty, Miss. A. Chakravarty and Miss R. Bhattacharjee for their wise and friendly counsel.

SHILLONG
Date...26:9:.....'86

Reba Bhattacharjee
REBA BHATTACHARJEE

CONTENTS

	Preface	i - ii
	Abbreviations used in figures	iii
Chapter I	FAUNISTIC STUDIES	1 - 45
	Introduction	1
	Materials and Methods	7
	Observations and remarks/discussion	8
	Family Lytocestidae Wardle and McLeod	
	Subfamily Lytocestinae Satpute and Agarwal	
	<i>Lytocestus indicus</i> Moghe, 1931	8
	<i>L. birmanicus</i> Lynsdale, 1956	13
	<i>L. filiformis</i> Fuhrmann and Baer, 1925	16
	<i>L. longicollis</i> Rama Devi, 1973	19
	<i>L. clariae</i> n.sp.	22
	<i>L. attenuatus</i> n.sp.	26
	<i>L. assamensis</i> n.sp.	31
	<i>L. heteropneustii</i> n.sp.	37
	Subfamily Djombanginae Satpute and Agarwal	
	<i>Djombangia penetrans</i> Bovien, 1926	42
Chapter II	SURFACE FINE TOPOGRAPHY	46 - 50
	Introduction	46
	Materials and Methods	47
	Observations and discussion	47
Chapter III	HISTOCHEMISTRY	51 - 71
	Introduction	51
	Materials and Methods	57
	Results and discussion	57
Chapter IV	SEASONAL INCIDENCE	72 - 87
	Introduction	72
	Materials and Methods	74
	Observations	74
	Discussion	79
Chapter V	HISTOPATHOLOGY OF THE HOST	88 - 104
	Introduction	88
	Materials and Methods	93
	Observations	93
	Discussion	97
	SUMMARY	105 - 108
	REFERENCES	109 - 127

PREFACE

The uniqueness of the caryophyllidean cestodes lies in their embodiment of a single set of reproductive organ within a non-segmented body. Utilising aquatic oligochaetes as their intermediate hosts, these caryophyllids are well known to have certain adverse effects on their hosts and are therefore considered to be economically important pathogens of their piscine hosts.

Caryophyllid fauna of India is yet poorly known. Of the 45 genera of caryophyllids which includes 126 described species distributed over all the zoogeographical regions, only 14 species belonging to 9 genera have so far been recorded from fish hosts (mostly catfishes) in India.

Clarias batrachus and **Heteropneustes fossilis**, the two most common food-fish of north-east India are frequently parasitized by the caryophyllid fauna. The first chapter records the spectrum of caryophyllid species harbouring these catfishes. A detailed morphological account and morphometric analyses accompany the description of each species dealt with herein.

Of all the caryophyllid species described herein, scanning electron microscopic studies were carried out for the two most commonly occurring types, viz., **Lytocestus indicus** and **Djombangia penetrans**. The second chapter is a brief account to elucidate the surface fine topography of these parasites.

Cestodes have so far been scantily dealt with from the histochemical point of view. To obtain an overview of cestode physiology, **Lytocestus indicus** and **Djombangia penetrans**, the apparently pathogenic species of **Clarias batrachus** were subjected to histochemical analyses. The third chapter of the present manuscript deals with the study on histochemical aspects in the two species.

Frequent occurrence of some caryophyllid species and not so common of others in their piscine hosts logically necessitated the study of incidence pattern of the various types recovered during the different times of the year. This aspect of study is dealt with in the penultimate chapter.

The economic importance of these fish hosts under consideration is naturally high as they constitute a popular diet for a considerable section of human population in this region of the country. The histopathology of the hosts could not therefore be neglected. The ultimate chapter notes the extent of damage at the intestinal tissue level, as caused by these parasites to their fish hosts.

LIST OF ABBREVIATIONS

FGL	Frontal glands
♀ GP	Female gonopore
♂ GP	Male gonopore
ILM	Inner longitudinal muscle
MGL	Mehlis' gland
PLM	Parenchymal Longitudinal muscles
SC	Scolex
STU	Subtegument
SUC	Sucker
T	Testes
TU	Tegument
UGL	Uterine glands
VIT	Vitellaria

Chapter I FAUNISTIC STUDIES

INTRODUCTION

Caryophyllids are widely distributed cestodes of the fresh-water siluriform and cypriniform fishes of the world. Comprising 126 species and 45 genera, they constitute approximately one-fourth of the cestode fauna of the fresh-water fishes (Mackiewicz, 1972).

Caryophyllidean cestodes are unique in having a single set of reproductive organs within a non-segmented body and in utilising aquatic oligochaetes as their intermediate hosts. These cestodes are considered a very ancient group that originated from acoelate turbellarian larvae and branched out at the beginning of Paleozoic era as parasites of aquatic invertebrates (Kulakovskaya and Demshin, 1978). Although caryophyllids have been known for more than 200 years, their taxonomy is not yet stable and there is disagreement regarding the genera and species within the group. This is because all the workers do not agree as to whether these unsegmented worms constitute a separate order or represent a pseudophyllidean family. However, from the time of their initial discovery, these tapeworms were being placed in a separate, non-pseudophyllidean, non-cestodarian group by few authors. Then, with the emergence of a more stable classification, Leuckart (1878) considered the group a separate family. Beneden (in Olsson, 1893)

raised its status to the rank of an order Caryophyllidea, after which Woodland (1923) placed the order within Cestodaria, a subclass of tapeworms established by Monticelli (1892). Later, Hunter (1927) treated Caryophyllaeidae as an independent family of Pseudophyllidea and he divided it into four sub-families, viz., Caryophyllaeinae (Nyebelin, 1922) Hunter 1927; Capingentinae Hunter, 1927; Lytocestinae Hunter, 1927; and Wenyoninae Hunter, 1927. Many years after this Wardle and McLeod (1952) raised the family Caryophyllaeidae to the rank of an order Caryophyllidea and the four sub-families, to the rank of families. However, there was disagreement regarding acceptance of their view because Fotedar (1958) followed the classification of Hunter (1927). Yamaguti (1959) suggested a single family Caryophyllaeidae under the order Caryophyllidea and included three subfamilies, instead of four within it — Caryophyllaeinae, Lytocestinae and Capingentinae, the subfamily Wenyoninae having been considered a synonym of Caryophyllaeinae. Wardle, McLeod and Radinovsky (1974) retained the four subfamilies of Hunter (1927) as the families under the order Caryophyllidea. Mackiewicz (1972) accepted the classification proposed by Wardle and McLeod (1952) but he regarded the three sub-families of Yamaguti (1959) as the only families under the order Caryophyllidea. Though the systematics of Caryophyllidea is complex, at present the group comprises four distinct families that have been erected on the basis of

the distribution of testes and vitellaria in relation to the cortical and medullary zones of the body proper (see Mackiewicz, 1972, 1981a and 1982). These are: Caryophyllaeidae Leuckart, 1878 (in Lühe, 1910) (= Caryophyllaeinae Nyebelin, 1922; Caryophyllaeinae Hunter, 1927) [includes Wenyoninae Hunter, 1927 and Wenyonidae Wardle and McLeod, 1952 fide Yamaguti (1959), Mackiewicz (1963a)], with small forms having varying shapes of holdfast, genital pores opening together on the last quarter of the ventral surface, utero-vaginal atrium present but within a sphincter muscle, parenchymal muscle in two layers, and yolk glands in the medulla; Capingentidae Wardle and McLeod, 1952 (= Pseudolytocestinae Hunter, 1929; Capingentinae Hunter, 1930) the members which have yolk glands cortical for only one third to one half of their bulk and the remainder in the medulla; Balanotaeniidae Mackiewicz and Blair, 1979, having the yolk glands and testes disposed in the cortex; and Lytocestidae Wardle and McLeod, 1952 (= Lytocestinae Hunter 1927) [includes Bovieninae Fuhrmann, 1931 and Lallidae Johri, 1959 fide Mackiewicz (1963a)] which includes forms having vitellaria disposed in the cortex, testes medullary, holdfast commonly undifferentiated, cirrus pouch and genital atrium opening separately, and ovarian wings in the cortex but ovarian bridges in the medulla. Later Satpute and Agarwal (1980a) suggested that the family Lytocestidae which accommodated 17 genera and 37 species be redivided into two subfamilies, viz., Lytocestinae and Djombanginae, the latter having

been erected by these authors as a new subfamily.

The subfamily Djombanginae shares the characters of the subfamily Lytocestinae such as cortical vitelline follicles and medullary testes, but it is distinctly different from the latter in possessing a feeble sucker at the tip of the holdfast, in the extension of the uterus throughout the testicular field and in having embryonated eggs in the uterus.

Of the various members of the subfamily Lytocestinae, the genus *Lytocestus* was erected by Cohn (1908) for the cestodes from the siluroid host, *Clarias fuscus*, in Hongkong. The generic diagnosis was given as: holdfast undifferentiated and not broader than the body; parenchymal muscles in a ring around the testes; and no post ovarian yolk glands present. To the genotype *adherens* Cohn, 1908, seven more species have been added to date. They are: *L. filiformis* (Woodland, 1923) Fuhrmann and Baer, 1925 [= *Caryophyllaeus filiformis* (Woodland, 1923) Woodland, 1937; *L. alestesi* Lynsdale 1956 fide Mackiewicz (1962)]; *L. indicus* (Moghe, 1925) Woodland, 1926 [= *Monobothrioides indicus* Moghe, 1925, according to Woodland (1937)]; *L. javanicus* (Bovien, 1926) Furtado, 1963 [= *Caryocestus javanicus* (Bovien, 1926)]; *L. birmanicus* Lynsdale, 1956 [= *L. alestesi* Lynsdale, 1956, according to Johri (1959)]; *L. parvulus* Furtado, 1963; *L. longicollis* Rama Devi, 1973; and *L. fossilis* Singh,

1975. The last-mentioned is the only species included in the genus which possesses post-ovarian vitelline follicles. Though its author placed this species under the genus **Lytocestus** the histological details for ascertaining the family or genus allocation are lacking in its account, thus raising a doubt for including the form with post-ovarian vitelline follicles in the genus. All the **Lytocestus** species, however, appear to be distributed in the Ethiopian and Oriental regions of the zoogeographical realm. Of these, three species, namely, **L. indicus**, **L. longicollis** and **L. fossilis**, have been represented from the Indian sub-continent.

Several other species under 6 genera also represent the subfamily Lytocestinae in India. These are: **Lucknowia fossilis** Gupta, 1961; **L. indica** Niyogi, Gupta and Agarwal, 1982a; **Crescentovitus biloculus** Murhar, 1963; **Lytocestoides aurangabadensis** Shinde, 1970, **L. paithenesis** Shinde and Deshmukh, 1975; **Bovienia serialis** (Bovien, 1926) Fuhrmann, 1931 (described by Mackiewicz and Murhar, 1972) and **Introvertus raipurensis** Satpute and Agarwal, 1980(b). Further, the genus **Hunteroides**, erected and placed under the subfamily Lytocestinae by Johri (1959), turned out to be a cestodarian (Joyeux and Baer, 1962; Mackiewicz, 1972).

To the genus **Djombangia** Bovien, 1926 (now under the subfamily Djombanginae) which includes **D. penetrans** recovered from the duodenum of **Clarias batrachus** from Brantas

river near Djombang in East Java, Satpute and Agarwal (1974a) added another species, *D. indica*, from India and provided its detailed description later (Satpute and Agarwal, 1980a). Sahay and Sahay (1977) also described a new species, *D. caballeroi*, from the stomach of *Heteropneustes fossilis* in India, but Mackiewicz (1981b) doubts the validity of this species as its description is based on flattened specimens only and there appears no significant difference from the type species, *D. penetrans*.

The family Capingentidae is also well represented in the Indian caryophyllid fauna. The contributions made for the family Capingentidae include the descriptions of several forms like: *Adenoscolex oreini* by Fotedar (1958); *Pseudolytocestus clarii*, *Capingentoides batrachii* and *Pseudocaryophyllaeus indica*, all by Gupta (1961); *P. ritai*, *P. mackiewiczzi*, *P. lucknowensis*, *Pliovitellaria osteobramensis* and *Pseudocapingentoides cameroini* by Gupta et al. (1984); *Capingentoides singhii* and *Pseudocapingentoides indica* by Verma (1971), and *Capingentoides moghei* by Pande (1973).

However, of the caryophyllids known from India, a single report of a member of Caryophyllaeidae is available. Mehra (1930) described in an abstract form *Caryophyllaeus kashmierensis* from *Schizothorax micropogon*. Leaving this record which also needs verification there is no other report of any representative of the family from India.

In the present study i.e., during the two years exploration of caryophyllids of the edible catfishes *Clarias batrachus* and *Heteropneustes fossilis*, collected from Guwahati (Assam) and its suburbs and brought alive to Shillong markets for sale, eight species of *Lytocestus* were recovered. While four of them represent the already known species, it is for the first time that their occurrence is being reported from the north-eastern region of India. The remaining four forms, which include three from *C. batrachus* and one from *H. fossilis*, appear to be new to science. One species of *Djombangia* was also frequently recovered from *C. batrachus*.

The present study includes a description of all these forms, with remarks on the already known species and a discussion on the validity of the new ones. The type specimens of the forms described as new species will be deposited in the helminthological collection of the Eastern Regional Station of the Zoological Survey of India, Shillong (Meghalaya).

Materials and Methods

The specimens comprising the present material were recovered from the intestine of the freshly killed fishes, *C. batrachus* and *H. fossilis*, from time to time during 1982-1984. The incidence of infection was high in *C. batrachus* and considerably low in *H. fossilis*.

The recovered worms were first stretched in hot water, and then flattened under the pressure of a cover-slip.

Bouin's fluid and 10% neutral formaldehyde were used as fixatives. Borax carmine and Gower's carmalum were used for staining the whole mounts. For histological studies, cross and sagittally cut series of 6-7 μ thick sections, stained with haematoxylin-eosin, were used.

Identification of the different types has been accomplished after Mackiewicz (1972), basing on the whole mounts and histological sections of the species. Morphometric analysis was based on the measurements of the various organs and their relation with the length of the body, taking a minimum of ten specimens, unless otherwise stated, for each species. All the measurements are in millimeters.

Observations

Family Lytocestidae Wardle and McLeod, 1952
Subfamily Lytocestinae Satpute and Agarwal, 1980(a)
Genus Lytocestus Cohn, 1908

Lytocestus indicus Moghe 1931
 (Syn. *Caryophyllaeus indicus* Moghe, 1925)
 (Plates 1.1 and 1.2)

A total of 455 specimens of *L. indicus* were collected.

Description

Body broad and flat, with traces of external segmentation; body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex unarmed, short and bluntly rounded, markedly narrower than the body and provided with longitudinal furrows in some specimens.

PLATE 1.1 *Lytocestus indicus*

Fig.1 Full worm (whole mount);

Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg.

PLATE 1.1

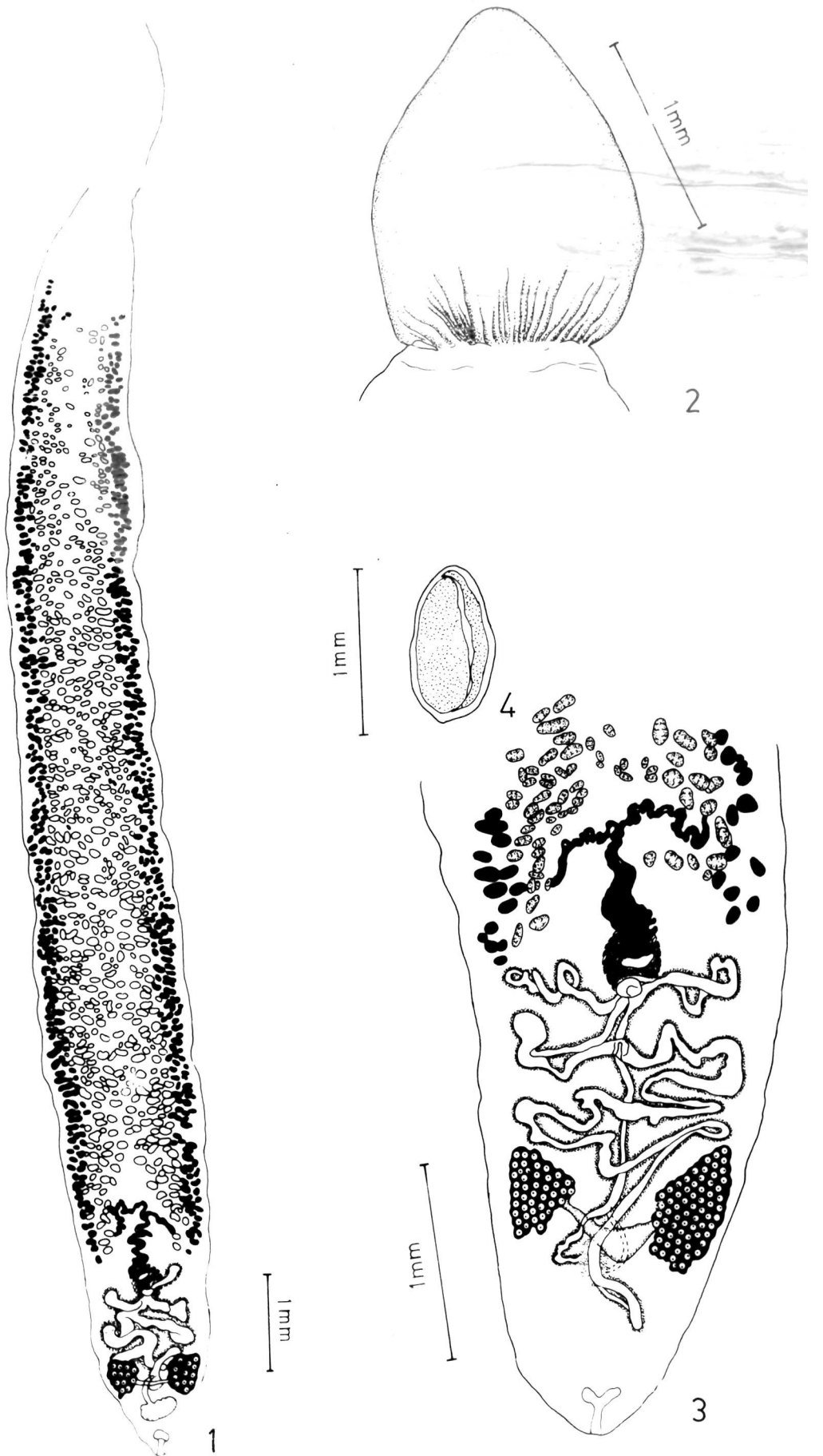
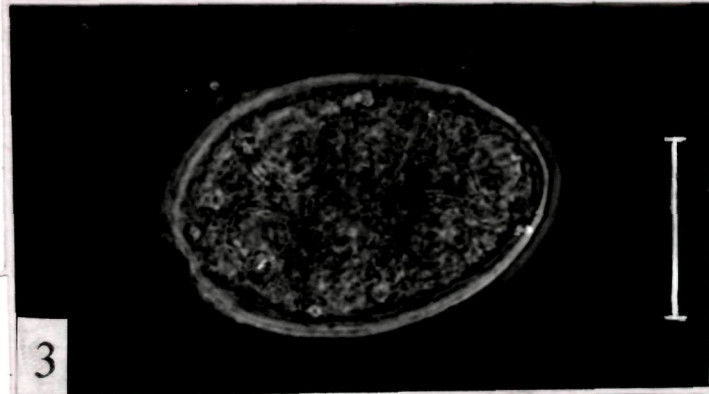
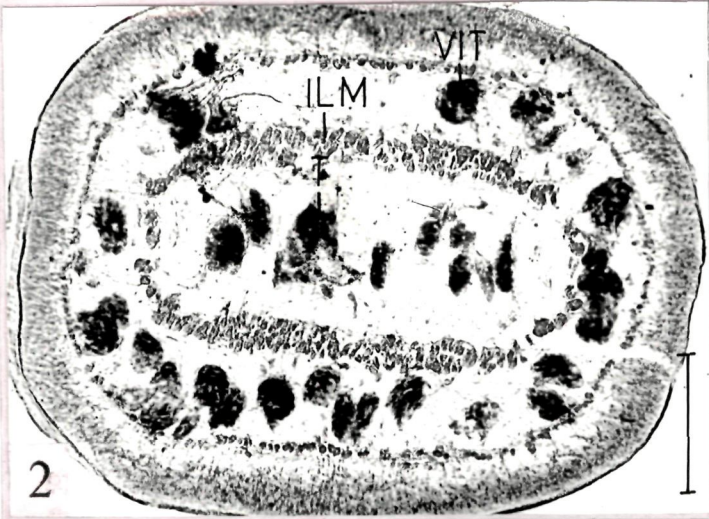
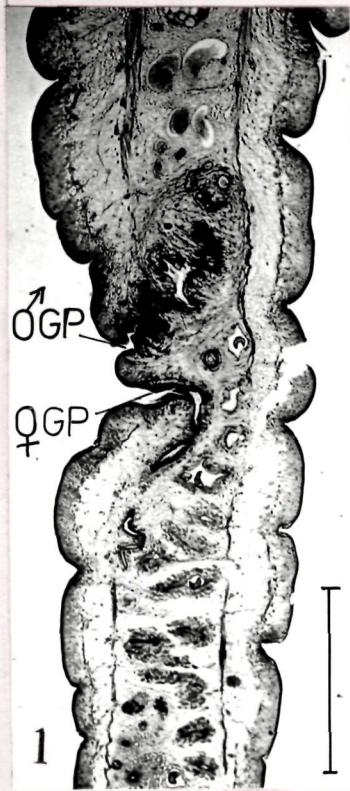


PLATE 1.22 *Lytocestus indicus* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.5 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Operculate egg as seen under phase contrast (scale bar = 0.05 mm);
- Fig.4** Specimen showing longitudinal division of the worm into two separate parts immediately posterior to the short neck and reunion of the same a little anterior to the cirrus sac (scale bar = 0.5 mm).
- Fig.5** The same at higher magnification (scale bar = 0.15 mm).

PLATE 1.2



Neck very short and indistinct. Testes numerous, 212-438 in number, occupying the medullary region of the body, ovoid in shape, larger than vitelline follicles and extending from the base of the neck to the cirrus sac region posteriorly; cirrus sac prominent, opening separately ~~before~~ the utero-vaginal pore. Ovary bilobed, wing like in shape, follicular, the two lobes of ovary joined to each other by an ovarian isthmus posteriorly; Mehlis' gland well developed, located behind ovarian isthmus; vagina distinct, joining the terminal end of the uterus to open unitedly to the exterior at the utero-vaginal pore. Vitelline follicles corticular, in a ring around the testes, no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs oval in shape, smooth, embryonated and operculated.

The measurement of the body and its organ are given in Table I.

Host: **Clarias batrachus**
 Location: Duodenum and intestine.
 Locality: **Guwahati** (Assam)

Remarks

The species, *L. indicus*, was first described by Moghe (1925) as *Caryophyllaeus indicus* from the common Indian siluroid, *Clarias batrachus*. Woodland (1926) raised a doubt regarding the presence of post-ovarian vitelline

TABLE I: *Lytocestus indicus* —
Morphometric measurements

Characters	Present observation		Moghe's (1931) observation
	(Range)	(S.D.)	
1. Length of the body	10.56 - 19.8	± 2.8	15 - 29
2. Maximum breadth of the body at the level of cirrus sac.	1.45 - 3.6	± 0.7	1.82 - 2.73
3. Length of the scolex	1.06 - 1.98	± 0.27	3
4. Length of the neck	0.46 - 1.32	± 0.25	-
5. Testicular follicles			
(a) Length	0.08 - 0.22	± 0.03	0.119
(b) Breadth	0.04 - 0.14	± 0.02	0.095
6. Ovarian lobes			
(a) Length	0.26 - 0.85	± 0.20	-
(b) Extent	0.99 - 2.11	± 0.03	-
7. Vitelline follicles			
(a) Length	0.07 - 0.19	± 0.02	0.077 - 0.088
(b) Breadth	0.04 - 0.13	± 0.01	0.088 - 0.112
8. Pre-testes distance (commencement of testicular follicles from anterior extremity)	1.99 - 3.66	± 0.22	-
9. Pre-vitellaria distance	1.58 - 3.36	± 0.4	-
10. Distance between anterior extent of testes and vitellaria	0.19 - 0.99	± 0.23	-
11. Position of the genital pore from the posterior extremity	1.43 - 3.63	± 0.75	-
12. Eggs (a) Length	0.06 - 0.08	± 0.001	0.088
(b) Breadth	0.02 - 0.03	± 0.002	0.40

[SD = Standard Deviation]

follicles and maintained that they were in reality ovarian follicles, suggesting thereby shifting of the species from *Caryophyllaeus* to *Lytocestus*. Moghe (1931) redescribed the species in view of Woodland's (1926) remarks and placed it under the genus *Lytocestus*.

The present observations are in conformity with those of Moghe (1931), except for minor deviations with regard to the measurements as shown in Table I.

Of all the specimens of *L. indicus* collected during the present investigation, a single specimen showed a longitudinal division of the worm immediately posterior to the short neck into two separate parts which reunited a little anterior to the cirrus sac. [Pl. 1.2; Fig.4-5).

Such anomalies amongst caryophyllids are rare (Janiszewska, 1954) but Simha and Rasheed (1981) reported an anomaly in *L. indicus*, in which the anterior end of the worm was duplicated for about one-fourth of the body length, into two complete bothria along with the testes and vitellaria.

Amongst other caryophyllidean anomalies, *Archigetes brachyurus* was reported to possess post-ovarian vitellaria by Mrazek (1908). Absence of post-ovarian vitellaria in *Caryophyllaeus laticeps* and *Glaridacris laruei* was observed by Janiszewska (1954) and Mackiewicz (1965a), respectively.

Similarly, Mackiewicz (1963b) reported presence of post-ovarian vitellaria in *Monobothrium hunteri*. Fusion of posterior lobes of ovary in *G. laruei*, isolated vitelline follicles in the neck of *G. catasomi* and shortened posterior ovarian lobes in *Isoglaridacris hexacotyle* were reported by Mackiewicz (1965a, 1968a). Duplication of reproductive system was recorded by Mackiewicz (1978) in the genus *Glaridacris* and *Penarchigetes*. Jones and Mackiewicz (1969) observed the testes of *Atractolytocestus huronensis* to be posterior to the ovary.

There are reports of anomalies occurring in polyzoic cestodes also. Therefore, Braun (1900), on summarizing the recorded cases of anomalies in polyzoic cestodes, attributed forking of the strobila to metabolic disturbance or fenestration in the region of proliferation. Chandler (1930) recorded an abnormal *Taenia pisiformis* with a normal scolex but with two chains of strobila. Clapham (1939) also reported duplication of the reproductive system along with the gonopores in *Taenia pisiformis* and *Diphyllidium caninum*.

The anomaly observed in the form under present investigation, can be attributed to a possible mechanical injury to the tegument which could have triggered secondary growth. This seems probable as caryophyllids are known to have a diffuse type of growth (Nyebelin, 1922) and are

regarded monozootic (Wardle et al. 1974).

The rare occurrence of anomalies amongst the caryophyllids, however, is indicative of a high degree of genetic stability in the group [Mackiewicz, 1972].

***Lytocestus birmanicus* Lynsdale, 1956**

(Plates 1.3 and 1.4)

362 no. of specimens of *L. birmanicus* were collected during the exploration.

Description

Body elongated, flattened, posterior end broader than the anterior, with traces of external segmentation present; body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex lanceolate, smooth and narrows to form the neck that gradually widens into the posterior part of the body. Testes numerous, 170-384 in number, medullary in disposition, spherical or oval in shape, extending a short distance from behind the anterior vitellaria to cirrus sac posteriorly; cirrus sac oval, lined by a thick muscular wall, opening slightly anterior to the utero-vaginal pore. Ovary bilobed, follicular, cortical and extends posteriorly behind the Mehlis' gland, united by a median isthmus; Mehlis' gland prominent behind ovarian isthmus; uterine coils glandular, extending up to the level of cirrus sac; vagina a straight tube, joins

PLATE 1.3 *Lytocestus birmanicus*

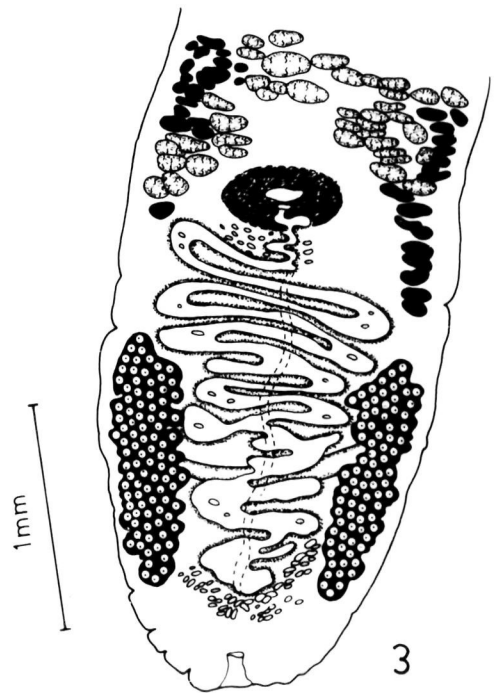
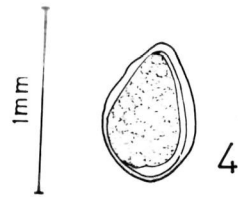
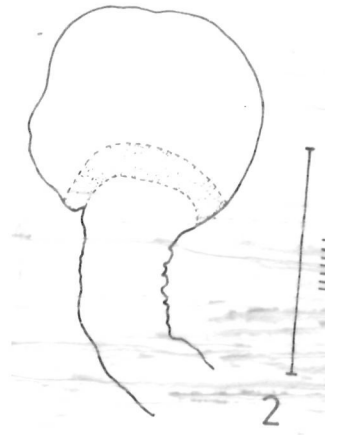
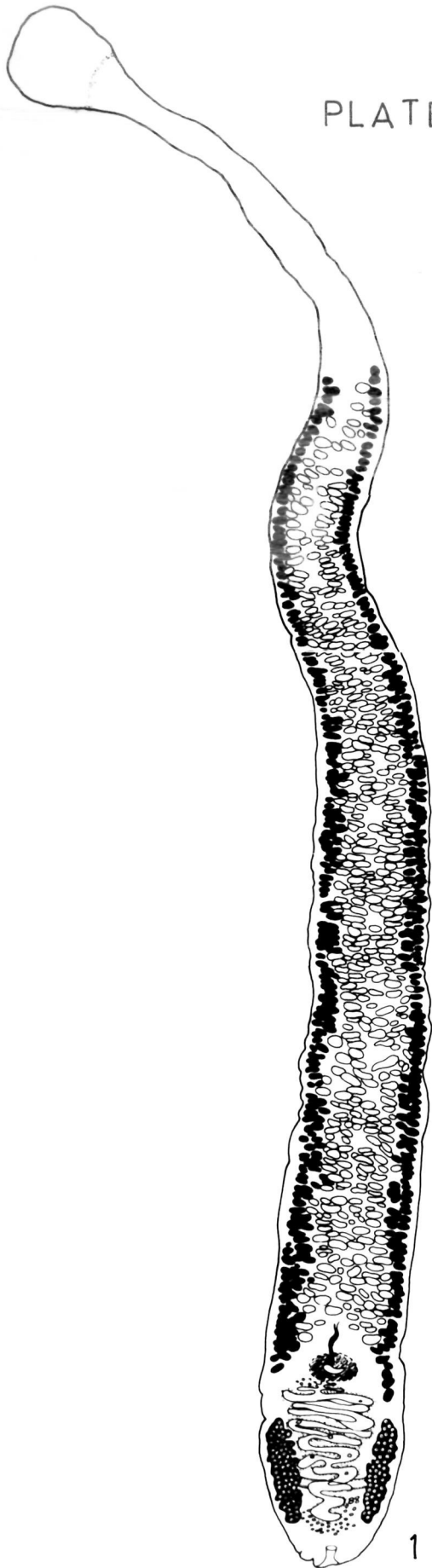
Fig.1 Full worm (whole mount);

Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg.

PLATE 1.3



1

2

4

3

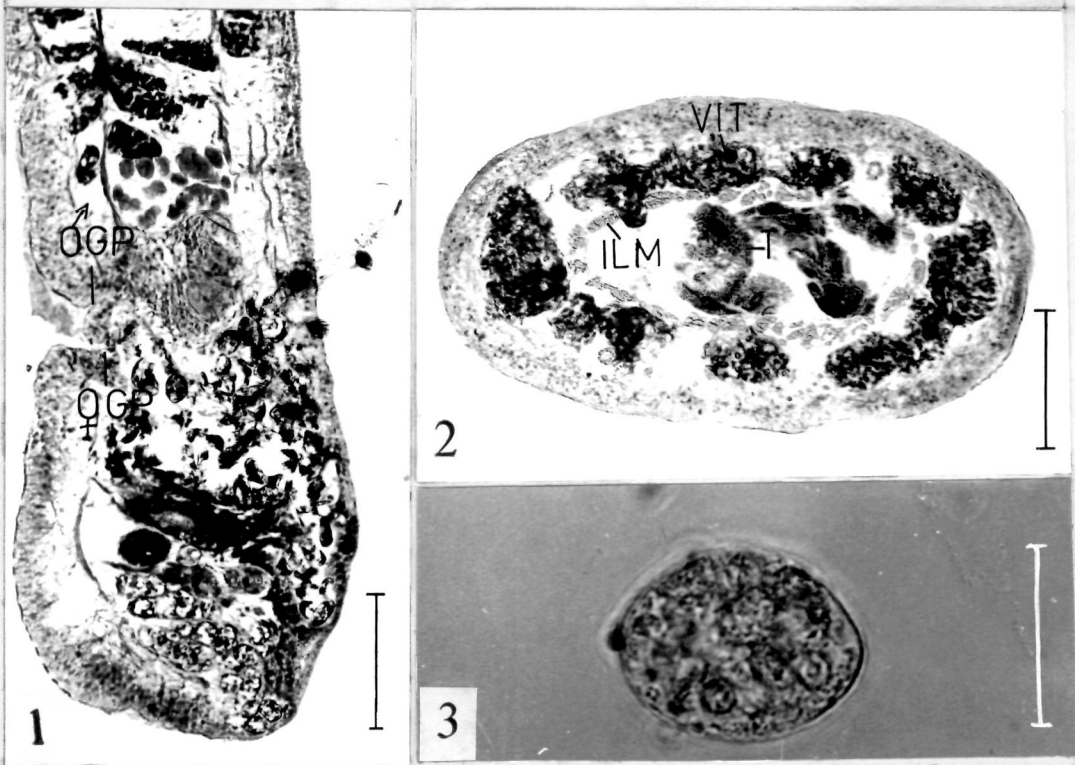
PLATE 1.4 *Lytocestus birmanicus* (Photomicrographs)

Fig.1 Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);

Fig.2 Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);

Fig.3 Egg as observed under phase contrast (scale bar = 0.05 mm);

PLATE 1.4



the uterus at the proximal part to open unitedly at the uterovaginal pore. Vitellaria transversely elongated, cortical, arranged in an annular manner in the space between the two longitudinal muscle layers, extend as far as the utero-vaginal aperture; the vitelline follicles appear concentrated in two lateral bands on either side of the body but some are also scattered in the median field, no post-ovarian vitelline follicles present. Eggs smooth and oval in shape.

The measurement of the body and its organs are given in Table II.

Host: *Clarias batrachus*
Location: Intestine
Locality: Guwahati(Assam)

Remarks

L. birmanicus was first described by Lynsdale (1956) from the intestine of *Clarias batrachus* in Rangoon, Burma. The present observations are in conformity with those of Lynsdale (1956) in all the aspects except for minor deviations with regard to the measurements of the various organs that are represented in Table II.

This species is being reported for the first time from India.

TABLE II: *Lytocestus birmanicus* —
Morphometric measurements

Characters	Present observation		Lynsdale's (1956) observation
	(Range)	(S.D.)	
1. Length of the body	5.28 - 16.36	± 3.76	10 - 12
2. Maximum breadth of the body at the level of cirrus sac.	0.53 - 1.45	± 0.28	0.9
3. Length of the scolex	0.46 - 1.18	± 0.22	0.8
4. Length of the neck	1.32 - 5.47	± 1.23	-
5. Testicular follicles			
(a) Length	0.06 - 0.21	± 0.04	0.150 - 0.180
(b) Breadth	0.02 - 0.11	± 0.02	0.100 - 0.130
6. Ovarian lobes			
(a) Length	0.33 - 1.25	± 0.29	-
(b) Extent	0.33 - 1.18	± 0.26	-
7. Vitelline follicles			
(a) Length	0.04 - 0.14	± 0.03	0.100 - 0.120
(b) Breadth	0.02 - 0.10	± 0.02	0.040 - 0.060
8. Pre-testes distance (commencement of testicular follicles from anterior extremity)	1.52 - 2.20	± 0.99	-
9. Pre-vitellaria distance	1.18 - 1.94	± 0.65	-
10. Distance between anterior extent of testes and vitellaria	0.01 - 0.06	± 0.35	-
11. Position of the genital pore from the posterior extremity	0.46 - 2.5	± 0.69	-
12. Eggs			
(a) Length	0.05 - 0.07	± 0.02	0.050
(b) Breadth	0.02 - 0.03	± 0.01	0.030

[SD = Standard Deviation]



Lytocestus filiformis (Woodland, 1923)
Furhmann and Baer, 1925

(Plates 1.5 and 1.6)

209 specimens of *L. filiformis* were collected during the two years study.

Description

Body flat, elongated, ribbon like, posterior end broader than the anterior end, longitudinal muscle fibres disposed in two distinct zones of cortex and medulla. Scolex smooth, undifferentiated, variable in shape, may be flat or pointed in some. Neck long, slender, Testes numerous, 232-532 in number, occupying the medullary region of the body, spherical or oval in shape extending from behind the neck up to the cirrus sac posteriorly; cirrus lined by a thin muscular wall, opening separately from the utero-vaginal pore. Ovary bilobed, follicular, cortical, the two lobes joined to each other by an ovarian isthmus; Mehlis' gland well developed, behind ovarian isthmus; uterine coils glandular, extending from behind the isthmus beyond the anterior horns of ovary up to the cirrus sac; vagina distinct, joins the uterus distally to open at the utero-vaginal pore. Vitellaria cortical, smaller than testes, spherical or oval in shape, form a crescent around the testes, no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs smooth, operculate and oval in shape.

PLATE 1.5 *Lytocestus filiformis*

Fig.1 Full worm (whole mount);

Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg.

PLATE 1.5

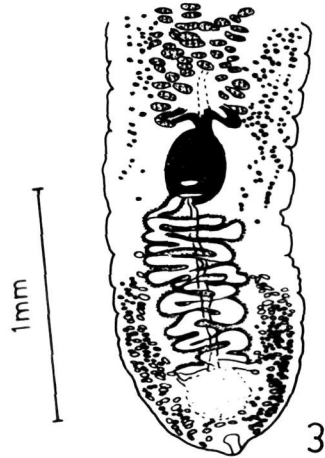
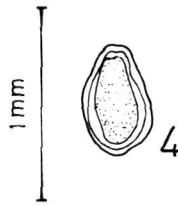
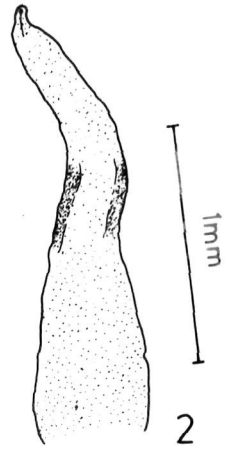
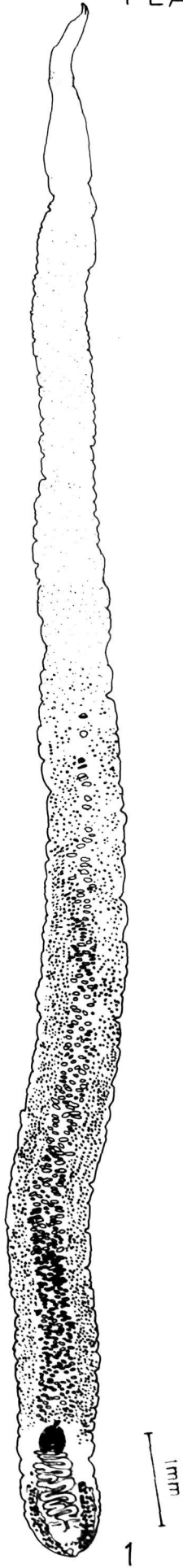
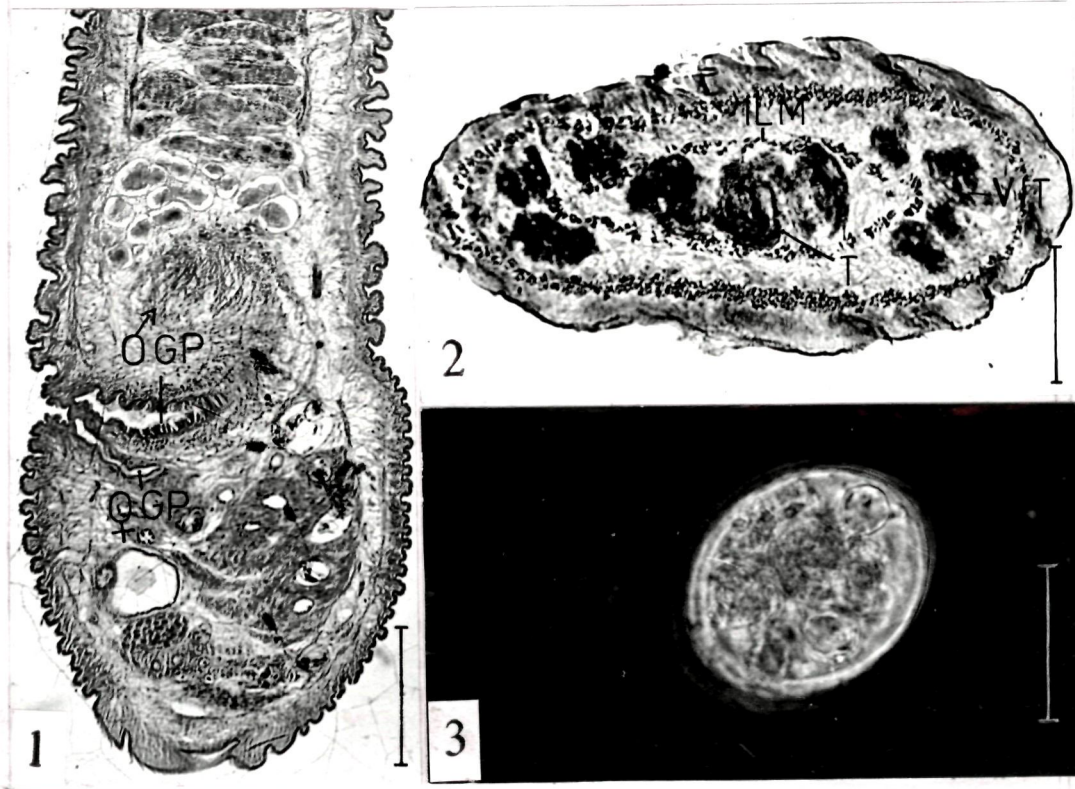


PLATE 1.6 *Lytocestus filiformis* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.6



The measurement of the body and its organs are presented in Table III.

Host: *Clarias batrachus*

Location: Intestine

Locality: Guwahati(Assam)

Remarks

L. filiformis was first described by Woodland (1923) as *Caryophyllaeus filiformis* from a mormyrid fish host, *Mormyrus coschive*, of river Nile at Khartoum. Later, Fuhrmann and Baer (1925), on the basis of cortical disposition of vitallaria and medullary disposition of testes, shifted it to the genus *Lytocestus*.

The present description supplements the original one by providing measurements of the various organs in Table III.

C. batrachus represents a new host for *L. filiformis*. Further, its occurrence from the north-eastern region of India constitutes a new locality report zoogeographically, i.e., from the Oriental region, in addition to the Palearctis realm, from where the species was originally described.

TABLE III: *Lytocestus filiformis* —
Morphometric measurements

Characters	Present observation		Woodland's (1923) observation
	(Range)	(S.D.)	
1. Length of the body	5.94 - 33.00	± 8.75	7.5 - 24
2. Maximum breadth of the body at the level of cirrus sac.	0.59 - 1.65	± 0.30	1 - 2
3. Length of the neck	1.98 - 12.54	± 2.79	-
4. Testicular follicles			
(a) Length	0.04 - 0.14	± 0.03	-
(b) Breadth	0.01 - 0.08	± 0.02	-
5. Ovarian lobes			
(a) Length	0.53 - 1.5	± 0.32	-
(b) Extent	0.46 - 1.32	± 0.24	-
6. Vitelline follicles			
(a) Length	0.02 - 0.07	± 0.008	-
(b) Breadth	0.01 - 0.04	± 0.008	-
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	2.31 - 16.50	± 3.73	-
8. Pre-vitellaria distance	2.11 - 14.19	± 3.18	-
9. Distance between anterior extent of testes and vitellaria	0.13 - 2.31	± 0.74	-
10. Position of the genital pore from the posterior extremity	0.73 - 2.3	± 0.44	-
11. Eggs (a) Length	0.03 - 0.05	± 0.006	0.0622 - 0.0695
(b) Breadth	0.01 - 0.03	± 0.001	0.0292 - 0.0329

[SD = Standard Deviation]

Lytocestus longicollis Rama Devi, 1973
(Plates 1.7 and 1.8)

The collection comprised 141 specimens of *L. longicollis*.

Description

Body long, slender, ribbon like; body proper divided into an outer cortex and an inner medulla by two distinct layers of parenchymal longitudinal muscles. Scolex undifferentiated, unarmed, but may vary in shape from spatulate or oblong in relaxed specimens to swollen and pear shaped in contracted ones. Neck long, slender, occupying one-third of the body length. Testes numerous, 213-520 in number, occupying the medullary region of the body, spherical in shape, extending from anterior narrow end of the body to the cirrus-sac posteriorly; cirrus sac oval, lined by thin muscular wall, enclosing the long ductus ejaculatorious that opens seperately from the utero-vaginal pore. Ovary bilobed, follicular, H-shaped, connected by band-like ovarian isthmus, corticular in disposition; Mehlis' gland situated posterior to isthmus in between the two ovarian lobes, uterine coils glandular, extending from behind ovarian isthmus to the level of cirrus pore beyond the anterior horns of ovary; vagina straight or slightly convoluted, opening unitedly with the uterus as utero-vaginal pore; receptaculum seminis a conspicuous sac, oval in shape,

PLATE 1.7 *Lytocestus longicollis*

Fig.1 Full worm (whole mount); (a) anterior portion, (b) posterior portion

Fig.2 Scolex end (enlarged);

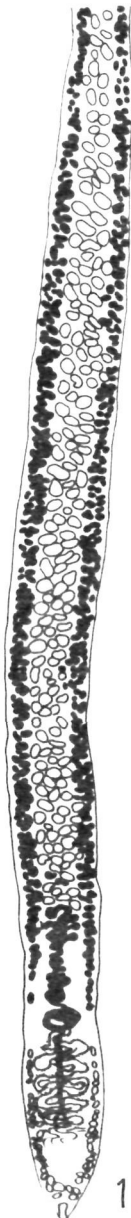
Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg.

PLATE 1.7

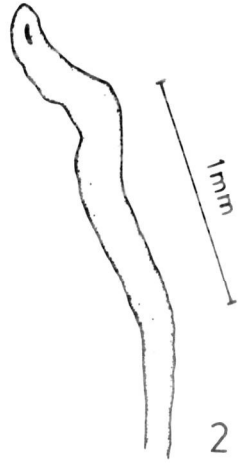


1a

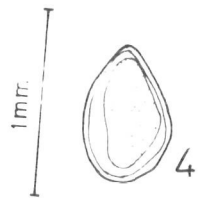


1b

1mm



2



4

1mm



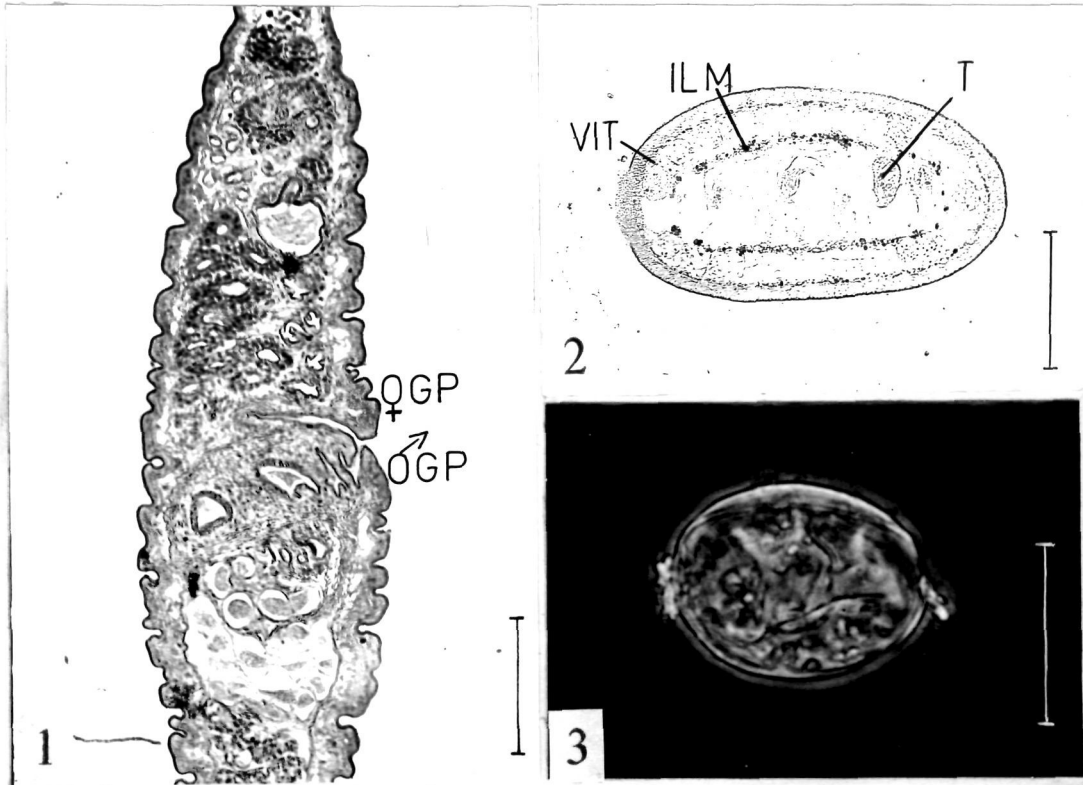
3

1mm

PLATE 1.8 *Lytocestus longicollis* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.5 mm);
- Fig.3** Egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.8



lying anterior to ovarian isthmus. Vitellaria cortical, in a ring around the testes, lobular, smaller than testes, extending from a few millimeters anterior to the testes up to the cirrus sac, no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs oval in shape, smooth, and operculate.

The measurement of the body and its organs are presented in Table IV.

Host: *Clarias batrachus*

Location: Intestine

Locality: Guwahati (Assam)

Remarks

L. longicollis was originally described by Rama Devi (1973) from *Clarias batrachus* in Visakhapatnam district of Andhra Pradesh. The species was distinguished from the rest of the lytocestiid types in having a receptaculum seminis, which is absent in the other species. The species derived its name from its long neck. The present observations are in conformity with those of Rama Devi in all the aspects except for minor deviations with regard to the measurements of the various organs as represented in Table IV.

TABLE IV: *Lytocestus longicollis* :-
Morphometric measurements

Characters	Present observation		Rama Devi's (1973) observation
	(Range)	(S.D.)	
1. Length of the body	14.52 - 32.20	± 5.22	10.8 - 20
2. Maximum breadth of the body at the level of cirrus sac.	0.53 - 1.45	± 0.23	0.5 - 0.84
3. Length of the neck	4.62 - 12.08	± 2.27	5.36 - 7.6
4. Testicular follicles			
(a) Length	0.06 - 0.16	± 0.03	-
(b) Breadth	0.03 - 0.12	± 0.02	0.10 - 0.16
5. Ovarian lobes			
(a) Length	0.72 - 1.10	± 0.32	0.46 - 0.78
(b) Extent	0.26 - 0.79	± 0.53	-
6. Vitelline follicles			
(a) Length	0.03 - 0.11	± 0.02	0.039 - 0.07
(b) Breadth	0.02 - 0.07	± 0.02	-
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	4.62 - 13.2	± 2.26	-
8. Pre-vitellaria distance	3.98 - 12.07	± 2.27	-
9. Distance between anterior extent of testes and vitellaria	0.64 - 1.19	± 0.19	-
10. Position of the genital pore from the posterior extremity	1.12 - 2.31	± 0.43	-
11. Eggs			
(a) Length	0.05 - 0.07	± 0.01	0.046 - 0.054
(b) Breadth	0.02 - 0.03	± 0.002	0.023 - 0.031

[SD = Standard Deviation]

***Lytocestus clariae* n.sp.**
(Plates 1.9 and 1.10)

112 specimens were collected during the two years exploration of cestode fauna from *Clarias batrachus*.

Description

Body elongate, flat with no trace of internal or external segmentation, tapering anteriorly, body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth and unarmed with bluntly tapering extremity, followed by a short neck that is devoid of any reproductive organs. Testes numerous, 270-495 in number, occupying the medullary region of the body, ovoid in shape, larger than vitelline follicles, extending from a little behind the anterior follicles of vitellaria posteriorly up to the cirrus sac; cirrus sac a compact parenchymatous bulb, enclosing the ductus ejaculatorius; cirrus opening joining distally the terminal part of the female ducts to open to the exterior by a common pore. Ovary bilobed, H-shaped, follicular, extending posteriorly behind Mehlis' gland, the lobes cortical in disposition and joined to each other by an ovarian isthmus which is medullary; Mehlis' gland present behind the ovarian isthmus; uterus glandular, extending from in front of the isthmus up to the cirrus sac; vaginal tube joining the uterus at its distal end to open unitedly at the shallow

PLATE 1.9 *Lytocestus clariae* n.sp.

Fig.1 Full worm (whole mount);

Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg

Fig.5 Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles.

PLATE 1.9

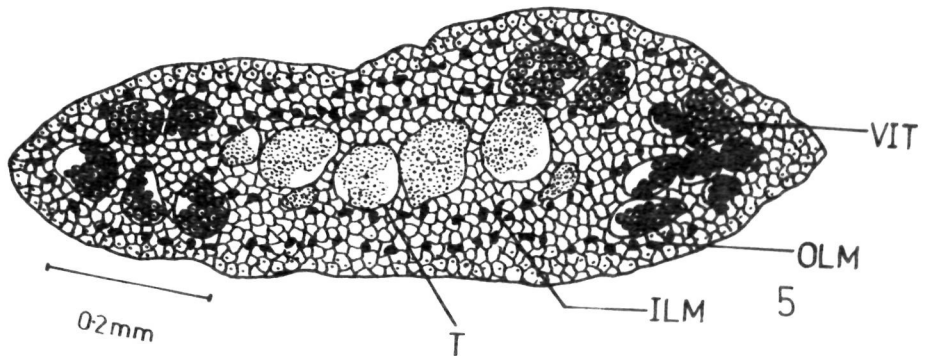
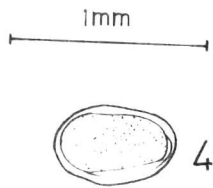
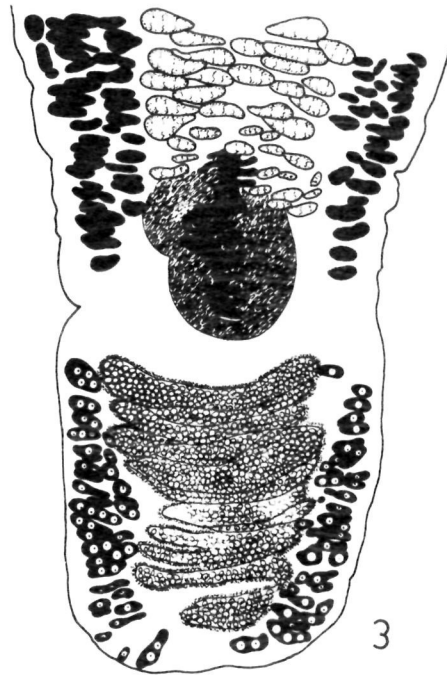
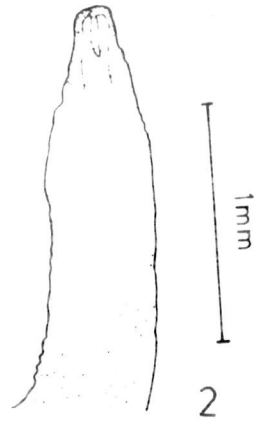
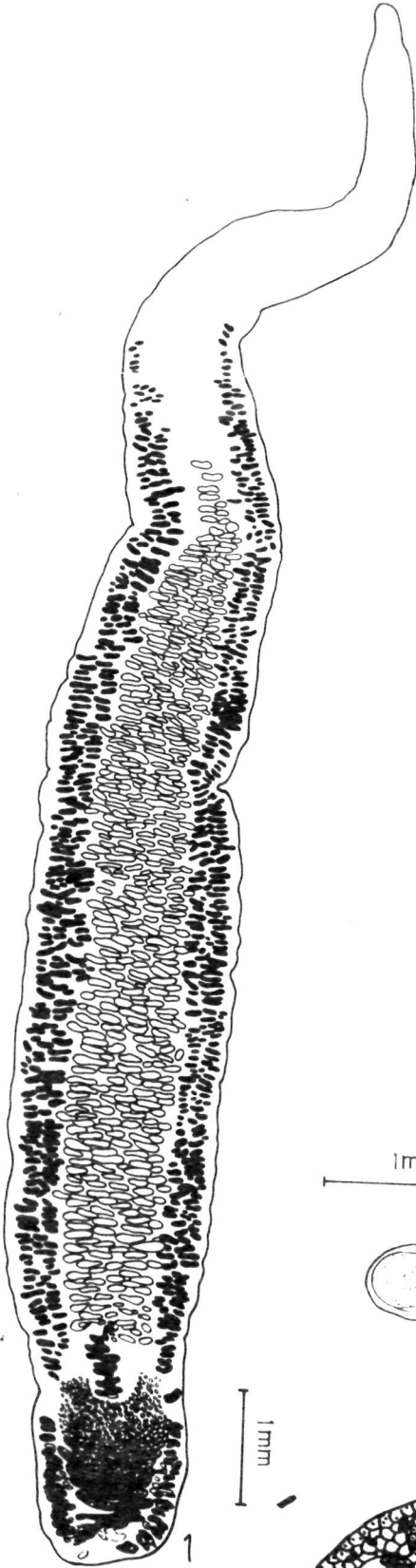
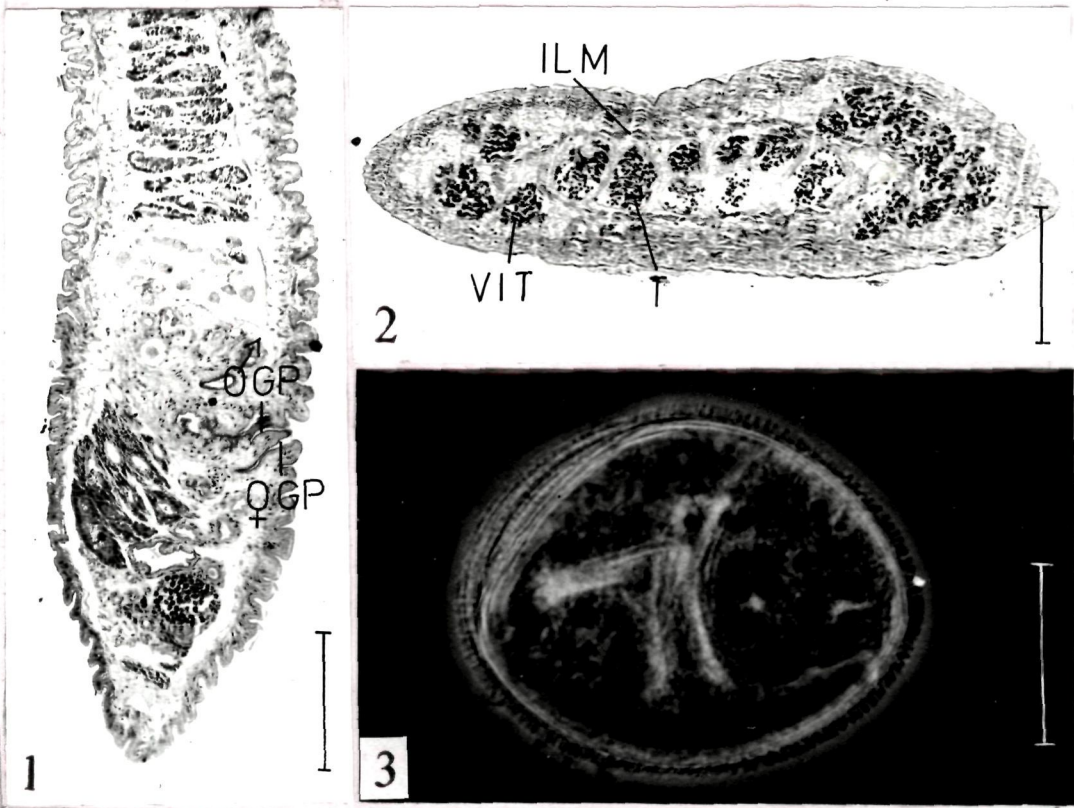


PLATE 1.10 *Lytocestus clariae*

- Fig.1** Sagittal section through the posterior region revealing confluent genital apertures (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Operculate and spinose egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.10



atrium. Vitelline follicles ovoid in shape, commencing from a short distance anterior to the testes up to the level of cirrus sac, arranged in two rows lateral to the testes; no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs oval in shape, spinuous and operculate, as observed under the phase contrast microscope.

The measurement of the body and its organs are presented in Table V.

Statistically the characters such as the length and extent of ovarian lobes, anterior extent of testes and vitellaria, length of the neck and scolex, and position of the genital pore have a positive correlation with the length of the body that is significant at $P < 0.5\%$ level. This implies that the characters are variable and can therefore be considered as intra-specific variations. However, the size of the testes, vitellaria and egg show a negative correlation with the length of the body, indicating thereby the non-variable nature of these characters; the latter thus could be genetic. The length of the neck, though variable, bears a definite proportion with the length of the body and is approximately 5 to 6 times less than the length of the body.

TABLE V: *Lytocestus clariae* n.sp. : morphometric measurements (based on ten mature specimens)

Characters	Range	S.D.
1. Length of the body	8.58 - 22.44	± 4.12
2. Maximum breadth of the body at the level of cirrus sac.	0.66 - 2.31	± 0.67
3. Length of the neck	0.05 - 0.29	± 1.34
4. Testicular follicles		
(a) Length	0.06 - 0.22	± 0.05
(b) Breadth	0.04 - 0.11	± 0.01
5. Ovarian lobes		
(a) Length	0.53 - 1.65	± 0.34
(b) Extent	0.46 - 1.32	± 0.27
6. Vitelline follicles		
(a) Length	0.05 - 0.18	± 0.04
(b) Breadth	0.02 - 0.08	± 0.05
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	1.38 - 8.05	± 1.81
8. Pre-vitellaria distance	1.18 - 6.93	± 1.54
9. Distance between anterior extent of testes and vitellaria	0.13 - 1.18	± 0.37
10. Position of the genital pore from the posterior extremity	0.04 - 0.09	± 0.38
11. Eggs		
(a) Length	0.03 - 0.05	± 0.01
(b) Breadth	0.02 - 0.03	± 0.01

[SD = Standard Deviation]

Discussion

The disposition of vitellaria in the cortex and testes in the medullary zone ascertains the inclusion of the present form in the family Lytocestidae Wardle and McLeod, 1952. Further, owing to the characters such as presence of undifferentiated scolex, absence of post-ovarian yolk glands, uterine coils covered with thick coat of accompanying cells and ejaculatory duct enclosed within compact parenchymatous bulb, the present form belongs to the genus *Lytocestus* Cohn, 1908.

On comparison with the known species of *Lytocestus*, the present form stands close to *L. javanicus*, *L. longicollis*, *L. filiformis*, *L. parvulus* and *L. fossilis* in possessing an undifferentiated scolex that tapers anteriorly, in the ovarian lobes behind the Mehlis' gland and uterine coils up to the cirrus sac and in the extent of testes, i.e., a little posterior to the anterior follicles of vitellaria. In having a short neck and also in the extent of testes and vitellaria, the present form comes close to *L. indicus* and *L. birmanicus* as well. However, it differs from all of them in having confluent genital apertures and spinuous eggs. All the species mentioned above have distinctly separated genital apertures and smooth-surfaced eggs.

The different shape and size of the body and the size of the eggs are characters which further differentiate

the present form from *L. adherens*; the eggs are much smaller in the former than the latter species. On the basis of statistical analysis also, the egg size has been found to be a non-variable character and hence can be assigned significant taxonomic importance in differentiating the species.

In view of the above differences, the present form stands out as a species distinct from the known species of *Lytocestus* and is, therefore, considered a new species.

Host: *Clarias batrachus*

Location: Intestine

Locality: Guwahati (Assam)

Specific Diagnosis:

Lytocestus clariae n.sp.

Elongated body, undifferentiated scolex, short neck, H-shaped ovary, the arms of which extend beyond the Mehlis' gland; confluent genital apertures; spiny eggs.

Etymology: The species is named after its host *C. batrachus*.

Lytocestus attenuatus n.sp.

(Plates 1.11 and 1.12)

98 specimens were recovered from the intestine of *Clarias batrachus*, during the two years study.

Description

Body thick, slender, elongated and flattened,

PLATE 1.11 *Lytocestus attenuatus* n.sp.

Fig.1 Full worm (whole mount);

Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg

Fig.5 Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles.

PLATE 1.11

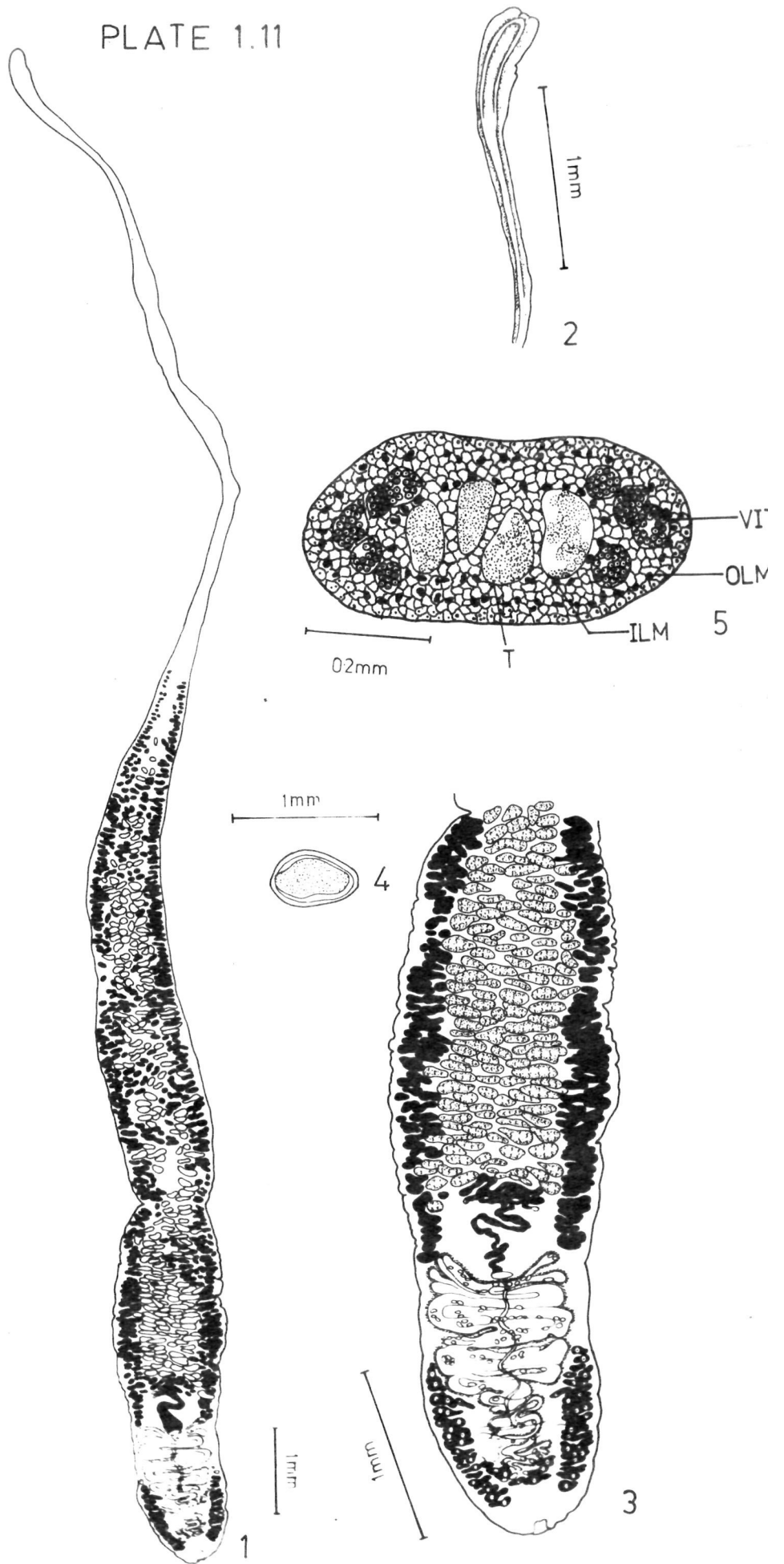
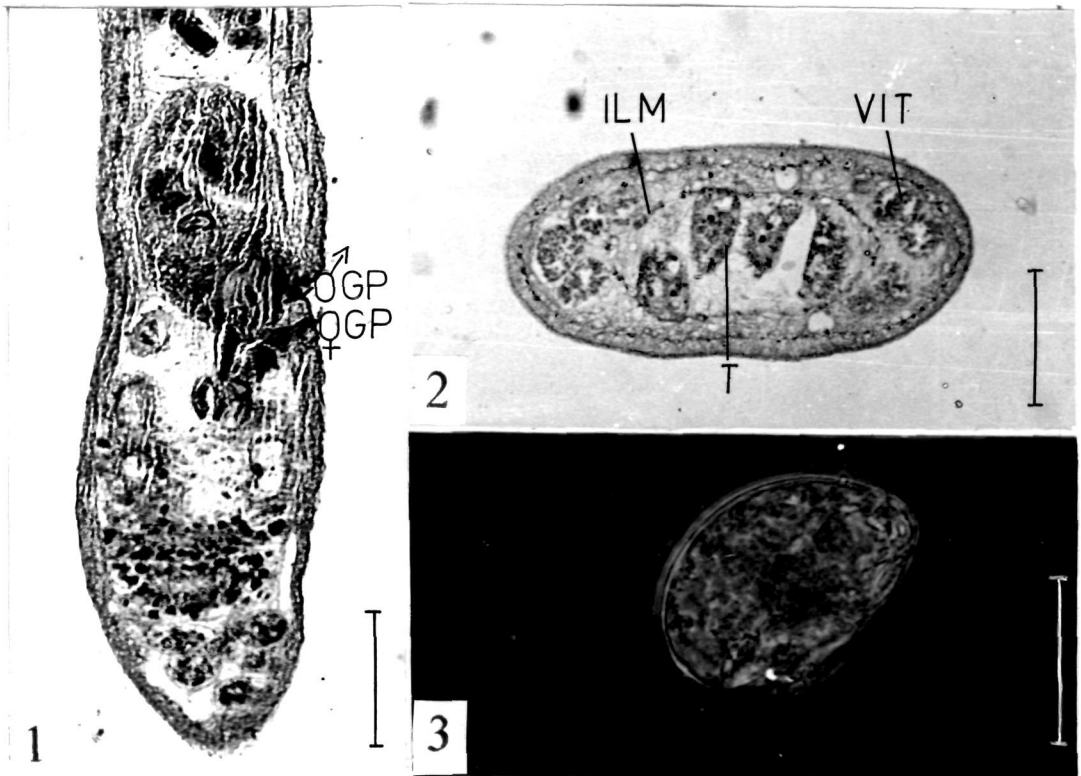


PLATE 1.12 *Lytocestus attenuatus* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Operculate egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.12



posterior end broader than the anterior end, body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex smooth, undifferentiated, unarmed with bluntly rounded extremity, followed by a long narrow neck. Testes ovoid, longer than vitelline follicles 155-398 in number, occupying the medullary region and extending from a little posterior to the anterior vitelline follicles caudad up to the cirrus sac; cirrus sac medullary, enclosing a thin winding ejaculatory duct and opening separately from, and anterior to, the utero-vaginal pore. Ovary bilobed, follicular, bent inwards, inverted 'A' shaped, the lobes extending to the posterior level of Mehlis' gland and joined to each other by an ovarian isthmus, ovarian lobes cortical and isthmus medullary; Mehlis' gland well developed, behind the ovarian isthmus; uterus glandular, extending from behind Mehlis' gland anteriorly beyond the lateral horns of the ovary and up to the cirrus pouch; vagina distinct, straight or slightly convoluted, joining the terminal end of the uterus to open at the utero-vaginal pore. Vitelline follicles ovoid, arranged in two rows lateral to the testes and extending from a little anterior to the testes up to the cirrus sac, no post-ovarian vitelline follicles present. Excretory pore terminal. Eggs smooth and operculate as observed under the phase contrast microscope.

The measurement of the body and its organs are presented in Table VI.

TABLE VI: *Lytocestus attenuatus* n.sp. : morphometric measurements (based on ten mature specimens).

Characters	Range	S.D.
1. Length of the body	11.88 - 35.44	±0.45
2. Maximum breadth of the body at the level of cirrus sac.	0.66 - 1.18	± 0.17
3. Length of the neck	6.14 - 7.06	± 1.30
4. Testicular follicles		
(a) Length	0.08 - 0.18	± 0.04
(b) Breadth	0.03 - 0.15	± 0.03
5. Ovarian lobes		
(a) Length	0.53 - 1.52	± 0.32
(b) Extent	0.53 - 0.92	± 0.93
6. Vitelline follicles		
(a) Length	0.05 - 0.12	± 0.04
(b) Breadth	0.01 - 0.07	± 0.05
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	6.79 - 21.05	± 4.76
8. Pre-vitellaria distance	6.14 - 13.00	± 3.30
9. Distance between anterior extent of testes and vitellaria	0.46 - 8.05	± 2.78
10. Position of the genital pore from the posterior extremity	0.79 - 1.52	± 0.31
11. Eggs (a) Length	0.04 - 0.06	± 0.01
(b) Breadth	0.02 - 0.03	± 0.01

[SD = Standard Deviation]

Statistically, the length and extent of the ovarian lobes, length of the neck, anterior extent of testes and vitellaria and position of the genital pore bears a positive correlation with the length of the body and is significant at $P < 0.5\%$ level. The size of the testes, vitellaria and eggs however, shows a negative correlation with the length of the body. The latter shows a definite proportion with the length of the neck, i.e., it is twice the length of the neck.

Discussion

The present species also belongs to the family Lytocestidae, owing to the presence of cortical vitellaria and medullary testes. Further, on account of having undifferentiated scolex, uterus with thick coat of accompanying cells, ejaculatory duct enclosed within a compact parenchymatous bulb, and the absence of post ovarian vitellaria, this form also comes under the genus *Lytocestus*.

In sharing the characters such as shape of the body, which is thin, slender and elongated, and undifferentiated scolex, the present form comes close to *L. longicollis*, *L. parvulus*, *L. filiformis*, *L. fossilis* and *L. javanicus*. However, it differs from each of them in certain characters: from *L. longicollis* in not possessing a receptaculum seminis; from *L. parvulus* in lacking a linear arrangement of vitelline

follicles in five rows; from *L. fossilis* in the absence of post-ovarian vitelline follicles; and from *L. filiformis* in having oval and large-sized vitelline follicles as compared to the small and globular ones occurring in the later species. As concluded from the statistical morphometric analysis, the size of the vitelline follicles appears to be a non-variable character. Thus, it can be used as a feature for inter-specific differentiation. *L. javanicus* differs from the present form in the absence of a long neck. The neck size, though variable in the latter form, bears a definite proportion with the length of the body and is found to be almost half of it. The present form also differs from the type species in characters such as shape and size of the body and size of the egg. While *L. adherens* is club shaped, the present form is slender, elongated and filiform. Further, the maximum size recorded for *L. adherens* is 1cm, but the same for the present form is 3.3 cm. While the egg in *L. adherens* is 0.08 x 0.03 mm, in the present form it is much smaller, i.e., 0.056 x 0.03 mm. Since the size of the egg is also proved to be a non-variable character statistically, it is further confirmatory for distinguishing it from the latter.

In lacking a prominent holdfast, which is distinct feature of *L. indicus* and *L. birmanicus*, the present form can be distinguished from these species. It also stands

apart from *L. clariae* n. sp. described herein, in having separate genital apertures and in possessing smooth-surfaced eggs; in *L. clariae*, the genital apertures are confluent and the egg surface is spinuous.

Therefore, considering all the above differences, it is proposed to assign the present form the rank of a new species.

Host: *Clarias batrachus*
Location: Intestine
Locality: (Guwahati (Assam))

Specific diagnosis:

***Lytocestus attenuatus* n. sp.**

Body filiform; scolex undifferentiated; long, slender neck; ovary inverted - 'A' shaped; testes medullary, vitellaria in two fields lateral to the testes; eggs smooth and operculate.

Etymology: The specific name is given on the basis of thin, tapering shape of the body.

***Lytocestus assamensis* n. sp.**
(Plates 1.13; 1.14 and 1.15)

65 specimens were recovered from *C. batrachus* during the two years study.

Description

Body very elongate, flat with no trace of internal

or external segmentation, slightly tapering anteriorly, creamish white in colour, body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth and unarmed with bluntly tapering extremity; well developed gland cells present, with a distinct zone of their denser aggregation 3-4 mm from the anterior extremity; this distinct glandular region provides the only clue for distinguishing and delimiting the scolex region from the neck; the limits of the latter are otherwise not well demarcated from the rest of the body proper following it. Testes numerous, 266-565 in number, occupying the medullary region of the body, ovoid in shape, larger than vitelline follicles; external seminal vesicle present; cirrus sac prominent, opening separately from the female genital pore at the level of the posterior seventh of the body length. Ovary bilobed, bent inwards in the shape of an inverted 'A', ovarian wings joined to each other by an isthmus, the whole ovary ~~cortical~~ in disposition; Mehlis' gland well developed, located behind isthmus; uterus glandular, extending from in front of the isthmus anteriorly beyond the lateral horns of the ovary; vagina distinct, joining the terminal end of the uterus and opening to the exterior at the utero-vaginal pore, the latter situated mid-ventrally immediately posterior to the male opening. Vitelline follicles partly cortical and partly medullary, commencing from pre-testicular region

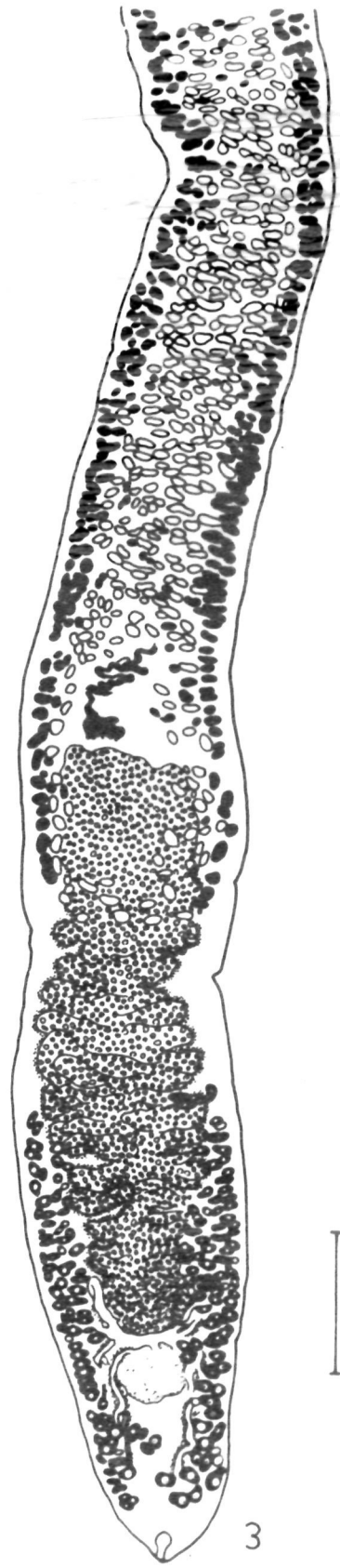
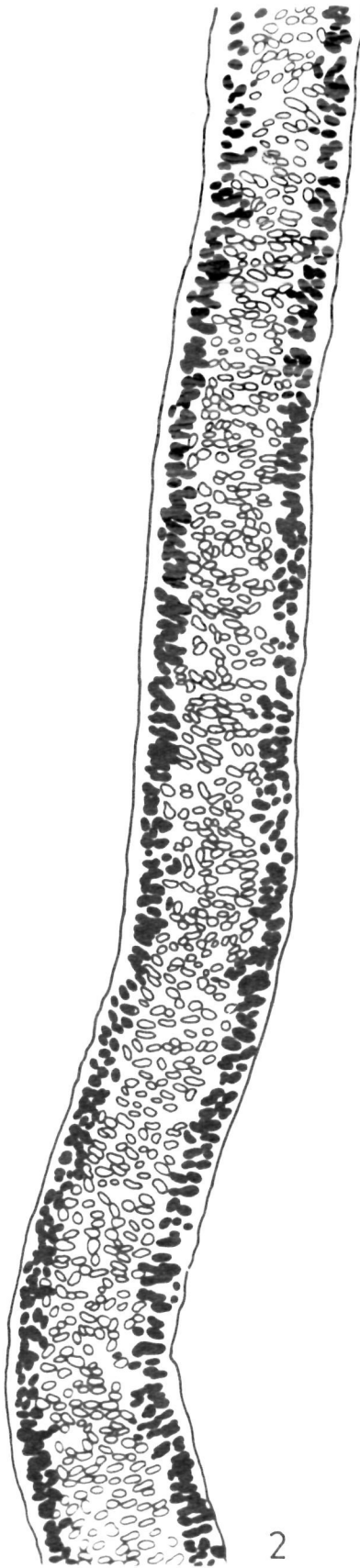
PLATE 1.13 *Lytocestus assamensis* n.sp.

Fig.1 Anterior portion of the full worm;

Fig.2 Middle third of the worm;

Fig.3 Posterior third of the body.

PLATE 1.13



1mm

PLATE 1.14 *Lytocestus assamensis*

- Fig.1** Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles;
- Fig.2** Egg;
- Fig.3** Scolex end (enlarged);
- Fig.4** Posterior end enlarged to show the disposition of the various components of the reproductive system.

PLATE 1.14

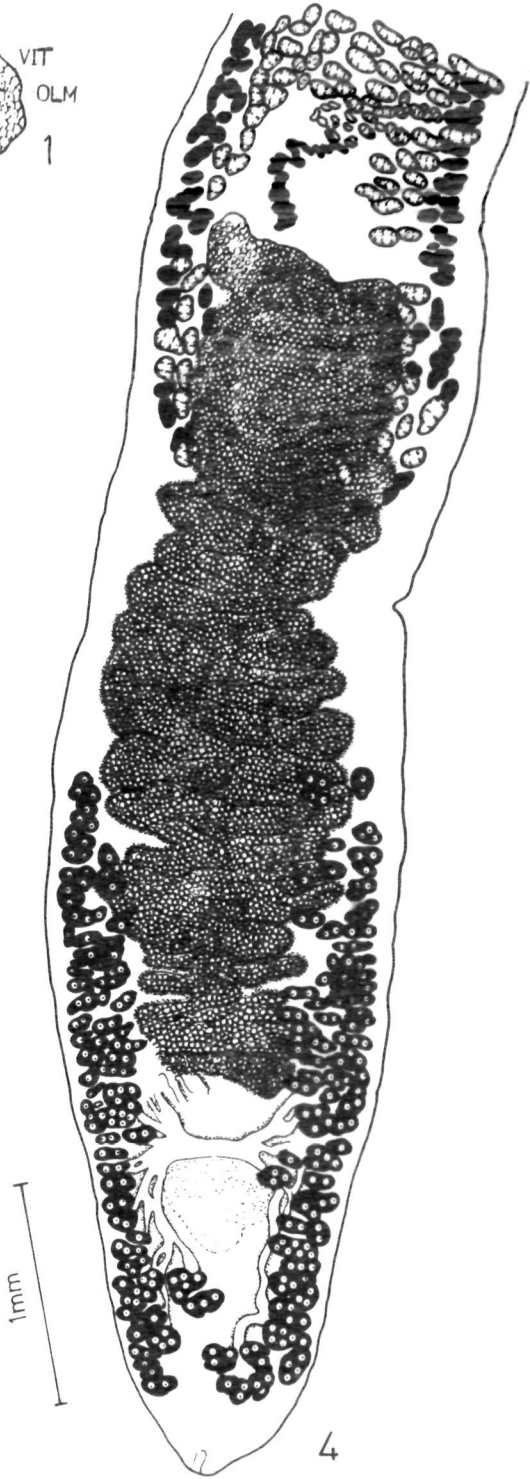
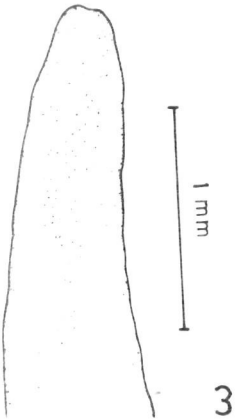
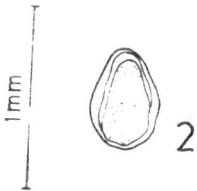
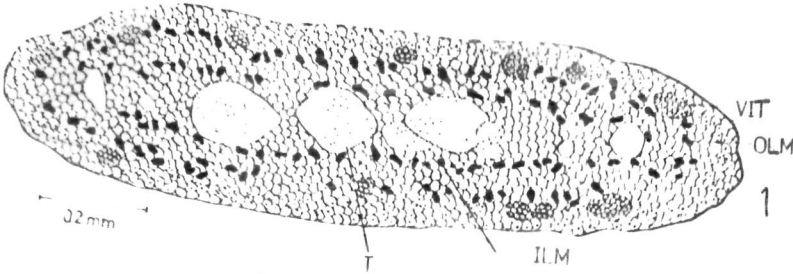
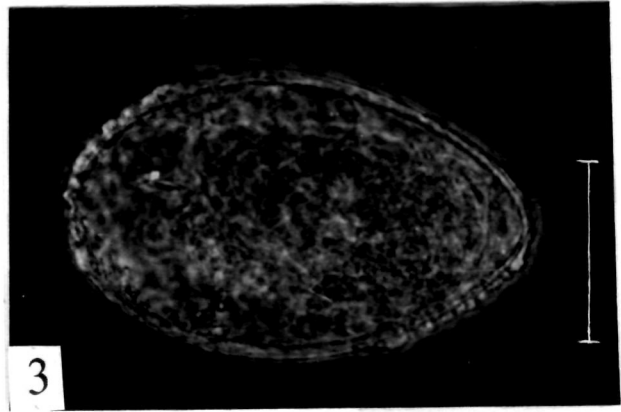
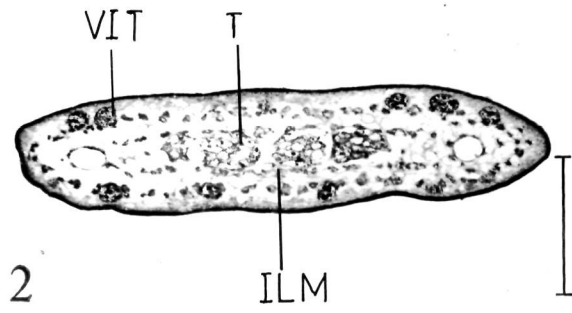
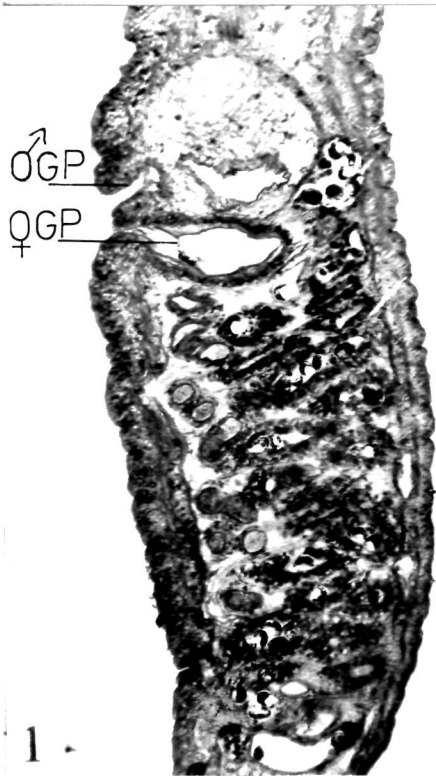


PLATE 1.15 *Lytocestus assamensis* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Operculate egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.15



0.66-0.82 mm from the anterior extremity and extending posteriad up to the level of the cirrus sac, no post-ovarian vitelline follicles present. Excretory pore at the terminal hind end. Eggs smooth, and operculate as confirmed from phase-contrast observations of eggs.

The measurement of the body and its organs are presented in Table VII.

Morphometric measurements when analysed statistically, reveal that the length and extent of ovarian lobes, anterior extent of testes and vitellaria, length of the neck and position of the genital pore are all directly proportional to the length of the body, i.e., bear a positive correlation which is significant at $P < 0.5\%$ level. The length of the body, however, has a definite proportion with the length of the neck; the neck and body-length ratio is 1:65. Certain other characters like the size of the testes, vitellaria and eggs bear a negative correlation with the length of the body, showing thereby the non-variability of these characters.

Discussion

On account of having cortically disposed vitellaria and medullary testes, the present form belongs to the family Lytocestidae. Further, owing to the presence of undifferentiated

TABLE VII: *Lytocestus assamensis* n.sp. : morphometric measurements (based on ten mature specimens).

Characters	Range	S.D.
1. Length of the body	25.54-50.82	± 8.97
2. Maximum breadth of the body at the level of cirrus sac.	1.32 - 4.62	± 1.24
3. Length of the neck	4.62-15.18	± 3.60
4. Testicular follicles		
(a) Length	0.10 - 0.53	± 0.20
(b) Breadth	0.06 - 0.15	± 0.05
5. Ovarian lobes		
(a) Length	1.52 - 5.08	± 1.09
(b) Extent	0.79 - 2.64	± 0.57
6. Vitelline follicles		
(a) Length	0.06 - 0.14	± 0.04
(b) Breadth	0.04 - 0.08	± 0.03
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	5.28 - 16.50	± 4.04
8. Pre-vitellaria distance	4.62 - 15.18	± 3.60
9. Distance between anterior extent of testes and vitellaria	0.66 - 0.82	± 0.01
10. Position of the genital pore from the posterior extremity	1.98 - 5.20	± 1.07
11. Eggs (a) Length	0.03 - 0.05	± 0.01
(b) Breadth	0.02 - 0.03	± 0.001

[SD = Standard Deviation]

scolex, absence of post-ovarian yolk glands, uterine coils covered with thick coat of accompanying cells and ejaculatory duct enclosed within a compact parenchymatous bulb, the present form belongs to the genus *Lytocestus*.

While ascertaining its specific status and on comparing it with the known forms of *Lytocestus*, the present form stands close to *L. longicollis* in sharing the characters such as undifferentiated scolex, inverted 'A'-shaped ovary and uterine coils extending beyond the anterior horns of ovary. However, it differs from the same in not possessing a receptaculum seminis which is the characteristic feature of *L. longicollis*. Further, the size of the body, testes and vitellaria appear to be much larger in the present form compared to *L. longicollis*. Also, the distribution of vitellaria within the testicular field in the present form further distinguishes it from *L. longicollis*, in which the vitellaria are confined to the lateral fields.

On comparison with *L. parvulus*, the present form appears distinctly different in not possessing five rows of vitelline follicles as in the latter. The size and shape of testes and vitellaria which are much larger and ovoid in the present form are the characters which differentiate it from *L. filiformis*. Because of the lack of post-ovarian yolk glands the present form is distinguished from *L. fossilis*

which is reported from a different host, i.e., *H. fossilis*. In general appearance and shape of the body, the present form stands apart from *L. javanicus*. While the present form is broad and flat occupying the full width and considerable length of the host's intestine, the body shape is much slender and elongated in the latter species. These characters and the egg size also differentiate the present form from the type species *L. adherens*, which is a club-shaped and small-sized worm having larger eggs.

In possessing an undifferentiated scolex end, the present form differs from *L. indicus* and *L. birmanicus*; in the latter two species the scolex end is prominent and distinct from the neck zone.

On comparison with the other new forms described herein, the present form appears to be different from *L. clariae* in not possessing confluent genital apertures and spinuous eggs; it differs from *L. attenuatus* in the distribution of testes and vitellaria that are intermingled and not confined to lateral fields, and in the extent of uterine coils limited only up to the ovarian isthmus and not beyond, as in *L. attenuatus*.

The various morphometric features used for the purpose of differentiating the present form from the other known species of *Lytocestus* are found consistent among

the numerous specimens studied. Statistically also, these are found as non-variable characters, hence appear to be interspecific differences and not intra-specific variations.

In view of the differences the present form has from the known species, it seems appropriate to assign it the status of a new species under the genus *Lytocestus*.

Host: *Clarias batrachus*

Location: Intestine

Locality: Guwahati (Assam)

Specific diagnosis:

Lytocestus assamensis n.sp.

Body very elongate; scolex undifferentiated, without any terminal introvert; long neck; inverted 'A'-shaped ovary; uterine coils not extending beyond ovarian isthmus; eggs smooth and operculate.

Etymology: The species has been named after the state Assam in N.E. India from where the fish hosts were collected.

Lytocestus heteropneustii n.sp.

(Plates 1.16 and 1.17)

The collection comprised 22 specimens of this form.

Description (based on measurements of 6 specimens and few series of histological sections).

Body elongate, flat with no trace of internal or external segmentation, tapering anteriorly; body proper

PLATE 1.16 *Lytocestus heteropneustii* n.sp.

Fig.1 Full worm (whole mount);

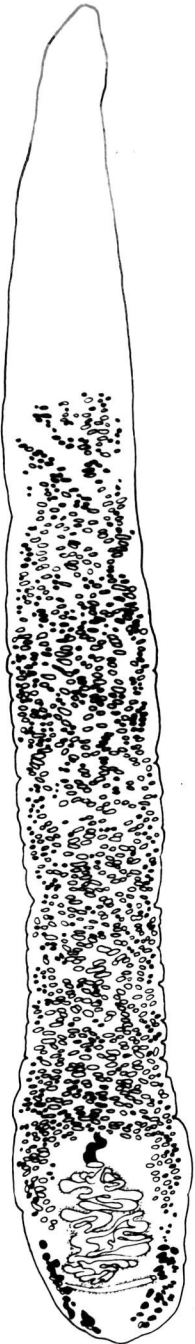
Fig.2 Scolex end (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg

Fig.5 Diagrammatic representation of the transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles.

PLATE 1.16



1mm

1

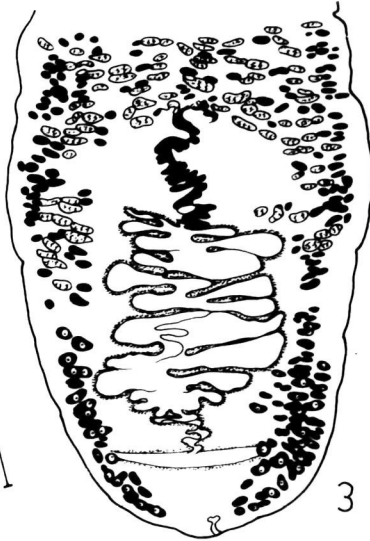


2

1mm

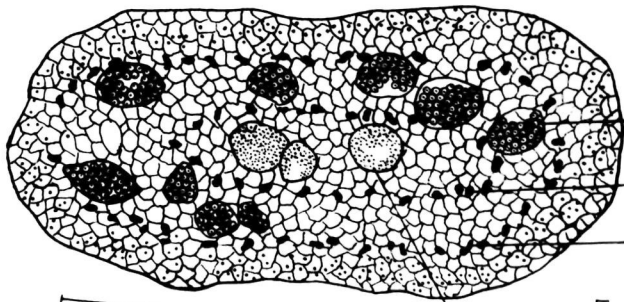


4



1mm

3



VIT
ILM
OLM

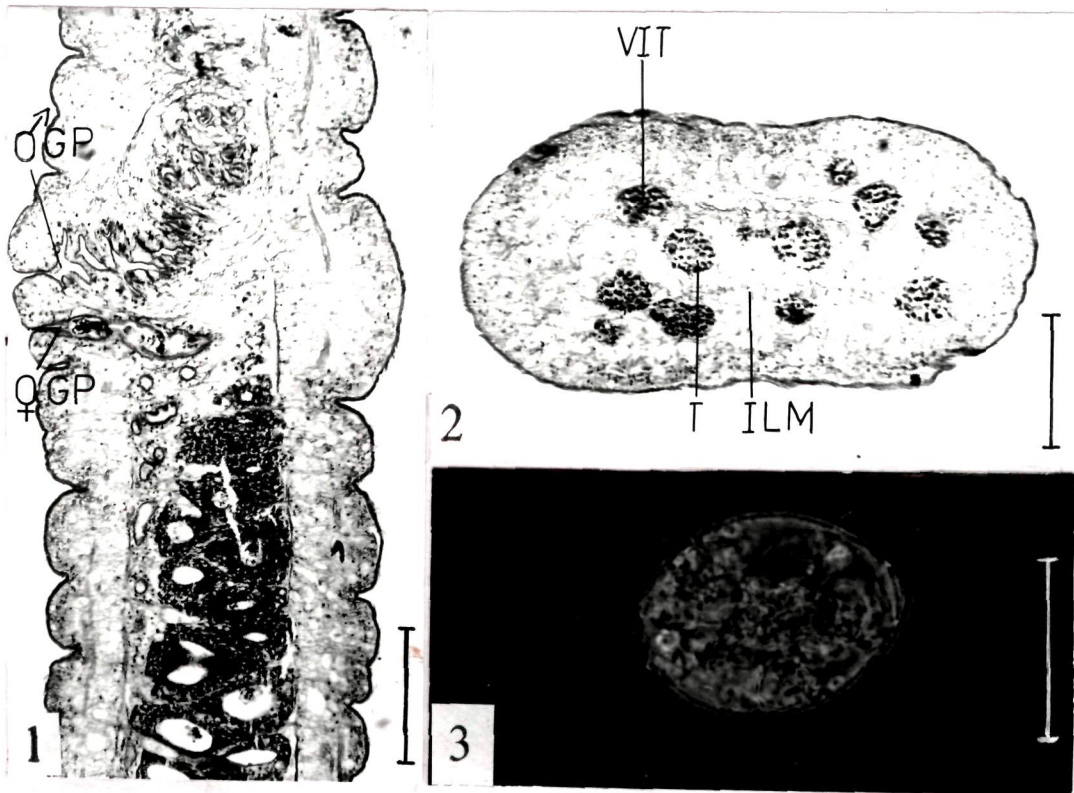
0.2mm

5

PLATE 1.17 *Lytocestus heteropneustii* (Photomicrographs)

- Fig.1** Sagittal section through the posterior region revealing separate male and female genital pores (scale bar = 0.15 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.15 mm);
- Fig.3** Operculate egg as seen under phase contrast (scale bar = 0.05 mm).

PLATE 1.17



divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex undifferentiated, smooth, unarmed with a conical base and bluntly tapering extremity followed by a short neck. Testes numerous, 235-340 in numbers, ovoid in shape, larger than vitelline follicles, commencing a short distance behind anterior vitellaria up to cirrus sac and occupying the medullary region; cirrus sac prominent, occupying the entire thickness of medulla, opening separately a little in front of the utero-vaginal pore. Ovary bilobed, follicular, H-shaped, ovarian lobes joined to each other by an ovarian isthmus, extending behind Mehlis' gland posteriorly; Mehlis' gland behind ovarian isthmus, uterus glandular, extending from in front of the isthmus anteriorly beyond the lateral horns of the ovary; no uterine coils behind ovarian isthmus; vagina distinct, joining the terminal end of the uterus to open unitedly at the utero-vaginal pore. Vitelline follicles spherical, cortical in disposition relative to longitudinal musculature, strewn in the mid-field of testicular region, commencing from the base of the neck and extending up to the anterior horns of the ovary, no post-ovarian follicles present. Excretory pore terminal. Eggs smooth, ovoid and operculate, as observed under the phase contrast microscope.

The measurement of the body and its organs are presented in Table VIII.

TABLE VIII: *Lytocestus heteropneustii* n.sp.: morphometric measurements (based on six mature specimens).

Characters	Range	S.D.
1. Length of the body	9.57-19.14	± 3.52
2. Maximum breadth of the body at the level of cirrus sac.	1.06 - 1.45	± 0.13
3. Length of the neck	1.98- 5.41	± 1.49
4. Testicular follicles		
(a) Length	0.11 - 0.19	± 0.03
(b) Breadth	0.03 - 0.08	± 0.02
5. Ovarian lobes		
(a) Length	0.99 - 3.10	± 0.86
(b) Extent	0.92 - 1.32	± 0.14
6. Vitelline follicles		
(a) Length	0.07 - 0.13	± 0.02
(b) Breadth	0.03 - 0.08	± 0.01
7. Pre-testes distance (commencement of testicular follicles from anterior extremity)	1.98 - 6.27	± 1.93
8. Pre-vitellaria distance	1.85 - 5.41	± 1.98
9. Distance between anterior extent of testes and vitellaria	0.12 - 1.12	± 0.43
10. Position of the genital pore from the posterior extremity	1.52 - 4.16	± 0.97
11. Eggs (a) Length	0.03 - 0.04	± 0.006
(b) Breadth	0.02 - 0.05	± 0.009

[SD = Standard Deviation]

The characters such as the length and extent of ovarian lobes, length of the neck, anterior extent of testes and vitellaria and position of the genital pore are statistically found to bear a positive correlation with the length of the body and significant at P 0.5% level. Further, though the length of the neck appears to be a variable character, it bears a definite proportion with the total length of the body, i.e., it is approximately 5.42 times less than the body length. However, the size of testes, vitellaria and eggs appear to be non-variable, since they bear a negative correlation with the length of the body.

Discussion

Cortically disposed vitellaria and medullary testes determines that the present form also belongs to the family Lytocestidae, and the presence of characters such as undifferentiated scolex, absence of post-ovarian yolk glands, uterine coils covered with thick coat of accompanying cells and ejaculatory duct enclosed within the parenchymatous bulb suggests that the present form belongs to the genus *Lytocestus*. It is, however, found distinctly different from the already known forms of the genus.

The present form stands apart from *L. indicus* and *L. birmanicus* in not possessing a holdfast distinct from the neck. In having the vitellaria intermingled with the

testicular follicles and not confined to the lateral fields, the present species deviates from *L. filiformis*, *L. parvulus*, *L. longicollis*, *L. clariae* n.sp. and *L. attenuatus* n.sp. It can further be differentiated from *L. longicollis* in the absence of a receptaculum seminis; from *L. parvulus* in the arrangement of vitelline follicles which in the latter are arranged in five rows; and from *L. clariae* n.sp. in not possessing confluent genital apertures and in having smooth-surfaced eggs. In having a short and stumpy body, the present form differs from *L. filiformis*, the long-necked *L. attenuatus* n. sp., *L. assamensis* n. sp. and *L. javanicus*. Since the neck length has been observed herein to bear a definite proportion with the total length of the body, it is considered a valid character for distinguishing the present form from all these species. It also stands distinctly apart from the type species *L. adherens* which has a club-shaped body, unlike its flat and elongated one. Besides, while the eggs in the present form is small (0.034 x 0.045mm), the same is larger in the type species *L. adherens* (0.08 x 0.03mm). Statistically also, the egg size is found to be a non-variable character, so it is considered valid for distinguishing the different species.

Further, on comparison with *L. fossilis*, the only other species of the genus described from the same host, i.e., *Heteropneustes fossilis*, the present form appears

to be different on account of the absence of post-ovarian yolk glands.

In view of the above differences, the present form is regarded as a new species of the genus.

Host: *Heteropneustes fossilis*

Location: Intestine

Locality: Guwahati (Assam)

Specific diagnosis:

Lytocestus heteropneusti n.sp.

Body elongate; short neck; undifferentiated scolex; H-shaped ovary; testes medullary; vitellaria strewn in the mid-field of testicular zones; eggs oval and smooth surfaced, operculate.

Etymology: The species is named after the host from which it has been recovered.

Family Lytocestidae Wardle and McLeod, 1952

Subfamily Djombanginae Satpute and Agarwal, 1980(a)

Genus *Djombangia* Bovien, 1926.

Djombangia penetrans Bovien, 1926.
(Plates 1.18 and 1.19)

398 specimens of *D. penetrans* were collected.

Description

Body short, broad and fleshy; body proper divided into an outer cortex and an inner medulla by two layers of longitudinal muscles. Scolex globular with a terminal

PLATE 1.18 *Djombangia penetrans*

Fig.1 Full worm (whole mount);

Fig.2 Scolex end showing sucker at the tip (enlarged);

Fig.3 Posterior portion of the worm enlarged to show disposition of the various components of the reproductive system;

Fig.4 Egg.

PLATE 1.18

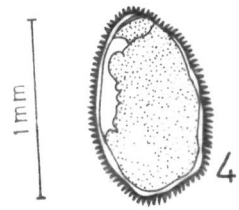
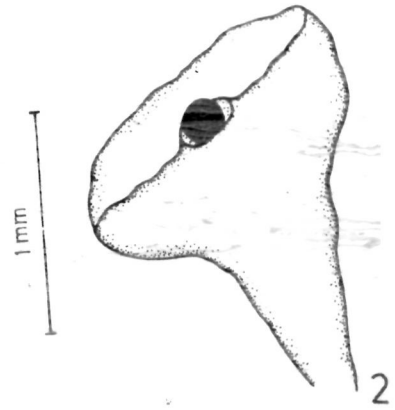
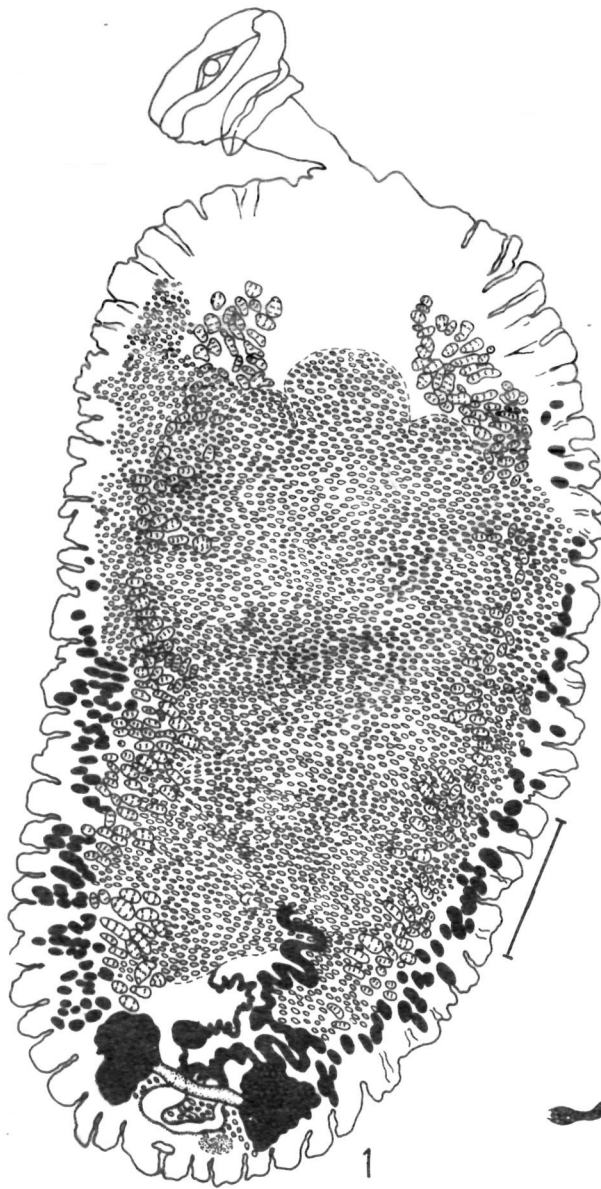
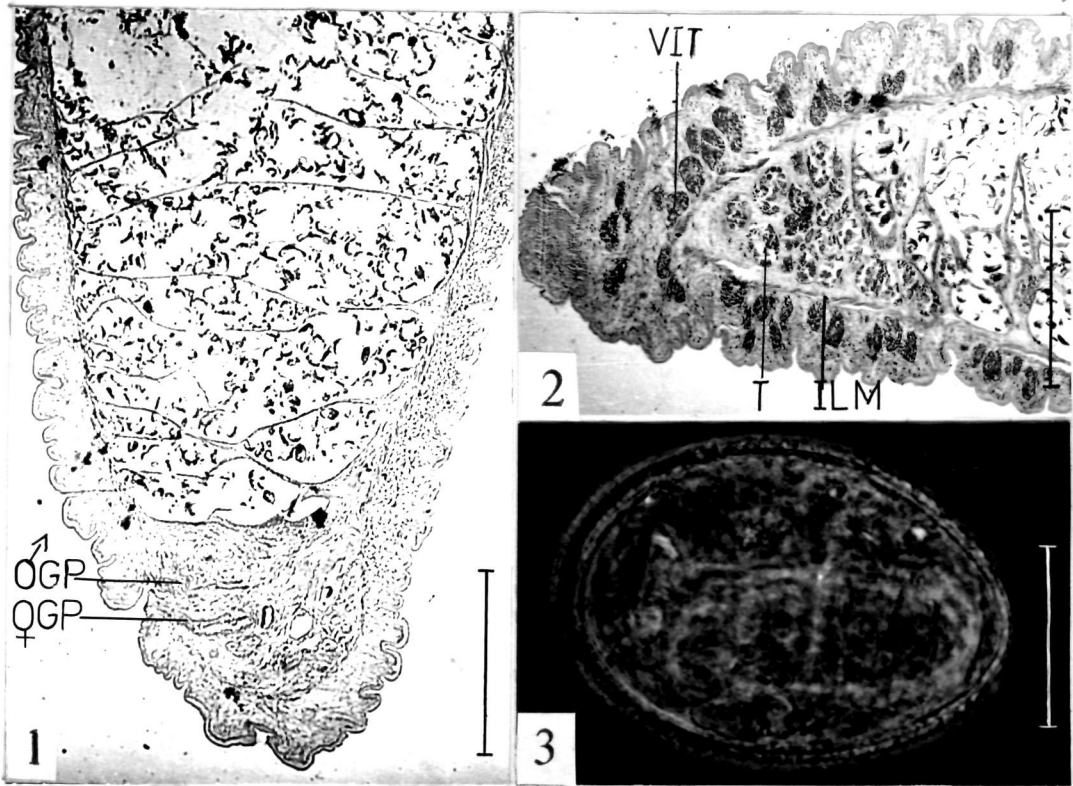


PLATE 1.19 *Djombangia penetrans*

- Fig.1** Sagittal section through the posterior region revealing confluent genital apertures (scale bar = 0.5 mm);
- Fig.2** Transverse section showing the distribution of testes and vitellaria in relation to the longitudinal muscles (scale bar = 0.5 mm);
- Fig.3** Operculate and spinose egg as seen under phase contrast (scale bar =0.05 mm).

PLATE 1.19



sucker. Neck marked off from the body. Testes 155-383 in number, spherical or ovoid, extending in two lateral rows from some distance behind the neck up to the level just in front of the ovary; cirrus pouch not well defined, opening into a common atrium immediately in front of the utero-vaginal pore; genital atrium close to posterior extremity, just in front of the ovarian isthmus. Ovary bilobed, at posterior extremity, follicular, the two lobes joined to each other by an ovarian isthmus; uterus partly glandular, its coils largely in the median field of medulla, and reaching cephalad up to the commencement of testicular region. Vitellaria globular, extending in cortical parenchyma of testicular and ovarian zone; no post-ovarian vitelline follicles present. Eggs oval, spiny and operculate.

The measurements of the body and its organs are presented in Table IX.

Host: *Clarias batrachus*
Location: Stomach and duodenum
Locality: Guwahati (Assam)

Remarks

The present form is identified to be *D. penetrans* Bovien, 1926 because it shares with the type species all the salient morphological features like a distinct sucker

TABLE IX: *Djombangia penetrans*: morphometric measurements and their comparison with the other *Djombangia* species described from India.

Characters	Present observation		<i>D. indica</i>	<i>D. cabellaro</i>
	(Range)	(S.D.)	Satpute & Agarwal's (1980) observation	Sahay & Sahay's(1977) observation
1. Length of the body	5.61 - 11.35	± 1.87	7.3 - 13.8	7.86 - 8.12
2. Maximum breadth of the body at the level of cirrus sac.	2.97 - 5.28	± 0.68	1.8 - 4.3	2.70 - 2.76
3. Length of the scolex	0.53 - 1.12	± 2.08	0.9 - 1.5	0.52 - 0.6
4. Length of the neck	0.26 - 0.99	± 0.29	0.6 - 1.7	1.00 - 1.22
5. Testicular follicles				
(a) Length	0.06 - 0.19	± 0.28	0.048 - 0.168	-
(b) Breadth	0.04 - 0.12	± 0.02	0.06 - 0.18	-
6. Ovarian lobes				
(a) Length	0.26 - 0.66	± 0.13	0.425 - 0.85	0.4
(b) Breadth	0.99 - 1.32	± 0.13	0.17 - 0.493	0.3
7. Vitelline follicles				
(a) Length	0.06 - 0.19	± 0.03	0.024 - 0.096	-
(b) Breadth	0.04 - 0.11	± 0.02	0.024 - 0.084	-
8. Pre-testes distance (commencement of testicular follicles from anterior extremity)	1.19 - 2.64	± 0.39	0.6 - 1.3	-
9. Pre-vitellaria distance	1.45 - 2.97	± 0.48	-	-
10. Distance between anterior extent of testes and vitellaria	0.0028-0.028	± 0.007	-	-
11. Position of the genital pore from the posterior extremity	0.79 - 1.32	± 0.19	-	-
12. Eggs				
(a) Length	0.056-0.073	± 0.005	0.014 - 0.081	0.08
(b) Breadth	0.028-0.039	± 0.003	0.043 - 0.068	0.04

[S.D. = Standard Deviation]

at the tip of the scolex, neck marked off from the body, no post-ovarian vitelline follicles, cirrus sac not prominent, and spinose eggs.

On comparison with the other known forms, the present form is found to be distinctly different from *D. indica* Satpute and Agarwal, 1980(a), described from *C. batrachus* in Raipur (M.P), in not having the post-ovarian vitelline follicles and in the absence of a prominent cirrus sac and receptaculum seminis, though it shares the characters such as the presence of a sucker at the tip of the holdfast and spinose eggs.

The present form also does not fit into the description of the other Indian species of *Djombangia*, i.e., *D. caballeri* Sahay and Sahay, 1977. The latter described from *H. fossilis* in Bihar is reported to have a conspicuous cirrus sac, non-operculate and aspinose eggs, the characters which distinguish it from *D. penetrans*. Till more material is collected and studied from various localities it appears that the genus *Djombangia* is represented by three species in India.

Chapter II **SURFACE FINE TOPOGRAPHY**

INTRODUCTION

Amongst the cestodes the majority of the studies carried out so far on surface topography pertains to the members of Pseudophyllidea and Cyclophyllidea (Lyons, 1977). All cestode species examined so far possess microtriches in the larval as well as the adult stage and these structures seem to be a universal feature of the group (Bråten, 1968a; Jha and Smyth 1969, 1971; Lumsden, 1966; Morseth, 1966; Yamane, 1968). Studies on some species of pseudophyllidean tapeworms indicate that there are differences in shape and density of microtriches between larvae and adult worms (Bråten, 1968b; Grammeltvedt, 1973) and between different species (Charles and Orr, 1968; Andersen, 1975).

The tegument of monozoic cestodes has been less intensively studied than that of strobilating species, the available accounts being restricted to some caryophyllids like *Hunterella nodulosa* (Hayunga and Mackiewicz, 1975), *Glaridacris catastomi* and *G. laruei* (Hayunga, 1979), and *Caryophyllaeus laticeps* (Richards and Arme, 1981a, b and 1982a,b).

In the present investigation surface fine topography of *Lytocestus indicus*, and *Djombangia penetrans*, the most

commonly occurring and apparently pathogenic species of the edible catfish, *Clarias batrachus*, was studied with the help of scanning electron microscopy.

Materials and Methods

Live specimens of *Lytocestus indicus* and *Djombangia penetrans* were obtained from the intestine of *C. batrachus*. After thorough washing the worms were fixed in 10% buffered formalin and preserved in 70% alcohol until further processing. They were dehydrated in a graded series of ethanol-amylacetate mixtures to pure amylacetate. Thereafter, the specimens were critical-point dried using liquid carbon dioxide, metal coated with gold palladium, and finally observed in a stereoscan Phillips 500 at 25 to 6000 x magnification at electron accelerating voltages ranging from 15 to 20 kv.

Observations and discussions

Scanning electron microscopic studies on *L. indicus* and *D. penetrans* reveal a similarity in the surface fine topography of the two species. In both, the body is divided into a body proper, a short neck and a distinct scolex. The tegument exhibits deep wrinkles and folds in the pre- and post neck region of both the species. (Pl. 2.1; Fig.1 and Pl. 2.3; Fig.1). A zone of finely wrinkled tegument which delimits the body of the worm from the scolex is distinguishable as the neck. However, the scolex in the two species

PLATE 2.1 Scanning electron micrographs of *Lytocestus indicus*

- Fig.1** Entire worm (scale bar = 1 mm);
- Fig.2** Scolex end; the smooth neck region is distinct from the finely wrinkled tegument of the distal end of the scolex (scale bar = 0.5 mm);
- Fig.3** Scolex tip showing deep wrinkles (scale bar = 30 u);
- Fig.4** The same under higher resolution revealing the cover of microtriches (scale bar = 30 u).

PLATE 2.1

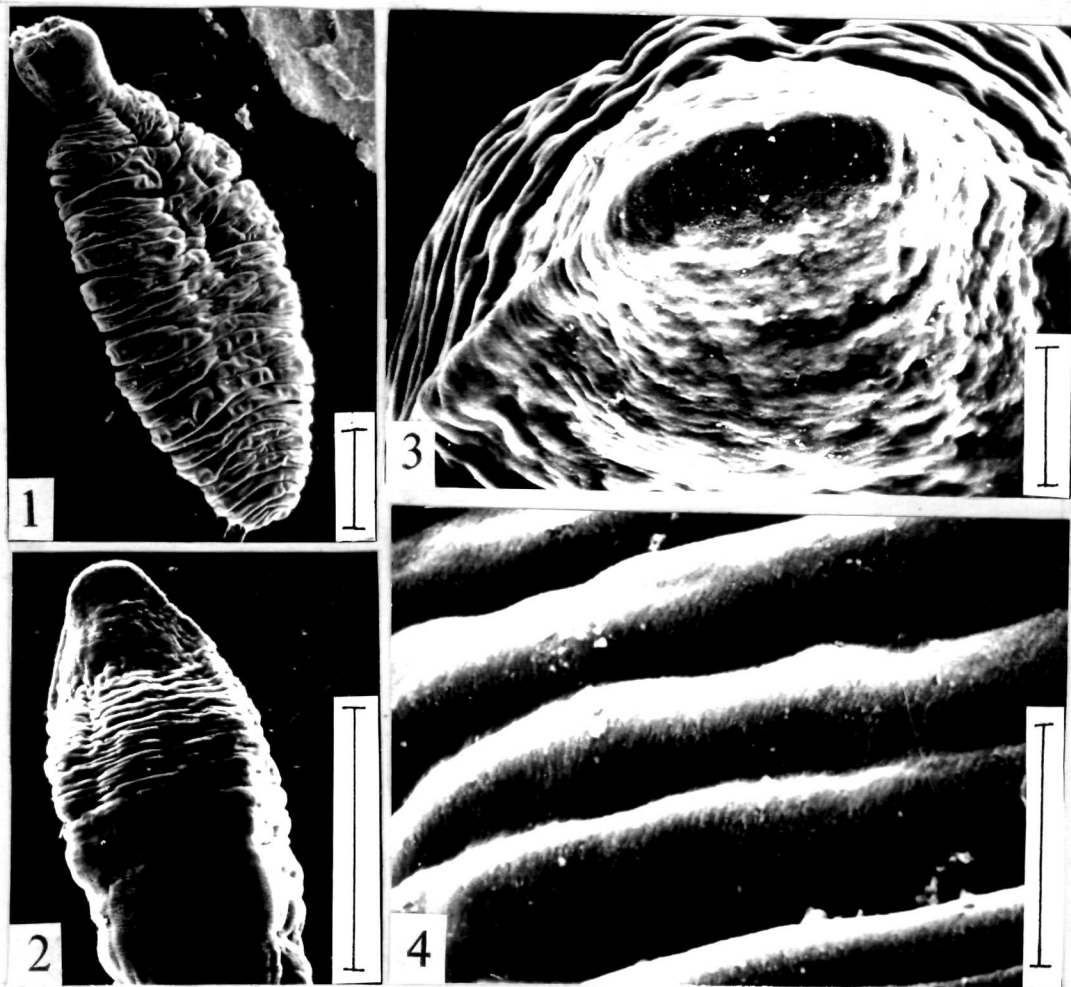


PLATE 2.2 Scanning electron micrographs of *Lytocestus indicus*

- Fig.1** Microtriches on the general tegument of the body (scale bar = 30 u);
- Fig.2** Tegument of the neck region; coarsely distributed pits occur in this (arrows) zone (scale bar = 30 u);
- Fig.3** Tegument in the post neck region. Note the conspicuous pits (arrows) and crackles (scale bar = 0.5 mm);
- Fig.4** A closer view of the surface pit (scale bar = 30 u).

PLATE 2.2

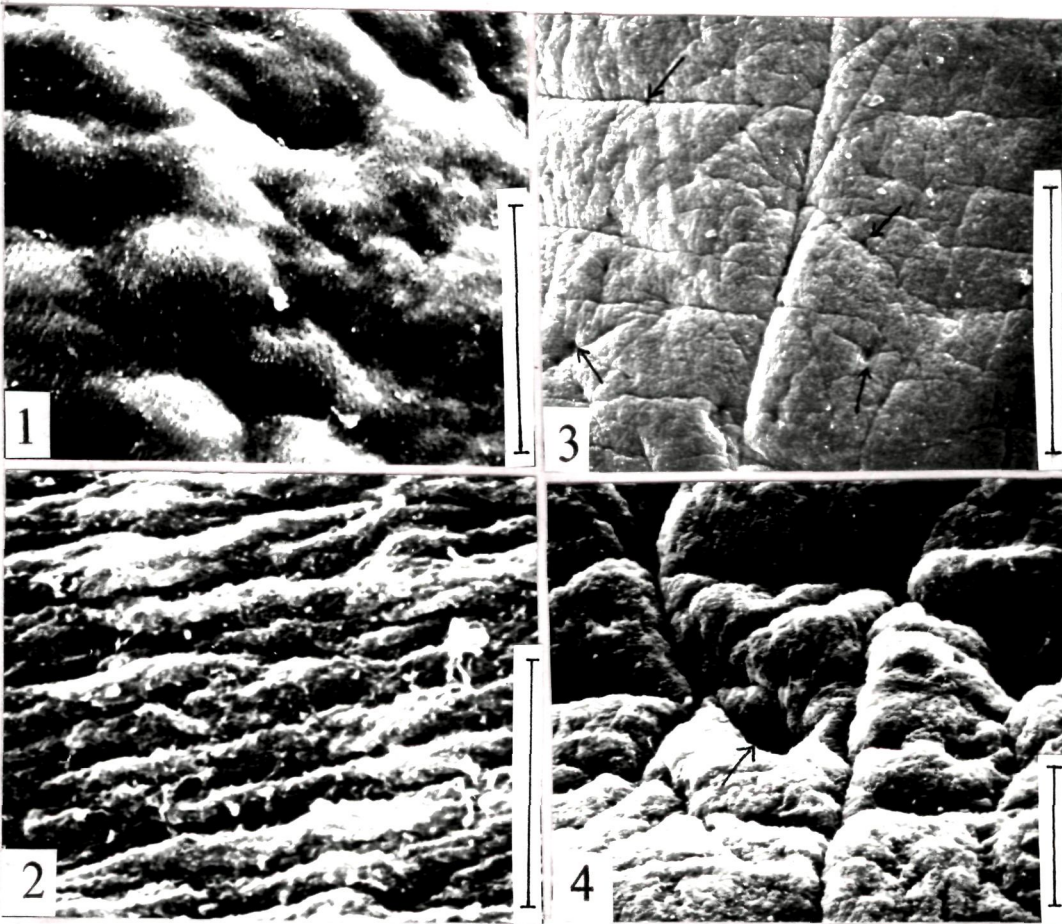
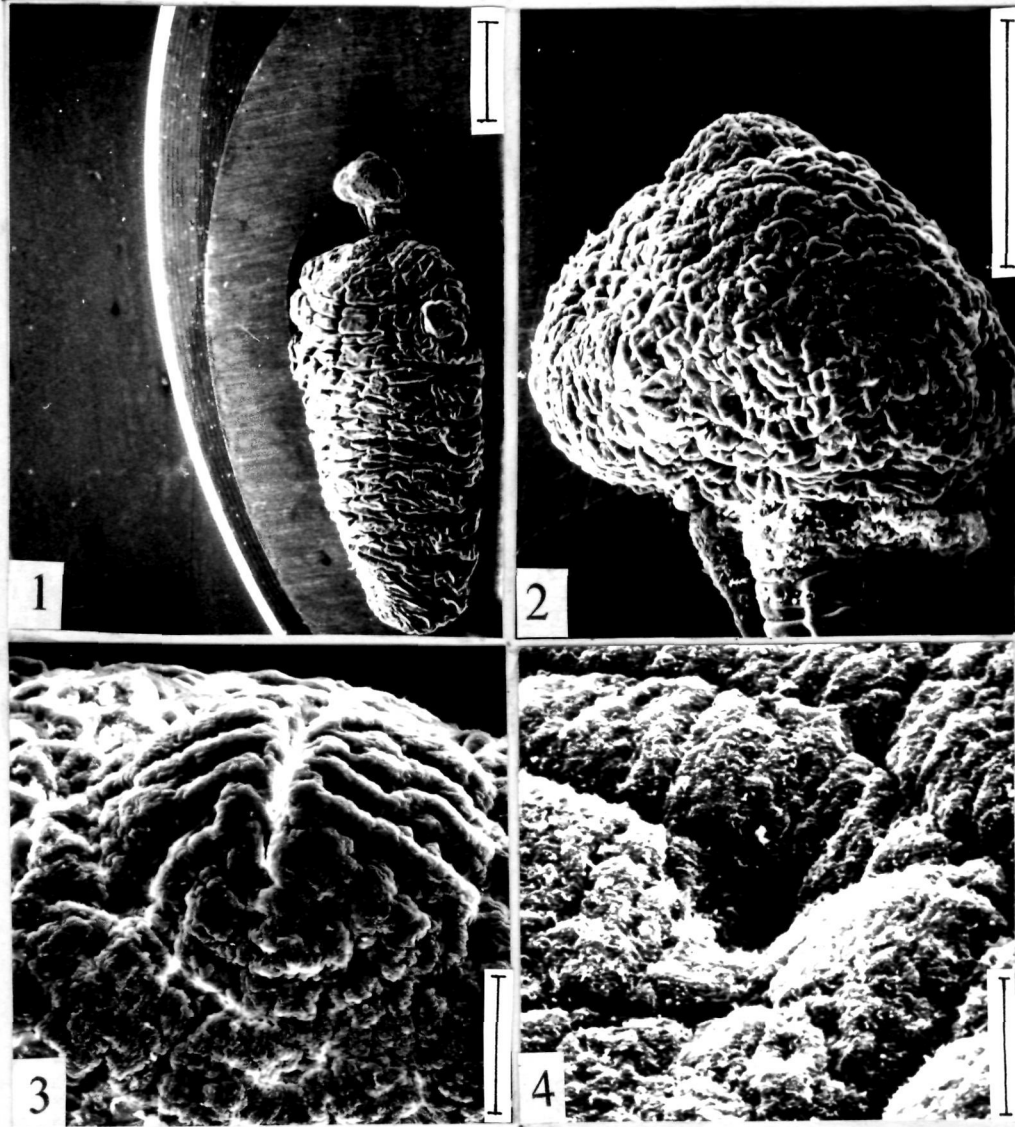


PLATE 2.3 Scanning electron micrographs of *Djombangia penetrans*

- Fig.1** Entire worm (scale bar = 1 mm);
- Fig.2** Scolex end showing deep folds and wrinkles (scale bar = 0.5 mm);
- Fig.3** Scolex tip under higher resolution revealing the deep wrinkles covered with microtriches (scale bar = 30 u);
- Fig.4** Genital pore (arrows) and the general tegument showing microtriches (scale bar = 30 u).

PLATE 2.3



is without any introvert, unlike in pseudophyllidean and cyclophyllidean cestodes, (Pl. 2.1; Fig. 2 and Pl. 2.3; Fig. 2). While, similar type of folds and wrinkles were observed in the tegument of the body proper of the two species, differences were distinct in the surface topography of their scolex. Thus, in *L. indicus*, only fine wrinkles were observed in the scolex surface (Pl. 2.1; Fig.3) but in *D. penetrans*, the scolex which is provided with a sucker at the tip showed intricate pattern of folds and wrinkles (Pl. 2.3; Fig. 2).

In both the cases, the whole body surface of the worm was revealed as carpeted with fine microtriches under higher resolution (Pl. 2.1; Fig. 4 and Pl. 2.3; Fig.3). These microtriches appeared to be uniform throughout the body without showing any regional differentiation in their morphology (Pl. 2.2; Fig. 1 and Pl. 2.3; Fig. 4). However, in other caryophyllidean cestodes, viz., *Hunterella nodulosa* and *Caryophyllaeus laticeps* as observed by Hayunga and Mackiewicz (1975), Hayunga (1979) and Richards and Arme (1981a,b and 1982a,b), the microtriches show distinct regional differentiation along the length of the worm. Three types of microtriches have been described from the tegument of *Hunterella nodulosa*: typical ones with well developed spines, another type with short filaments and the third type with no spines. Microtriches with spines are found only on the anterior part of the worm; those on the posterior part are

aspinose and between these two regions, in the transitional zone all the three types of microtriches abound. In *Caryophyllaeus laticeps*, the scolex-neck syncytium differs from that of the mid- and posterior body surface in the morphology of the microtriches.

The surface topography of pseudophyllidean and cyclophyllidean cestodes also reveals a variety of microthrix form. Along the length of *Hymenolepis* spp. and on the surface of adult *Taenia hydatigena*, polymorphism of microtriches was observed by Berger and Mettrick (1971) and Featherstone (1975). While in the larvae of *Diphyllobothrium ditremum* and *D. dendriticum* regional differentiation of microthrix occurs, the same is not apparent in the adult worms (Andersen, 1975).

Throughout the extent of the body in *L. indicus*, and *D. penetrans*, the tegument did not reveal any papillate or dome-shaped structures. However, in *L. indicus*, the tegument was interrupted by irregularly scattered pits, (Pl.2.2; Fig.2, Pl. 2.2; Fig. 3 and Pl. 2.2; Fig.4) which were absent in *D. penetrans*. These pits show a resemblance to the openings of the pore canals of some diphyllobothriid cestodes (Yamane et al. 1975). However, the functional significance of these pits in *L. indicus* could only be elucidated after the ultrastructural studies of the tegument in this species or other caryophyllids have been carried out.

The dense distribution of microtriches all over the general tegument in *L. indicus* and *D. penetrans* is also suggestive of the well accepted functional attributes of microtriches, i.e., absorption, secretion and anchorage to the hosts tissue, for other groups of cestodes (Lumsden, 1966; Morseth, 1966; Lee, 1972; Smyth, 1969 and Smyth, 1972).

Chapter III HISTOCHEMISTRY

INTRODUCTION

The studies carried out so far on cestode physiology mainly pertain to cyclophyllidean and pseudophyllidean groups and are based on biochemical observations (Smith, 1969). A look into the literature shows that relatively few histochemical studies have been made, still lesser concerning the adult worms.

In cestodes histochemical studies reveal that glycogen is mainly present in the parenchyma and muscles and is absent in the reproductive, nervous and excretory systems (Hedrick and Daugherty, 1957; Kilejian et al., 1961). In *Hymenolepis microstoma* and *H. nana*, a strongly positive reaction was observed in the cuticle of gravid segments of the worm (Burton and Bogitsh, 1963). Acid mucopolysaccharides were also reported to be present in the outer edge of the cuticle, but no metachromasia was seen. However, presence of red metachromasia was seen by Chowdhury et al. (1962) in *Taenia saginata*.

The distribution of glycogen in the cestodes, namely, *Anoplocephala plicata*, *A. perfoliata*, *A. mamillana*, *Calliobothrium coronatum*, *Caryophyllaeus mutabilis* and *Moniezia expansa* was studied by various workers like Brault and Leoper (1904a, b); Busch (1905a,b); Ortner-Schönbach (1913); von Brandt (1933); Wardle (1937); Smyth (1947,1949) and Yamao

(1952a,b) who reported the presence of traces of glycogen in the subcuticle and rich deposits in the parenchymatous tissue. Moczon (1975) found the highest content of glycogen in the testes and vitellaria in addition to parenchyma in *H. diminuta*. Baugh and Singh (1979) also observed the occurrence of this metabolite in various components of the reproductive system in *Raillietina* species. According to Gupta and Kapoor (1979) the cuticle, sub-cuticle, cortical parenchyma, and longitudinal and transverse muscles show the presence of 1:2 glycol groups, acid-mucopolysaccharides and traces of -metachromasia. All the structures contained glycogen excepting the cuticle but maximum concentration of it in the form of droplets was found in the parenchyma.

With regard to caryophyllideans, the histochemical studies done in the past relate mainly to glycogen and to some extent to lipid distribution in the worm. Ortner-Schonbach (1913) first reported the presence of glycogen in the "Faserzellenstränge" of *Caryophyllaeus laticeps*. Later, Mackiewicz (1968b) confirmed these observations in *C. laticeps* and also in *C. fennica*. However, Ginetsinskaya and Upenskaya (1965) reported in *C. laticeps* the maximum concentration of glycogen as occurring in the medullary parenchyma of the posterior part of the body surrounding the sex glands and that the latter were free of glycogen.

Studies by Mackiewicz (1968b) in vitellogenesis of *C. laticeps* and *C. fennica* showed that while the contents of the nuclear vacuole of the vitelline cells were mainly glycogen, the nucleus had decreased DNA content and the cytoplasm, increased RNA. Swiderski and Mackiewicz (1976) with their electron microscopic studies on the vitellogenesis of the caryophyllid *Glaridacris catastomi*, confirmed the occurrence of a single large non-membrane bound glycogen vacuole in the nuclei of mature vitelline cells and supported Mackiewicz's (1968b) conjecture that this glycogen vacuole could be a functionally significant food reserve in the egg.

Such nuclear vacuoles are a characteristic feature of the caryophyllidean cestodes (Mackiewicz, 1968b), and are not found among any other cestode groups. Thus, studies of Swiderski et al. (1978) confirm the lack of glycogen vacuole in the proteocephalan cestode, *Proteocephallus longicollis*. It was also found lacking in the cyclophyllidean cestodes like *Catenotaenia pusilla*, *Inermicapsifer madagascariensis* and *Hymenolepis diminuta* by Swiderski et al. (1970), and also in the tetraphyllid, *Echeneibothrium beauchampi* by Mokhtar-Maamouri and Swiderski (1976). Similarly, studies of earlier workers like Schauinsland (1885) Wisniewski (1932) and those of Smyth (1956) and Swiderski and Mokhtar (1974) revealed that pseudophyllideans are also devoid of such

glycogen vacuoles. The same also holds true for other platyhelminths like trematodes (Halton et al., 1974) or free living turbellaria (Domenici and Gremigni, 1974).

Protein histochemistry was studied in *Hymenolepis teniaeformis* and *Dipylidium caninum* by Waitz et al. (1964). Their observations revealed that the greatest concentration of the sulphhydryl groups was found in the cuticular and sub-cuticular regions. Christina James (1968) found the disulphide groups to be absent in the sub-cuticle of *Diphyllobothrium dendriticum*. However, Gupta and Kapoor (1979) observed that the cuticle, sub-cuticular cells, longitudinal and transverse muscles of *Cotugnia digonopora* were rich in proteins with the end groups like tyrosine and arginine; the parenchyma of this species was found to contain droplets of these proteins scattered here and there, while the dorsoventral muscles contained only their traces. The results obtained were in support of the earlier works of Monne (1959) and Chowdhury et al. (1962) on *H. teniaeformis* and *T. saginata*, respectively. The cuticle and sub-cuticle of *H. microstoma* was also reported to contain arginine and tyrosine.

Muthukrishnan (1974) observed that histochemically, the tegument of adult *T. hydatigena* comprised two layers, i.e., the outer "epicuticle" and the inner "maincuticle" which differed from each other in their chemical composition;

the "epicuticle" contained non-sulfated acid mucopolysaccharides of carboxyl type in combination with proteins. Thereafter, while studying the nature of protein components in the tegument of gravid proglottids in *T. hydatigena*, Muthukrishnan (1975a,b,c) observed the transformation of -SH groups into -S-S bonding and also reported the "main-cuticle" to be feebly PAS positive, compared to the other regions of the body.

Detailed histochemical studies of caryophyllids have not been carried out so far, but enzyme histochemistry has drawn relatively better attention in respect of this group. Non-specific phosphomonoesterases were studied in three species of caryophyllids, viz., *Lytocestus indicus*, *Introvertus raipurensis* and *Lucknowia indica* from *Clarias batrachus* by Gaur and Agarwal (1981) and also by Rasheed Unnisa and Simha (1982) in *L. indicus*. The studies revealed that the highest alkaline phosphatase activity was recorded in *I. raipurensis*. The cuticle of *L. indicus* was found to be slightly positive for acidic, and strongly positive for basic, phosphomonoesterase activity. Both acid and alkaline phosphatase activities were detected in *L. indica*. Further, studies of Rasheed Unnisa and Simha (1982) suggested that the tegument acts as a functionally digestive or absorptive surface, since non-specific phosphomonoesterase aids in hydrolysing the contents of the host's duodenum before absorption.

Lipid distribution in cyclophyllidean cestodes such as *Moniezia expansa*, *M. denliculata*, *T. saginata*, *T. marginata*, *Dipylidium caninum*, *Raillietina cesticillus*, *Hymenolepis microstoma* and *H. diminuta* was studied by earlier workers like Schiefferdecker (1874), Brault and Leoper (1904a,b), Pinter (1922), Arndt (1922), Coutelen (1931), von Brand (1933), Smyth (1947, 1949) and Hedrick (1958), respectively. While Parshad et al. (1981) and Vykhostyuk et al. (1981) studied the lipid distribution in the *Raillietina* spp., the same was studied in *Cotugnia digonopora* by Gupta and Kapoor (1979).

Studies on nucleic acids by Christina James (1968) showed that a cap of RNA activity is located in some of the nuclei of *Diphyllobothrium dendriticum*, although general parenchymatous strands, cuticle, basement membrane and calcareous corpuscles were found to be negative. DNA, however, was found in all the nuclei. In *H. microstoma*, abundant RNA was found to be located in sub-cuticular and parenchymal region of neck and immature proglottids by Burton and Bogitsh (1963) and the presence of only a small amount of basic protein was found in the egg-shell globules, vitellaria and yolk masses, and cytoplasm within the oocytes. Observations of Gupta and Kapoor (1979) on *Cotugnia digonopora* revealed the presence of DNA in the nuclei of subcuticular

and parenchymatous cells and also in the transverse and dorso-ventral muscles.

In view of the fore-going, further investigation into the caryophyllidean group seems desirable, as the information received would be an asset in obtaining an integrated picture of cestode physiology.

Material and Methods

Of the various types of caryophyllidean cestodes recovered from the host, *Clarias batrachus*, histochemical studies have been carried out in the two most commonly occurring and apparently pathogenic species, viz., *Lytocestus indicus* and *Djombangia penetrans*. For localization of carbohydrates, proteins, lipids and nucleic acids in the various tissues and organs of the parasite, specific fixatives were used and specific histochemical tests performed (after Pearse, 1968) as mentioned in Table X - XIII. All the paraffin sections were cut at 6-7 μ thickness.

Results and Discussion

Based on the specific histochemical tests the various metabolites could be demonstrated (Pls. 3.1 - 3.7). Their distribution in respect of the various parts of the body is present in the Tables X - XIII.

TABLE X: *Lytocestus indicus*: Summary of histochemical tests performed and their results in the various parts of the body.

Metabolites	Reaction	Author	Fixative	Tegument	Scolex	Frontal glands	Neck	Parenchyma	Subtegumental layer	Longitudinal muscles	Ovary	Mehlis' gland	Uterus	Uterine glands	Testes	Vitel-laria	Eggs	Vitel-line cells
Carbohydrates	PAS	Mc Manus 1946	Formol alcohol	+++	++	-	++	+++	+++	+	-	-	++	-	-	-	++	+++
Glycogen	Best's carmine method	Best, 1905	"	-	++	-	++	+++	+	-	-	-	++	-	-	-	++	+++
Mucosubstances	Mucicarmine	Mayer 1896; modified by Southgate, 1927	Bouin's fluid	++	-	-	-	+	+	+	-	-	+	-	-	-	-	-
Acid mucosubstances	Alcian Blue method	Scott and Dorling 1965	"	+++	+	+	+	+	-	-	+	+	+	+	+	+	++	+
"	Azur 'A'	Hughesdon 1949	"	+++	+	+	+	+	-	-	+	+	+	+	+	+	++	+
"	Toluidine Blue	Kramer & Windrum 1955	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins	Mercuric Bromophenol Blue method	Mazia et al. 1953	10% buffered formalin	+++	++	+++	+	+	+++	+++	++	+++	+++	+++	+++	+++	+	+
Disulphides	Perfoimic acid Alcian Blue method	Adams & Sloper, 1955-1956	"	++	+	+	+	+	-	-	+	+	+	+	+	+	++	+

+ = Weakly Positive Reaction; ++ = Strongly Positive; +++ = Very Strongly Positive; - = Negative.

TABLE XI: *Lytocestus indicus* —*Summary of histochemical tests performed and their results in the various parts of the body.*

Metabolites	Reaction	Author	Fixative	Tegument	Scolex	Frontal glands	Neck	Parenchyma	Subtegumental layer	Longitudinal muscles	Ovary	Mehlis' gland	Uterus	Uterine glands	Testes	Vitel-laria	Eggs	Vitel-line cells
Arginine	Sakaguchi method (1925)	Baker, 1947	10% Neutral buffered formalin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tyrosine	Millon's (1849)	Baker, 1956	"	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Histones	Alkaline Fast Green	Alfert & Geschwind (1953)	"	+++	+	+++	+	+	+++	+++	+++	+++	+++	+++	+++	+++	++	+++
Lipids (general)	Sudan Black B	Lison & Dagnelie, 1935	Formol Calcium	+++	++	+++	+	+	++	++	+++	+++	+++	+++	+++	+++	+	+
Unsaturated lipids	Performic Acid-Schiff's	Lillie, 1951	"	-	-	-	-	-	-	-	+++	-	-	-	+++	+++	-	-
Glycolipids	PAS	Mc Manus 1946	"	+++	+	-	+	+	-	-	-	-	-	-	-	+	++	++
DNA	Feulgen nuclear	Feulgen & Rossenback 1924	Carnoy's	+	+	+	+	+	+	+	+++	+	+	+	+++	+++	+	+
RNA	Methyl Green Pyronin Y	Modified by Kurnick 1955	"	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Haemoglobin	Benzidine Method	Pickworth 1934-'35	10% Neutral Formalin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = Weakly Positive Reaction; ++ = Strongly Positive; +++ = Very Strongly Positive; - = Negative.

TABLE XII: Djombangia penetrans

Summary of histochemical tests performed and their results in the various parts of the body.

Metabolites	Reaction	Author	Fixative	Tegument	Scolex	Sucker	Frontal Neck glands	Parenchyma	Subtegumental layer	Longitudinal muscles	Ovary	Mehlis' gland	Uterus	Testes	Vitel-laria	Eggs	Vitel-line cells
Carbohydrates	PAS	Mc Manus 1946	Formol Alcohol	+++	++	++	-	+++	++	+	-	-	++	-	-	+++	+++
Glycogen	Bests' carmine method	Best, 1905	"	-	++	++	-	+++	+	-	-	-	+	-	-	+	+
Mucosubstances	Mucicarmine	Mayer 1896; Bouin's modified by Southgate, 1927	Bouin's fluid	+++	-	-	-	+	+	+	-	-	+	-	-	+	-
Acid mucosubstances	Alcian Blue method	Scott and Dorling 1965	"	+++	+	+	+	++	-	-	+	+	+	+	+	+++	+
"	Azur 'A'	Hughesdon 1949	"	++	+	+	-	++	-	-	+	+	+	+	+	+	+
"	Toluidine Blue	Kramer & Windrum 1955	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins	Mercuric Bromophenol Blue method	Mazia et al. 1953	10% buffered formalin	Neutral+++	++	++	+++	++	++	+++	+++	+++	++	++	++	+	++
Disulphides	Performic acid Alcian Blue Method	Adams & Sloper, 1955-'56	"	+++	+	+	+	+	-	-	+	+	+	+	+	+	+

+ = Weakly Positive Reaction; ++ = Strongly Positive; +++ = Very Strongly Positive; - = Negative.

TABLE XIII: Djombangia penetrans —

Summary of histochemical tests performed and their results in the various parts of the body.

Metabolites	Reaction	Author	Fixative	Tegu- ment	Scolex	Sucker	Frontal glands	Paren- chyma	Subtegu- mental layer	Longitu- dinal muscles	Ovary gland	Mehlis' Uterus	Testes	Vitel- laria	Eggs	Vitel- line cells
Arginine	Sakaguchi method (1925)	Baker, 1947	10% Neutral buffered formalin	-	-	-	-	-	-	-	-	-	-	-	-	-
Tyrosine	Millon's (1849)	Baker, 1956	"	+	+	+	+	+	+	++	+	+	++	++	-	-
Histones	Alkaline Fast Green	Alfert & Geschwind (1953)	"	+++	++	+++	+++	+	+++	+++	+++	++	+++	+++	+++	+++
Lipids (general)	Sudan Black B	Lison & Dagnelie, 1935	Formol Calcium	+++	+	++	+	+	++	+	+++	+++	+++	+++	+++	++
Unsaturated lipids	Performic Acid- Schiff's	Lillie, 1951	"	-	-	-	-	-	-	-	++	-	++	++	-	-
Glycolipids	PAS	Mc Manus 1946	"	+++	+	+	-	+	-	-	-	-	-	-	++	++
DNA	Feulgen nuclear	Feulgen & Rossenback 1924	Carnoy's	+	+	+	+	+	+	+	++	+	+++	+++	+++	++
RNA	Methyl Green Pyronin Y	Modified by Kurnick 1955	"	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Weakly Positive Reaction; ++ = Strongly Positive; +++ = Very Strongly Positive; - = Negative.

PLATE 3.1 *Lytocestus indicus* - Localisation of carbohydrates
(Photomicrographs of longitudinal sections)

- Fig.1** Scolex showing positive reaction for PAS (scale bar = 0.15 mm);
- Fig.2** Tegument showing strong intensity for PAS (scale bar = 0.15 mm);
- Fig.3** Vitelline lobes showing glycogen masses (arrows). Best carmine (scale bar = 0.15 mm);
- Fig.4** Glycogen masses in vitelline lobes under higher resolution (scale bar = 0.02 mm);
- Fig.5** Strong reaction for glycogen in the vitelline cells within the egg. The area appearing dark in the photomicrograph stained deep red and the cell nuclei did not pick up haematoxylin stain (scale bar = 0.02 mm);
- Fig.6** Tegument showing positive reaction for acid mucopolysaccharides. Alcian Blue (scale bar = 0.15 mm).

PLATE 3.1

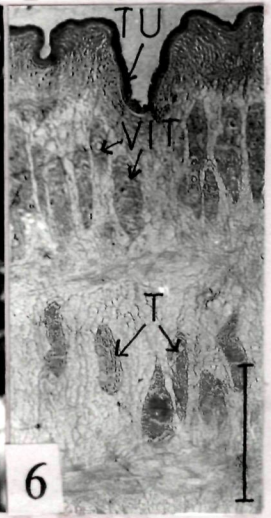
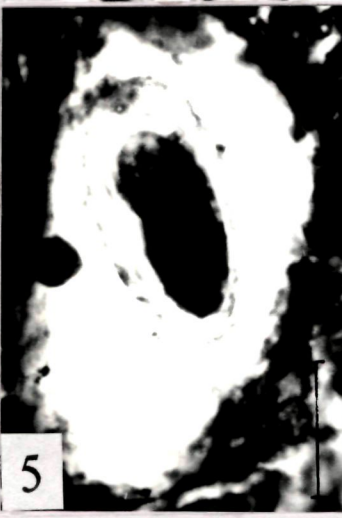
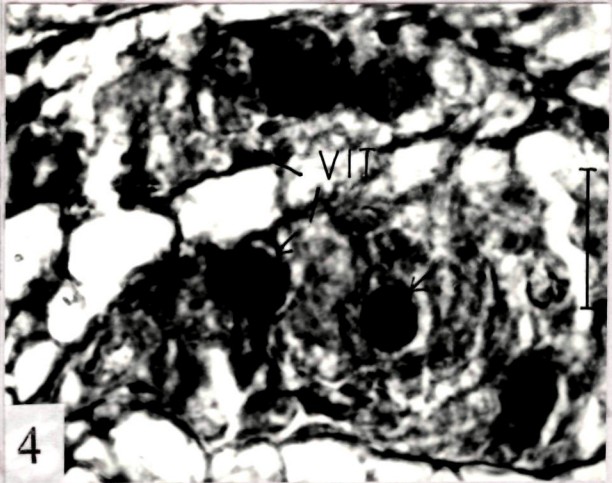
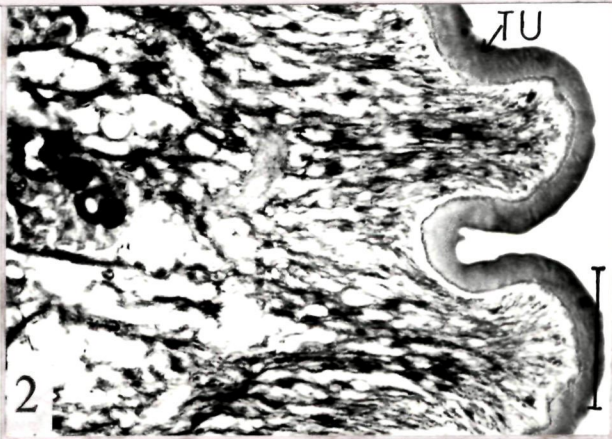
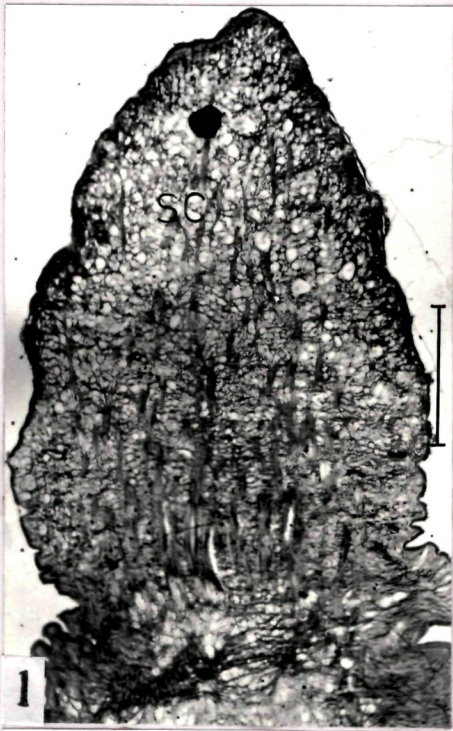


PLATE 3.2 *Lytocestus indicus* - Localisation of lipids
(Photomicrographs of longitudinal sections)

- Fig.1** General parenchyma of the scolex region showing sudanophilic lipids (scale bar = 0.15 mm);
- Fig.2** Vitelline cells within the eggs showing positive reaction (arrows). Sudan Black B (scale bar = 0.02 mm);
- Fig.3** Subtegument, parenchymal longitudinal muscles, testes and vitellaria showing intense reaction. Sudan Black B (scale bar = 0.15 mm);
- Fig.4** Testes and vitellaria showing strongly positive reaction for unsaturated lipids. Performic Acid-Schiff's reaction (scale bar = 0.15 mm).

PLATE 3.2

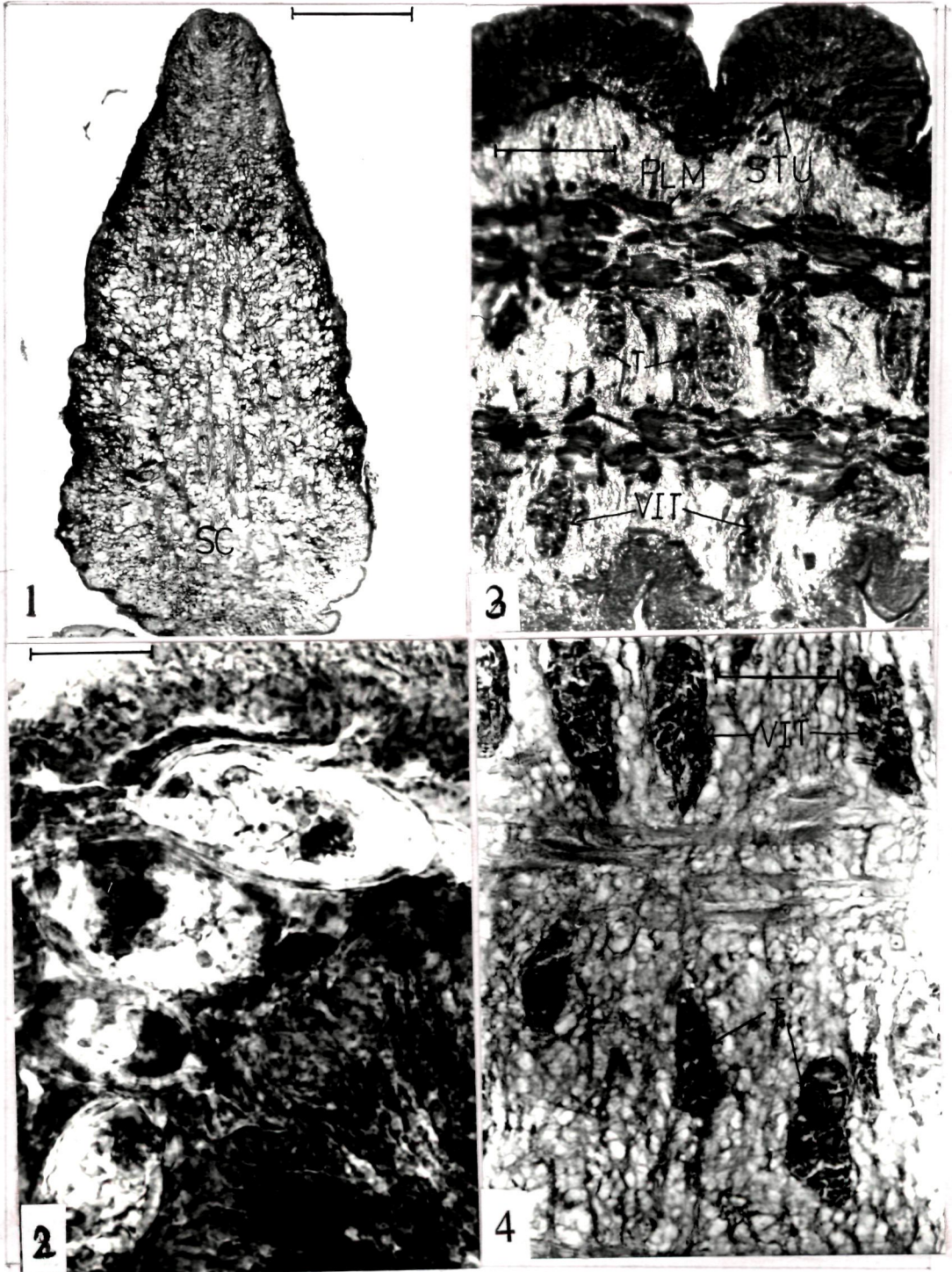


PLATE 3.3 *Lytocestus indicus* - Localisation of proteins
(Photomicrographs of longitudinal sections)

- Fig.1** Frontal gland cells (arrows) in the scolex region showing proteinaceous contents. Bromophenol Blue (scale bar = 0.05 mm);
- Fig.2** The same at higher magnification (scale bar = 0.02 mm);
- Fig.3** Tegument, muscles, testes and vitellaria showing positive reaction. Bromophenol Blue (scale bar = 0.15 mm);
- Fig.4** A portion of the mid-body showing positive reaction for tyrosine, in testes, vitellaria and parenchymal longitudinal muscles. The latter stain intensely. Millon's reaction (scale bar = 0.15 mm);
- Fig.5** The posterior third of the body showing a positive reaction for disulphides in the reproductive components. The Mehlis gland, uterine glands and the eggs in the uterus exhibit deep staining. Performic acid Alcian Blue method (scale bar = 0.15 mm).
- Fig.6** Tegument, testes, vitellair and parenchymal longitudinal musculature, showing strong reaction for basic proteins. Alkaline Fast Green (scale bar = 0.15 mm).

PLATE 3.3

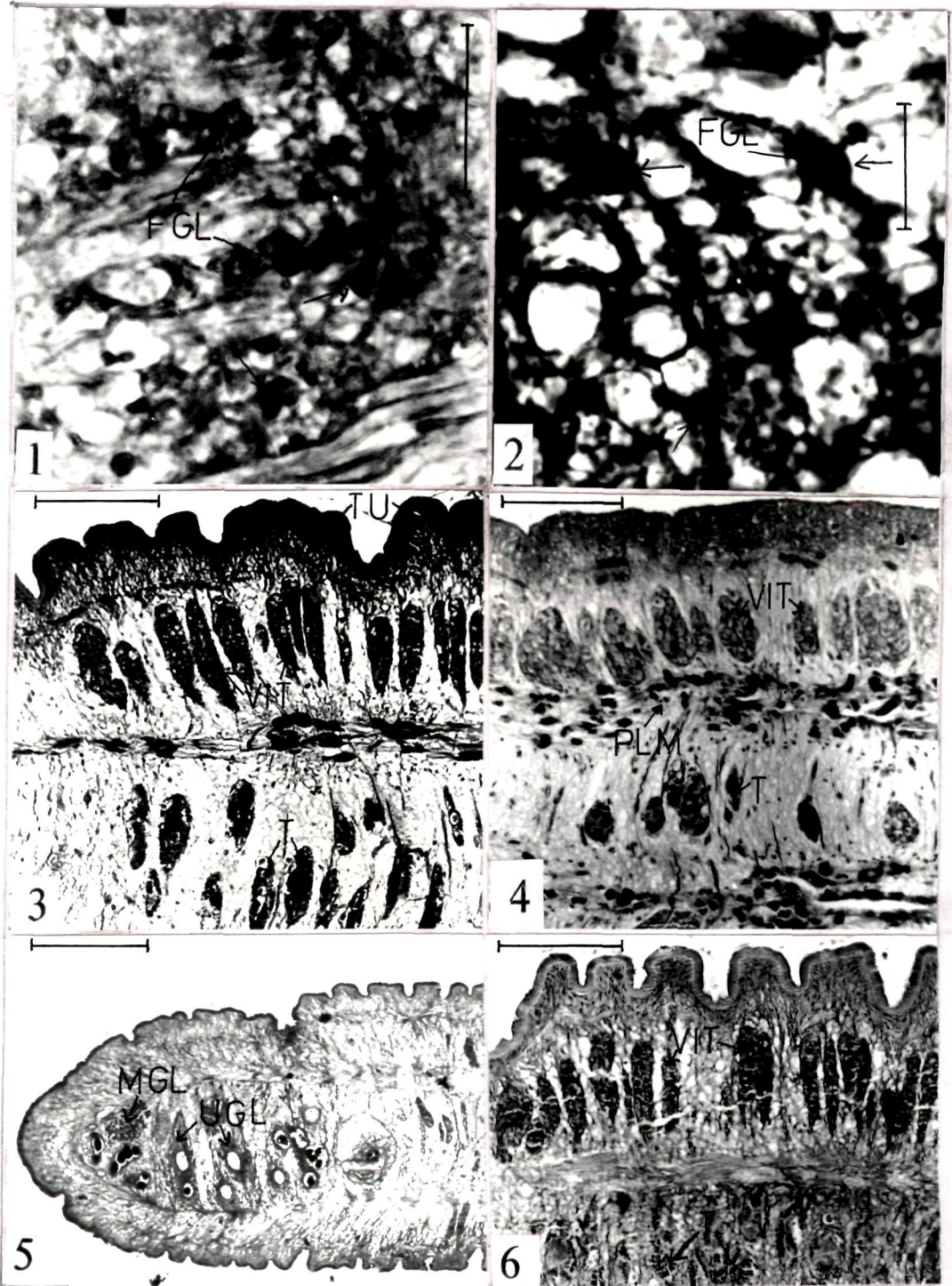


PLATE 3.4 *Djombangia penetrans* - Localisation of carbohydrates
(Photomicrographs of longitudinal sections)

- Fig.1** The sucker region in the scolex. PAS (scale bar = 0.05 mm);
- Fig.2** Tegument showing positive reaction for PAS (scale bar = 0.1 mm);
- Fig.3** General parenchyma showing high concentration of carbohydrates. PAS (scale bar = 0.15 mm);
- Fig.4** Rich deposits of glycogen in the parenchymatous tissue. Best carmine (scale bar = 0.15mm);
- Fig.5** Vitellaria showing glycogen rich nuclei (arrows). Best's carmine (scale bar = 0.15 mm);
- Fig.6** An enlarged view of a vitelline gland cell. The nucleus shows a positive reaction for glycogen (scale bar = 0.02 mm);
- Fig.7** Egg under oil immersion. While most of the dark area in the photomicrograph represents intense haematoxylin staining only a few spots (arrows) stain for glycogen (scale bar = 0.02 mm);
- Fig.8** Tegument and eggs showing positive reaction for acid mucopolysaccharides. Alcian Blue method (scale bar = 0.15 mm).

PLATE 3.4

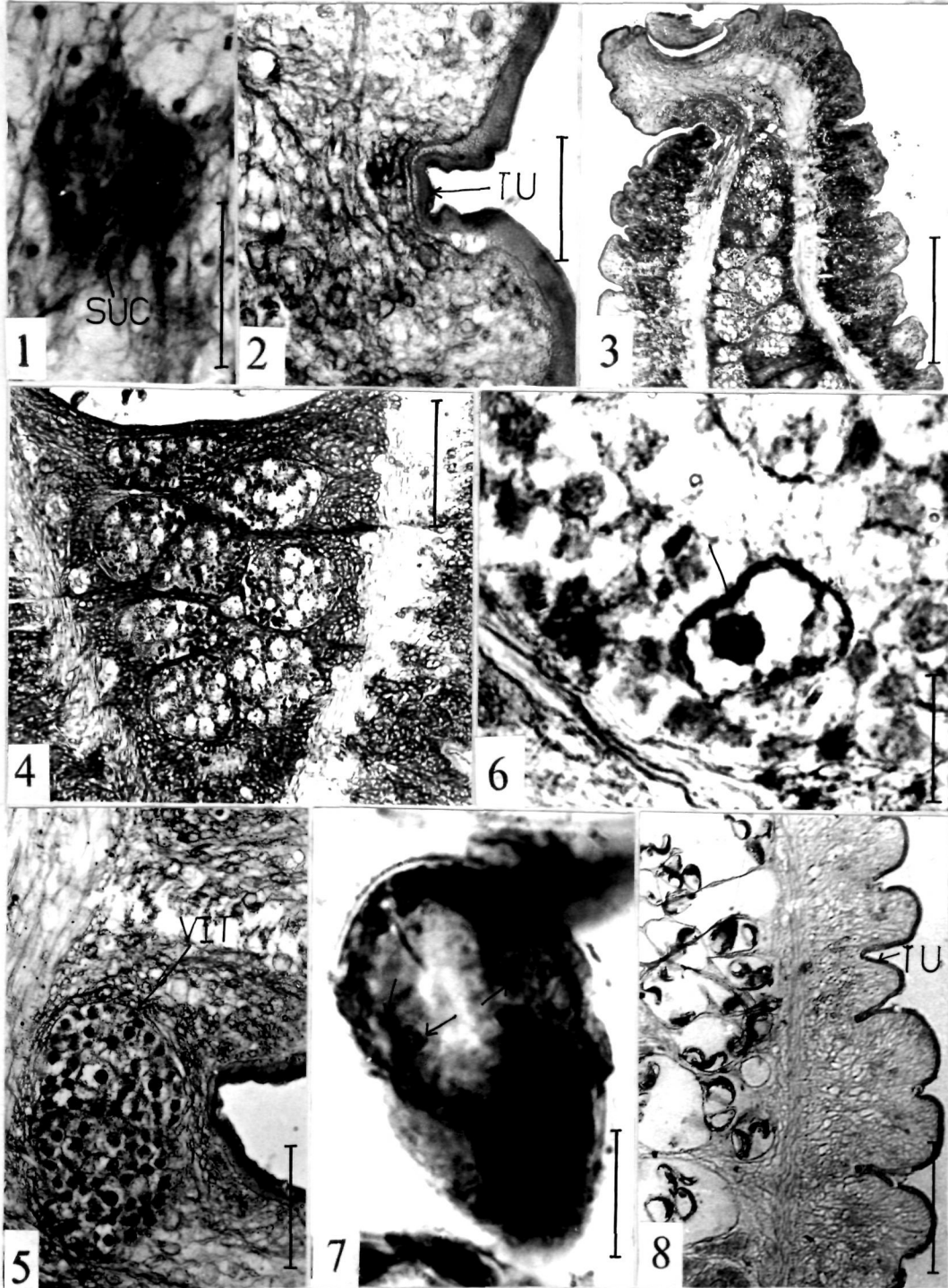


PLATE 3.5 *Djombangia penetrans* - Localisation of lipids
(Photomicrographs of longitudinal sections)

- Fig.1** Scolex region. The sucker at the tip of the scolex shows a strong reaction for Sudan Black B (scale bar = 0.1 mm);
- Fig.2** The lipid distribution in the various regions of the body. Tegument parenchymal musculature, ovary and eggs exhibit sudanophilic reaction (scale bar = 0.15 mm);
- Fig.3** Egg under oil immersion showing high concentration of lipid in the vitelline cells (scale bar = 0.02 mm);
- Fig.4** Testes and vitellaria showing a mild reaction for unsaturated lipids. Performic acid Schiff's reaction (scale bar = 0.15 mm).

PLATE 3.5

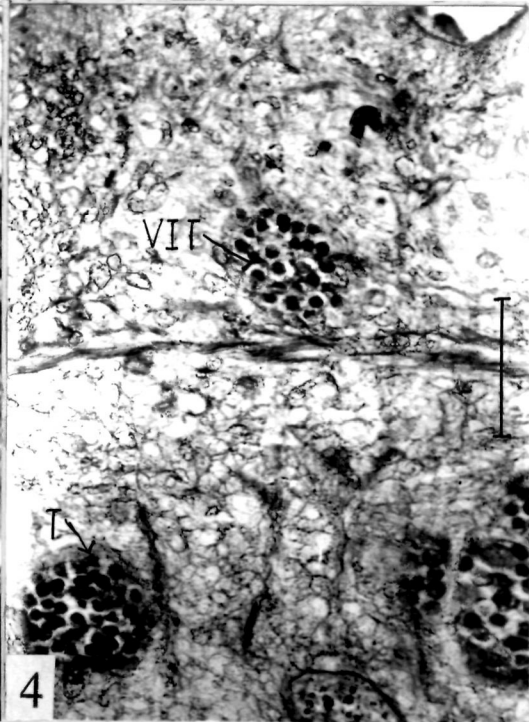
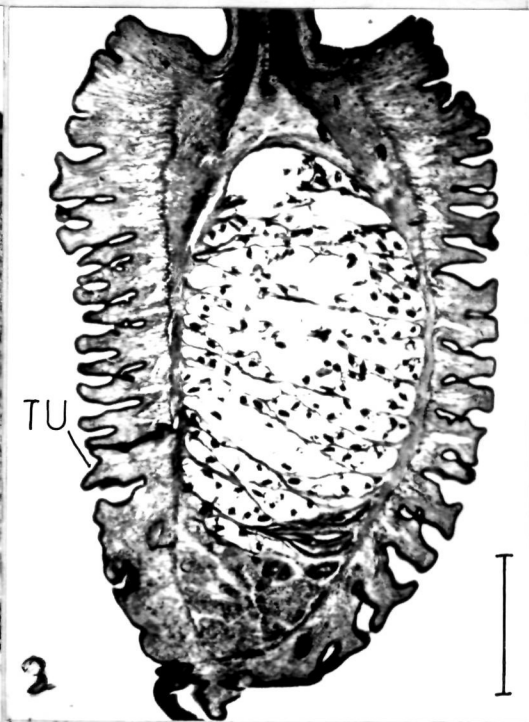
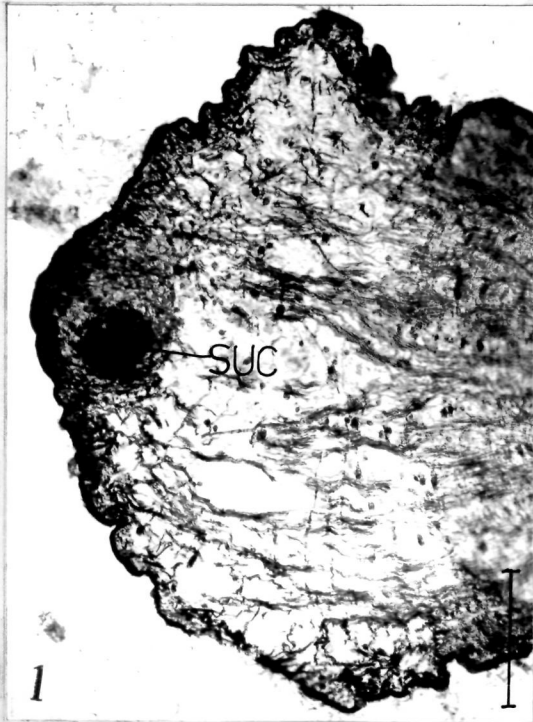


PLATE 3.6 *Djombangia penetrans* - Localisation of proteins
(Photomicrographs of longitudinal sections)

- Fig.1** . Scolex region with sucker at its tip. Bromophenol blue (scale bar = 0.15 mm);
- Fig.2** Frontal glands in the scolex showing intense reaction. Bromophenol Blue (scale bar = 0.02 mm);
- Fig.3** Tegument, testes, vitellaria and parenchymal longitudinal musculature showing intense staining. Bromophenol Blue (scale bar = 0.15 mm);
- Fig.4** Testes, vitellaria and eggs showing positive reaction for tyrosine Millon's reaction (scale bar = 0.15 mm);
- Fig.5** The same structures showing a positive reaction for disulphides. Performic acid Alcian Blue Method (scale bar = 0.15 mm);
- Fig.6** A portion of the posterior half of the body, tegument, parenchymal longitudinal musculature, ovary and eggs show a strong reaction for basic proteins. Alkaline Fast Green (scale bar = 0.15 mm).

PLATE 3.6

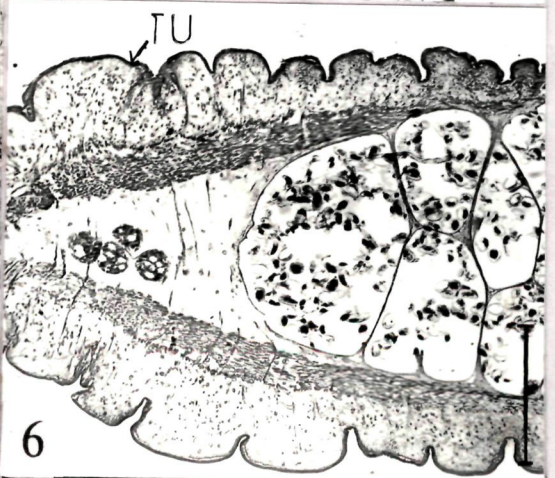
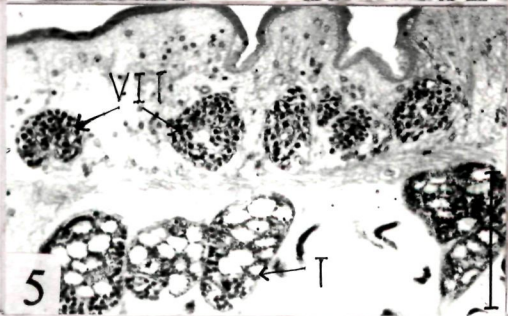
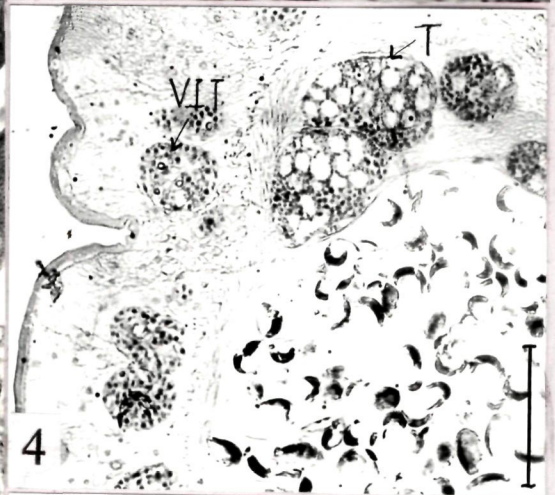
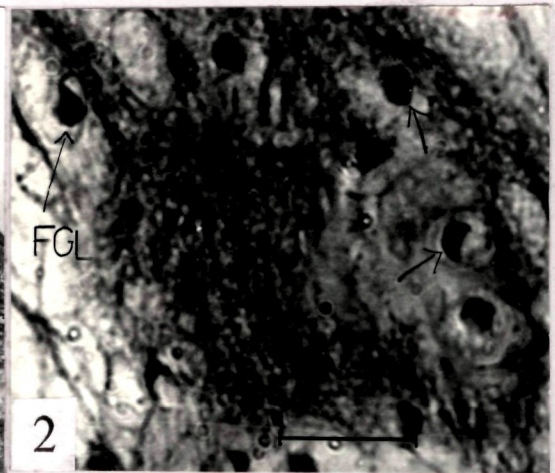
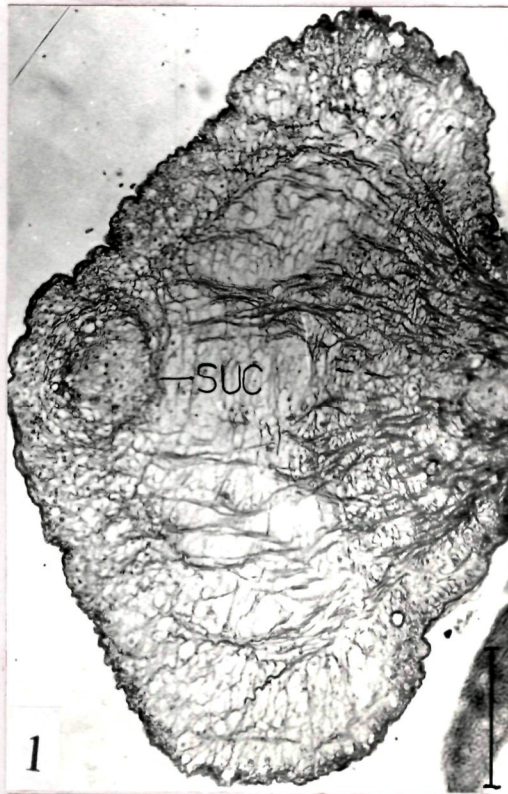


PLATE 3.7 Localization of nucleic acids

(Fig. 1 & 2 *Lytocestus indicus* - Photomicrographs of longitudinal sections)

Fig.1 Reproductive organs of *L. indicus* showing high concentration of DNA. Feulgen nucleal reaction (scale bar = 0.15 mm);

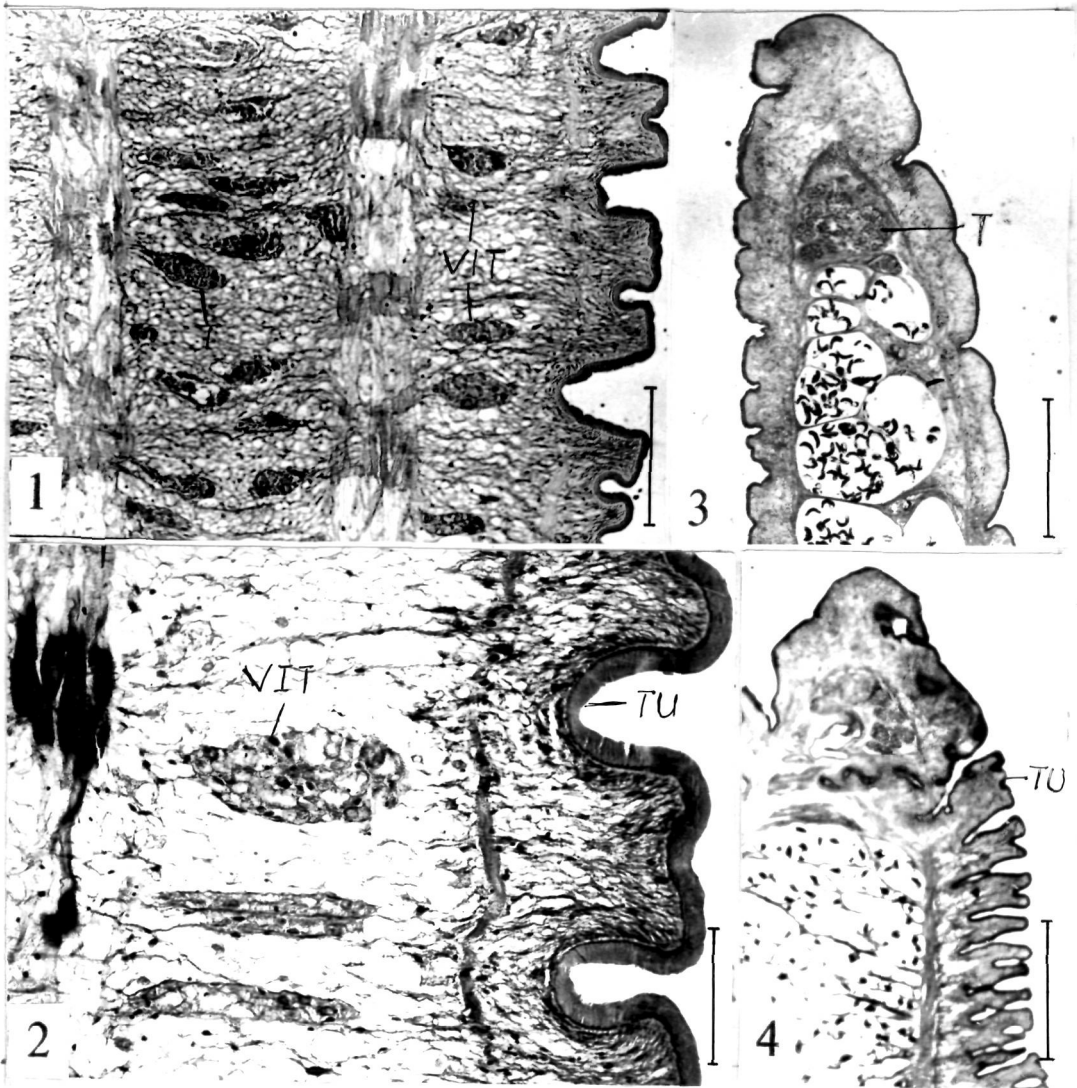
Fig.2 A portion of the body shows intense reaction for RNA. Methyl Green Pyronin Y (scale bar = 0.15 mm);

(Fig. 3 & 4 *Djombangia penetrans* - photomicrographs of longitudinal sections)

Fig.3 DNA in the various organs. Feulgen nucleal reaction (scale bar = 0.15 mm);

Fig.4 A portion of the body showing strong intensity for RNA. Methyl Green Pyronin Y (scale bar = 0.15 mm).

PLATE 3.7



These results indicate towards a similarity in the distribution of the different metabolites in the various organs of the two species.

The tegument scolex, neck, parenchyma, subtegmental muscle layer, vitelline cells of the egg, and uterus in both the species stained positively for PAS reaction, though the reaction was mild in the parenchymatous longitudinal muscles (Pl. 3.1, Fig. 1-2 and Pl. 3.4, Fig. 1-3). This observation of the occurrence of rich deposits of carbohydrates in the parenchymatous tissue, tegument and subtegmental layer of *L. indicus* and *D. penetrans* is in conformity with those of Gupta and Kapoor (1979) who also found the same tissues of *Cotugnia digonopora* to be rich in carbohydrates.

It is known that in the adult parasitic helminths carbohydrate is the major energy reserve and has manifold functions in the tissues, but carbohydrate levels in the worm may show alterations in accordance with the nutritional status or phase of the life cycle and among different cestode species. This metabolite is known to exhibit diurnal and annual cyclic changes as well. Yet carbohydrates are a major energy reserve, form important structural components and as phosphorylated intermediates, are most important for energy metabolism. Further, they are also important as constituents of nucleotides, glycolipids and glycoproteins

(Barrett, 1981). However, of the carbohydrates, the main reserve polysaccharide in helminths is glycogen (Barrett, 1981).

In *L. indicus* and *D. penetrans*, maximum concentration of glycogen was observed in the parenchyma. Presence of glycogen was also seen in the scolex and neck region, but it was absent in the tegument, sub-tegument, muscles and the gonads (Pl. 3.1, Fig. 3; Pl. 3.4, Fig. 4). This observation is in accordance with that of Ginetsinskaya and Upenskaya (1965) in *Caryophyllaeus laticeps*.

As a characteristic feature of caryophyllids (Mackiewicz, 1968b) a large glycogen vacuole in the nuclei of mature vitelline cells in the egg was observed in both the species; (Pl. 3.1, Fig. 4 and Pl. 3.4, Fig. 5) while it was more prominent and ubiquitously occurring in *L. indicus* eggs, not all the vitelline cells nuclei of the eggs in *D. penetrans* showed such a character. However, regarding the whole vitelline follicle, while in *L. indicus* glycogen was observed as conspicuous intercellular globular masses, 2-5 in number, in *D. penetrans* it appeared to be confined to the nuclei of only a few cells (Pl. 3.1, Fig. 5 and Pl. 3.4, Fig. 6-7).

Depending on the properties of glycogen, namely, its non-diffusible nature, low osmotic pressure and its

highly branched structure which provides large number of chain ends for enzymes to work on, glycogen as a reserve polysaccharide is advantageous (Barrett, 1981).

An enhancement of glycogen content without having any increase in egg size or in the number of vitelline cells per egg seems to have a selective value and adaptational significance (Mackiewicz, 1968b). Further, as hypothesized by Swiderski and Mackiewicz (1976), the partitioning of glycogen content into intranuclear and cytoplasmic compartments would lead to more efficient use of this energy reserve. Such a situation occurs among vertebrates like starving tadpoles where the nuclear glycogen is utilized after the depletion of the cytoplasmic glycogen (Himes and Pollister, 1962).

Besides the presence of glycogen, the eggs in *L. indicus* showed a mild occurrence of lipids whereas in *D. penetrans* eggs this metabolite was more prominently present (Pl.3.2; Fig.3 and Pl.3.5; Fig.3). Eggs containing both glycogen and lipid have been reported in *Diphylobothrium latum* and those containing only lipid, in other pseudophyllideans, like *Ligula columbi* and *Triaenophorus nodulosus*, by Ginetsinskaya et al. (1971). According to Calow and Jennings (1974) the lipids are a major energy source in free-living platyhelminths, whereas the enteric parasites have an energy

source rich in glycogen. While Jennings (1973) looked upon high glycogen as of adaptive value towards low or enhancing oxygen tensions prevailing in their habitat (i.e., gut), Jennings and Calow (1975) regarded this factor to be related to the high fecundity of the worm. However, in respect of caryophyllideans, they being monozoic cestodes, the requirement of large quantities of glycogen, instead of lipids, as an energy source seems to be related more to the biology of the egg and life cycle of the worm than to its fecundity. All the caryophyllids for which the complete life cycle has been worked out are known to utilize benthic intermediate hosts like tubificid annelids (see Mackiewicz, 1981a). Glycogen being heavier than lipid and also because it can be metabolized under anaerobic conditions, caryophyllidean eggs with more glycogen content seem to be better adapted to be approached by the intermediate hosts (Mackiewicz, 1981a).

The difference regarding the lipid and glycogen content in the eggs between *L. indicus* and *D. penetrans* can be explained on the basis of differences in their fecundity. The number of eggs in *D. penetrans* is much more compared to that in *L. indicus*. The uterine coils of *D. penetrans*, which run almost throughout the body, remain full of eggs, whereas in *L. indicus*, eggs whenever present are found in a limited number within the uterine coils that occupy only

a few millimeters of length in the posterior extent of the body. The fecundity rate seems much higher in *D. penetrans* than in *L. indicus* and since the number of eggs is more in *D. penetrans*, glycogen storage does not seem really essential for prolonging the period of infectivity as in other caryophyllid cestodes. However, the occurrence of intranuclear glycogen in a few vitelline cells in *D. penetrans* appears to be related to the low or variable oxygen tension in the gut of the host (Mackiewicz, 1981a).

Apart from the vitelline cells of the egg, the sucker at the tip of *D. penetrans*, the frontal, Mehlis' and uterine glands, and the gonads of *L. indicus* and *D. penetrans* all stained positively for lipids. Droplets of lipids were also found scattered throughout the parenchyma, and subtegumental muscle layer (Pl. 3.2; Fig.1; Pl.3.5; Fig.1-2). Occurrence of lipid in the tegument and subtegumental layer of the two species is in conformity with the observations of Waitz (1963) and Gupta and Kapoor (1979) in *Hydatigera taeniaeformis* and *Cotugnia digonopora*, respectively. von Brand (1952) also observed the scattered droplets of lipids in parenchyma of *Moniezia expansa*, *M. denticulata*, *T. saginata*, *T. marginata* and *D. caninum*. Unsaturated lipids, however, were found in the gonads and vitelline lobes of the two species (Pl. 3.2; Fig. 4; Pl.3.6; Fig.4) and glycolipids were present in the tegument, parenchyma, scolex and neck.

A variety of function is performed by the lipids in the tissues. Thus, lipid forms a major structural component of the cell membrane and as activators or glycosyl carriers lipids are also found associated with enzyme reactions. In the cytochrome chain and membrane transport mechanisms, lipid forms a component part and acts as important energy reserve (Barrett, 1981). Though, in most caryophyllidean eggs glycogen acts as major energy reserve, in forms like *D. penetrans*, lipid also seem to assume this function. However, the distribution of lipid in the eggs of helminths is found to be extra-embryonic, occurring in the space between the embryo and the egg shell and is discarded when the eggs hatch.

Another important constituent of the body is proteins. Almost all the organs excepting the vitelline cell nuclei showed the presence of proteins, though their maximum concentration was seen in the tegument and the various gland cells of the two species (Pl. 3.3; Fig. 1-3; Pl.3.6; Fig.1-3). Considerable concentration of protein is found in most of the endoparasites because proteins form a substantial part of the normal diet of a vertebrate, which as a host provides an environment rich in proteins and their related break down products for the cestode parasites (Smyth, 1969). Thus, along with the general proteins, the tegument, scolex, neck, parenchyma and the eggs showed the presence of disulphides

(Pl. 3.3; Fig. 4; Pl.3.6; Fig.4). These tissues also showed the presence of tyrosine, though in traces, but a large concentration of it was found in the gonads (Pl.3.3; Fig.5; Pl.3.6; Fig.5). Rich concentration of proteins in the tegument, subtegument and the muscle layers are in conformity with the observations of Gupta and Kapoor (1979) in *Cotugnia digonopora*. Also the occurrence of tyrosine in the species under present investigation is in accordance with the findings of Monne (1959), Chowdhury et al. (1962) and Gupta and Kapoor (1979) who made similar observations in *H. teniaeformis*, *T. saginata* and *Cotugnia digonopora*, respectively.

Several biological functions are attributed to proteins. In general, proteins are associated in contractile system, in transport, as protective agents, toxins, hormones, amino-acid reserves and as important structural components (Barrett, 1981). In *L. indicus* and *D. penetrans*, the frontal glands of the scolex and the uterine and Mehlis' gland cells appeared to be rich in proteins, indicating thereby the proteinaceous nature of their secretion.

The occurrence and distribution of the basic proteins like histones has not been studied in parasitic helminths (Barrett, 1981). However, histones being nucleoproteins, in the present investigation also, the nuclei of all the cells showed the presence of this basic protein in the two

species (Pl.3.3; Fig. 6; Pl. 3.6; Fig.6). Similarly, DNA appeared to be present in the nuclei of all the cells, but a high concentration of it was found in the reproductive organs (Pl.3.7, Fig.1; Pl.3.7 Fig.3). The eggs of *D. penetrans* had considerable amount of DNA in the vitelline cells but a very less concentration of it was found in the eggs of *L. indicus*. Burton and Bogitsh (1963) and Christina James (1963) showed that DNA is confined to the nuclei of all the cells in *H. microstoma* and *D. dendriticum*, respectively. However, because of the presence of glycogen in the vitelline cell nuclei in *L. indicus* the concentration of DNA was less. This observation seems in agreement with those of Mackiewicz (1968b) on *C. laticeps* and *C. fennica*. Comparatively higher concentration of DNA was found in the vitelline cells of the eggs of *D. penetrans* because it had only a few cells with intranuclear glycogen vacuole, most of them being rich in lipid.

Abundance of RNA was recorded in the tegument, muscles and reproductive organs of the species under investigation (Pl.3.7, Fig.2; Pl.3.7, Fig.4). Similar observations were made by Burton and Bogitsh (1962), and Gupta and Kapoor (1979). Besides tegument, muscles and reproductive organs, all the other organs and tissues of the two species also had positive reaction for RNA. This seems quite usual in

view of the well established functions, like the storage, transmission and translation of the genetic material, by these nucleic acids.

In gut parasites like cestodes, the tegument is the most important interface since it comes into immediate contact with the host tissue. In both the species under present investigations the tegument was observed to be highly positive for acid-mucopolysaccharides (Pl.3.1; Fig.6; Pl.3.4; Fig.7). On the basis of what is known of the biochemistry and function of the mucopolysaccharides, a protective function from the digestive enzymes of the host can be assigned to them (Barrett, 1981).

The eggs of *L. indicus* and *D. penetrans* were also found to be positive for acid-mucopolysaccharides. Such mucopolysaccharides are found to be widely distributed in the tegument and egg membranes of trematodes, cestodes and nematodes also (Barrett, 1981). Since mucopolysaccharide molecules can contain upto 500 times their own weight of water, such a property of mucopolysaccharide molecule is used in egg hatching in trematodes (Barrett, 1981). Suggestedly, the mucopolysaccharides perform a similar function in *L. indicus* and *D. penetrans*, as well.

A single specimen with pink colouration of the body of *L. indicus* was obtained during the present investigation.

As the investigation did not reveal it to be due to haemoglobin, this colouration might possibly be on account of vitamin^{B₁₂}. Such pink colour was earlier reported for some specimens of *Biacetabulum infrequens* and *Glaridacris laruei* by Mackiewicz (1972) on which assays for vitamin^{B₁₂} had been done. Vitamin B₁₂ was obtained from *H. nodulosa* and *G. laruei* also after conducting radio-isotope assays (Mackiewicz, 1981a). Based on these findings Mackiewicz considered that caryophyllidean cestodes have high concentrations of vitamin B₁₂ like the pseudophyllidean cestodes. It is known that cestodes with vitamin B₁₂ are capable of forming propionate from succinate as an end product of anaerobic energy metabolism, which leads to an increased energy yield from their substrates (Thachuck et al., 1977). Therefore, since the presence of vitamin B₁₂ is recorded from the caryophyllids, it may be assumed that caryophyllids also form propionate which functions to increase the energy available for their egg production which is otherwise low because of their monozoic body plan (Mackiewicz, 1981a).

The present histochemical observations on *L. indicus* and *D. penetrans* lend further support to Barrett's (1981) opinion that basically all living organisms are made of the same kinds of molecules having similar chemical compositions.

Chapter IV SEASONAL INCIDENCE

INTRODUCTION

The monozoic cestode parasites of freshwater teleostean fishes with poorly differentiated scolex and single set of reproductive organs are found to be highly pathogenic causing disease and death of pond fishes (Bauer et al., 1969). Since many of these fish hosts constitute a part of the diet for a considerable section of human population, this parasitic invasion poses some economic problems as well.

A review of existing literature reveals that there is scanty information available on the seasonal fluctuations and population dynamics of caryophyllids. However, the most important studies in this respect are those of Calentine and Fredrickson (1965), Kanaev (1956a,b), Kennedy (1968, 1969), Kulakovskaya (1962, 1964), Lawrence (1970) and Wunder (1939). The studies carried out so far positively indicate towards a distinct seasonal incidence cycle in some forms like *Caryophyllaeus laticeps* in carp of Poland (Wunder, 1939), *Archigetes brama* in USSR (Dubinina, 1949), *A. iowensis* in carp (Calentine, 1964) and *Monobothrium hunteri* and *Biacetabulum microcephalum* in *Catostomus commersoni* of USA (Calentine and Fredrickson, 1965). Lawrence (1970) found that comparatively less fluctuations occurred in *Glaridacris catastomi*, *G. laruei* and *Isoglaridacris commersoni*, all of which parasitize

C. commersoni. Similarly, Williams and Ulmer (1970) found *Spartoides wardi* in *Carpionodes carpiodes* to lack definite seasonal periodicity. No seasonal changes in incidence and intensity were observed by Dwight and Arai (1973) in *Hunterella nodulosa* infecting *Catostomus commersoni*. The studies of Patrick (1980) reveal that *Isoglaridacris bulbocirrus* and *Glaridacris laruei* were non-seasonal in prevalence and mean intensity, in Oyster and Bellamy River suckers, respectively. Seasonal periodicity of *Lytocestus lativitellarium* and *L. parvulus* infecting *C. batrachus* was studied by Furtado and Tan (1973). Also, the incidence pattern of *L. indicus* infecting *C. batrachus* was studied by Satpute and Agarwal (1980c). *L. indicus* and some other species like *Pseudocaryophyllaeus indica*, *Djombangia indica*, *Introvertus raipurensis* and *Lucknowia indica*, all parasitizing *C. batrachus* either singly or concurrently, were observed by Niyogi et al. (1982b) to show seasonal periodicity. Mackiewicz (1972) indicated however, that the period of highest incidence for many of these worms is in late winter or more commonly in early spring.

The present study aims at an investigation of the seasonal fluctuations in the occurrence of caryophyllids as observed during the period of two years study.

Materials and Methods

Clarias batrachus and *Heteropneustes fossilis* were explored for their cestode parasitic fauna, consecutively for a period of two years from April 1982 to March, 1984. A total of 210 *C. batrachus* and 289 *H. fossilis* were examined in the first year and their number was 312 and 268, respectively. However, a minimum number of 15 fishes of each of the two species was autopsied every month during this period of investigation.

The intestine of each fish was slit open and thoroughly scraped for search of parasites. Observations were made on the distribution of the parasites within the intestinal tract and the number of each type was recorded with reference to the sex of the host.

Student's-'t'-test was performed to analyse statistically the significance of parasitization in relation to the sex of host.

Observations

The two-year study revealed that 8 species of caryophyllids parasitize *C. batrachus*, of these seven representing the genus *Lytocestus* under the subfamily Lytocestinae and one, the genus *Djombangia* of the subfamily Djombanginae. In contrast, none of these species were ever recovered from

H. fossilis, which was found to harbour only a single species of *Lytocestus*, that too different from the one occurring in *C. batrachus*.

The seasonal incidence patterns studied showed differences in the time of occurrence and periods of highest incidence amongst the various species.

Few of the *Lytocestus* spp. showed distinct seasonal periodicity, but it was observed that most of these had a similar trend of occurrence; during summer months of June and July, their incidence was low but with the fall in temperature, i.e., during cold winter months (December to February) and during early springs (March to April) they again showed a remarkable increase in incidence.

L. indicus, *L. birmanicus*, *L. filiformis* and *D. penetrans* were found to occur throughout the year and are therefore considered the most common types. Though these species did not exhibit any significant change of seasonal occurrence during different months of the year, they showed relatively pronounced peaks during cold winter months and early spring.

L. indicus, which emerged as the most predominant species in *C. batrachus* did not show a regular pattern of incidence and instead, had a very high degree of fluctuation.

The peaks and falls occurred throughout the year. While on an average the incidence was low during the months of April, June, August and December, it showed the peaks in the months of May, July, September, October, January and February. The maximum incidence recorded however was in February, i.e., late winters (Pl. 4.1; Fig.1).

Lytocestus birmanicus also occurred throughout the year, but the highest incidence was recorded in the early spring (March and April). Peaks were prominent in the months of June, November and December but the maximum incidence attained was in March or April, with a comparatively lower incidence during rest of the months (Pl.4.1; Fig.2).

The seasonal pattern of *L. filiformis* was not regular. Fluctuation in the pattern of incidence was observed throughout the year. While the incidence was low during the wetter months like May, June, July, August and September, a slight peak was seen in October after which it gradually declined to rise up again in February to reach the peak period of incidence. In March again, a sharp decline was noticed. Thus, the highest incidence recorded was in the late winters (Pl.4.1, Fig.3).

A distinct seasonal incidence pattern was depicted by *L. longicollis*, *L. clariae* n.sp., *L. attenuatus* n.sp.

PLATE 4.1 Seasonal fluctuations in the occurrence of the various caryophyllid species over a period of two years (1982-1984)

Fig.1 *L. indicus*.

Fig.2 *L. birmanicus*

Fig.3 *L. filiformis*

These three species occur throughout the year.

Fig.4 *L. longicollis*. Low incidence during winter months and high incidence during early spring.

Fig.5 *L. clariae* n.sp. A low incidence during summer and a relatively high incidence during winter months is evident.

Fig.6 *L. attenuatus* n.sp. A somewhat similar pattern, as that of *L. clariae*, is exhibited.

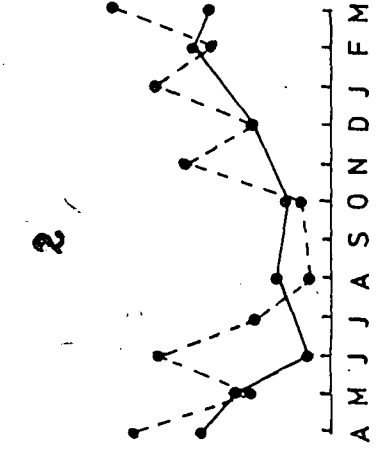
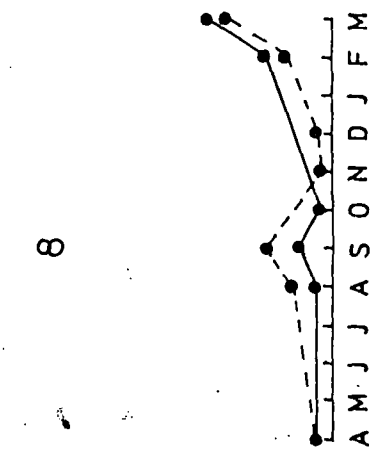
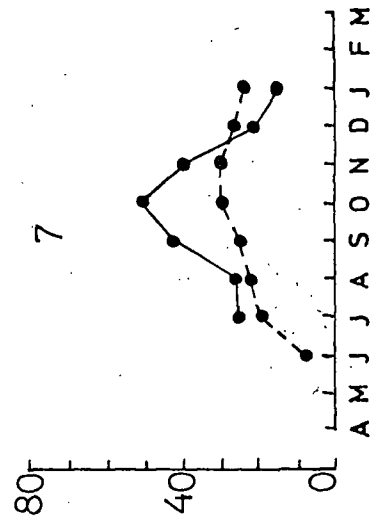
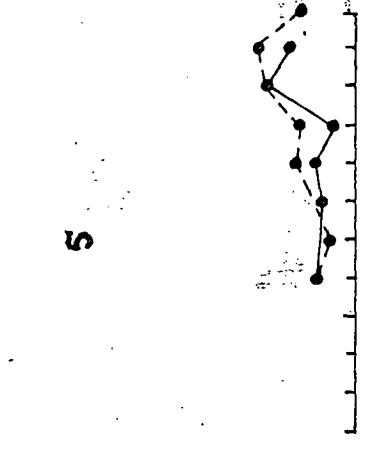
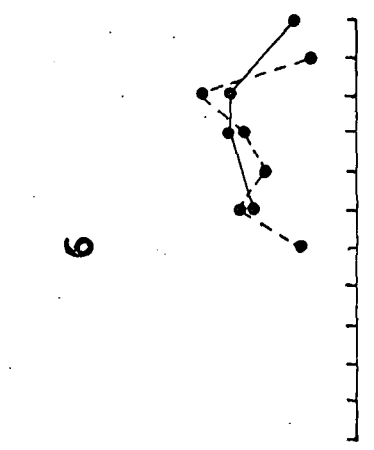
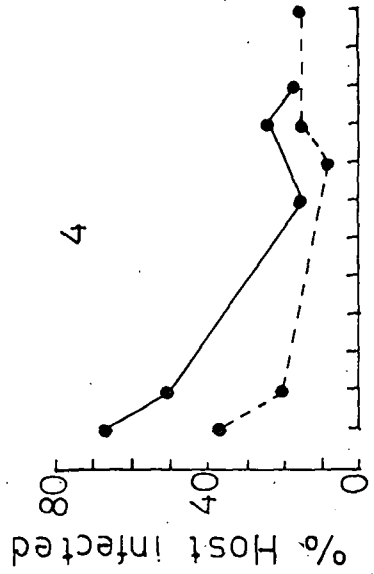
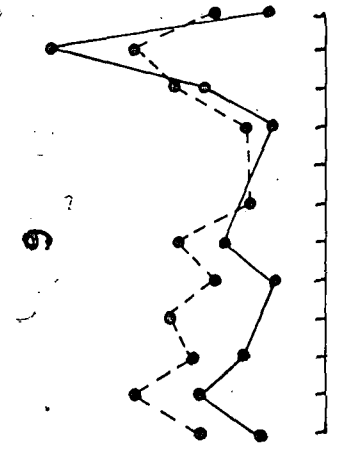
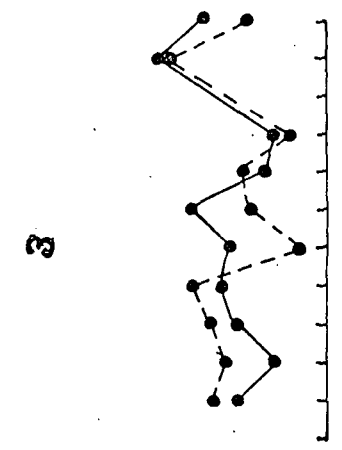
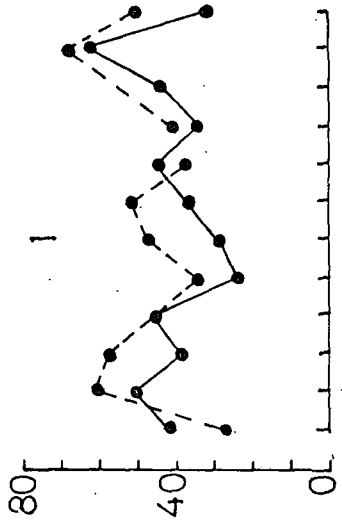
Fig.7 *L. assamensis* n.sp. The only *Lytocestus* species showing an autumn peak.

Fig.8 *L. heteropneustii* n. sp., showing a low incidence during summer but a prominent in early spring.

Fig.9 *D. penetrans*. Occurs throughout the year.

PLATE 4.1

—●— 1982-83
 - -●- - 1983-84



Months

and *L. assamensis* n.sp. recovered from *C. batrachus*, along with *L. heteropneustii* n.sp. from *H. fossilis*.

L. longicollis showed its maximum occurrence in the month of April or during spring, which sharply declined thereafter till October or November. With the onset of winter the occurrence of the species was reported though at a low incidence and then depicted a slight peak in December to be followed by a gradual decline again up to March (Pl.4.1; Fig.4).

The occurrence of *L. clariae* n.sp. was first reported during the autumn months of September and October, after which it showed a fall in incidence consecutively for a few months to follow. However, the peak period of incidence of *L. clariae* was also recorded in March or early spring (Pl.4.1; Fig.5).

L. attenuatus n.sp. also occurred during autumn, i.e., August or September, but had its peak with the onset of winter during the month of December. In the months to follow, i.e., February and March, the incidence declined sharply. However, the species made its appearance again in August or September (Pl.4.1; Fig.6).

L. assamensis n.sp. is the only *Lytocestus* species to show its highest period of incidence during autumn, i.e.,

October. Though the species occurred at a very low incidence during the wet season of June and July, a gradual increase in its incidence was recorded during the months of August and September, attaining the peak in October. Then onwards, the pattern of incidence depicted a sharp decline which continued up to the months of January, after which it reappeared again in June and July (Pl.4.1; Fig.7).

The only caryophyllidean recovered from *H. fossilis* was *L. heteropneusti* n.sp.; which occurred only during a few months of the year and showed a very low incidence of infection. This species could never be recovered during the period from May to July and in January, and made a sporadic appearance during August to November or December. January onwards its incidence pattern exhibited a slight rise with the peak occurring in March that too dropped to a minimum low in April (Pl.4.1; Fig.8).

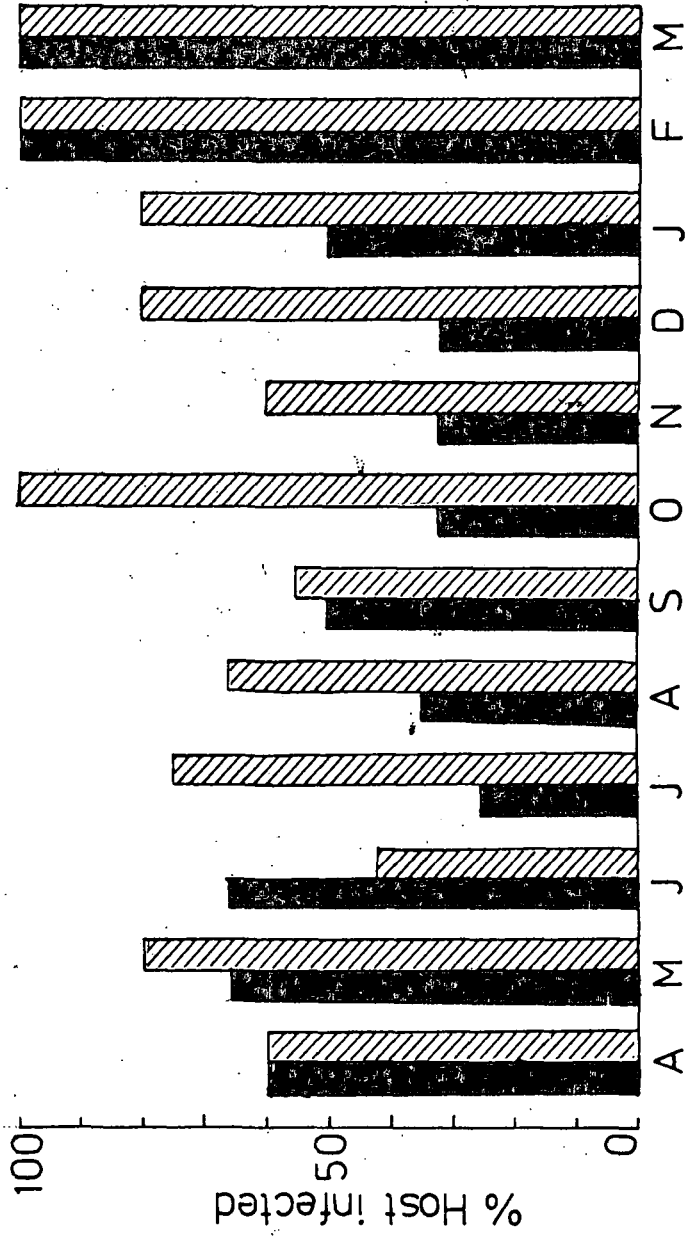
D. penetrans, representing the other genus in the present collection, is also a common type occurring throughout the year. The incidence of occurrence recorded was comparatively low during the months of March, April, August and December; peaks were prominent in the months of May, July and September but the highest peak was recorded in late winter i.e., February (Pl.4.1; Fig.9).

PLATE 4.2 Incidence pattern with regard to the sex of the host

Fig.1 Seasonal incidence pattern of caryophyllid infection with regard to the sex of the host, *Clarias batrachus*, depicting comparatively high prevalence in the males than the females for the year 1982-83.

PLATE 4:2

■ Female host
▨ Male host



Months (1982-83)

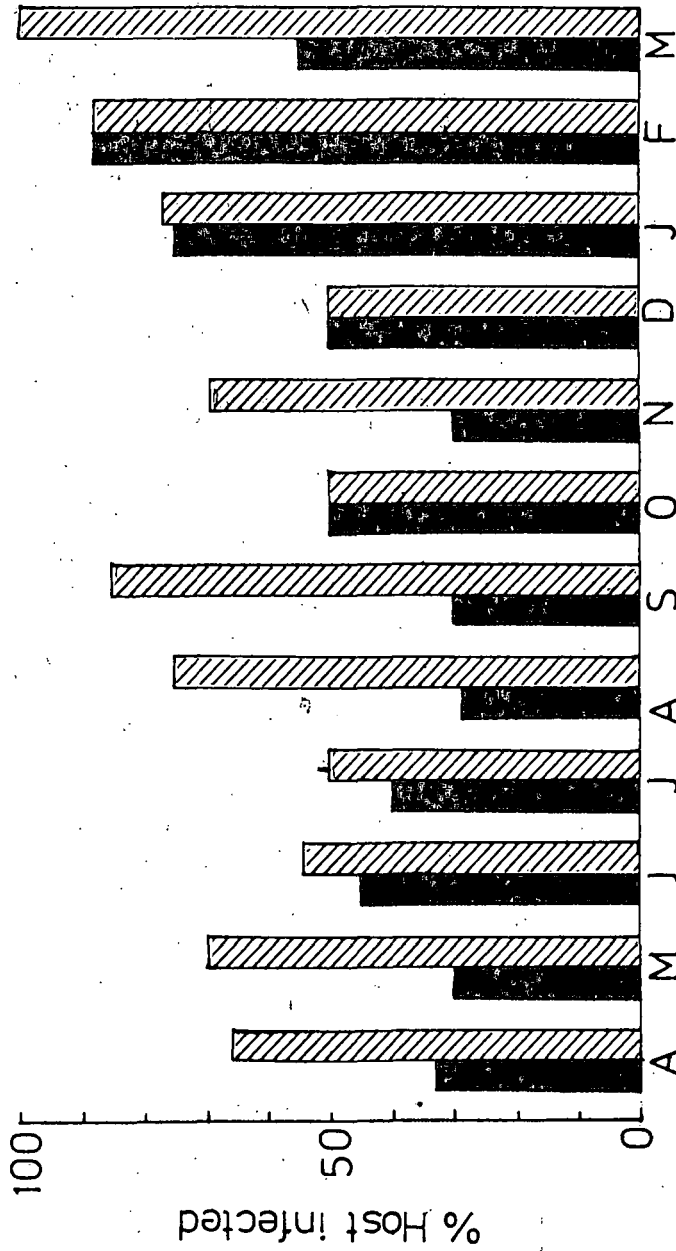
Fig 1

PLATE 4.3 Incidence pattern with regard to the sex of the host

Fig.1 Histogram showing higher prevalence of cayophyllid infection in the male hostfish *C. batrachus*, than the female for the year 1983-84.

PLATE 4.3

■ Female host
▨ Male host



Months (1983-84)

Fig 1

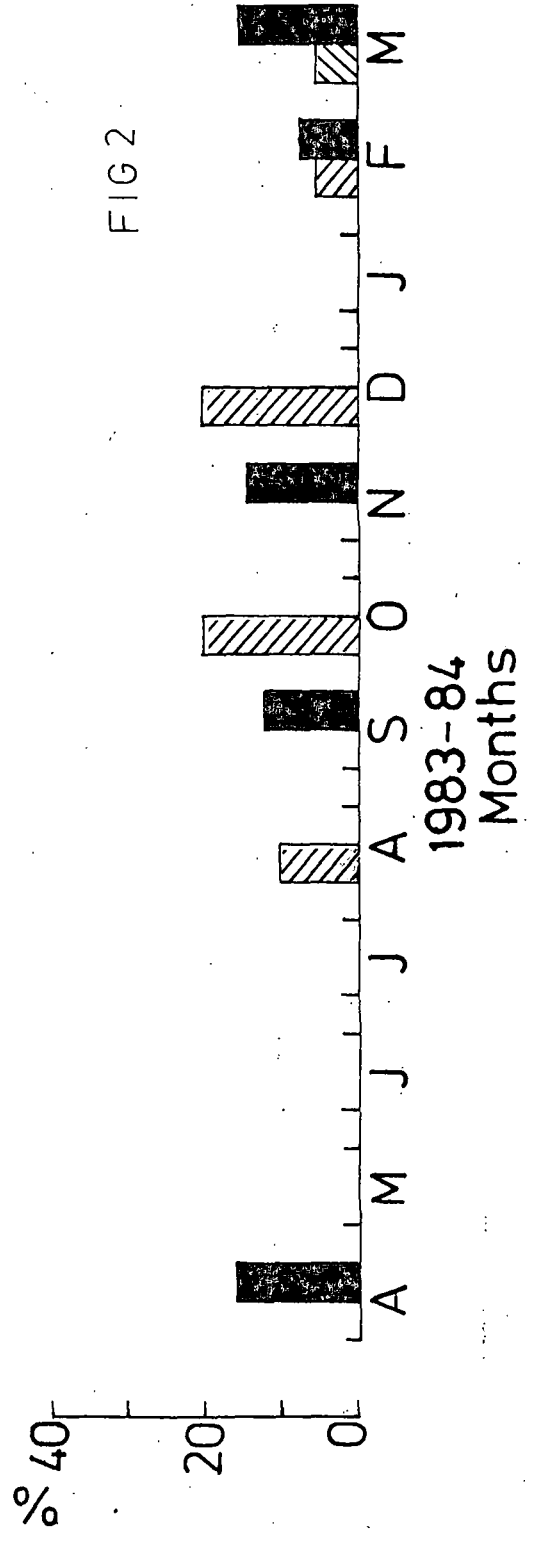
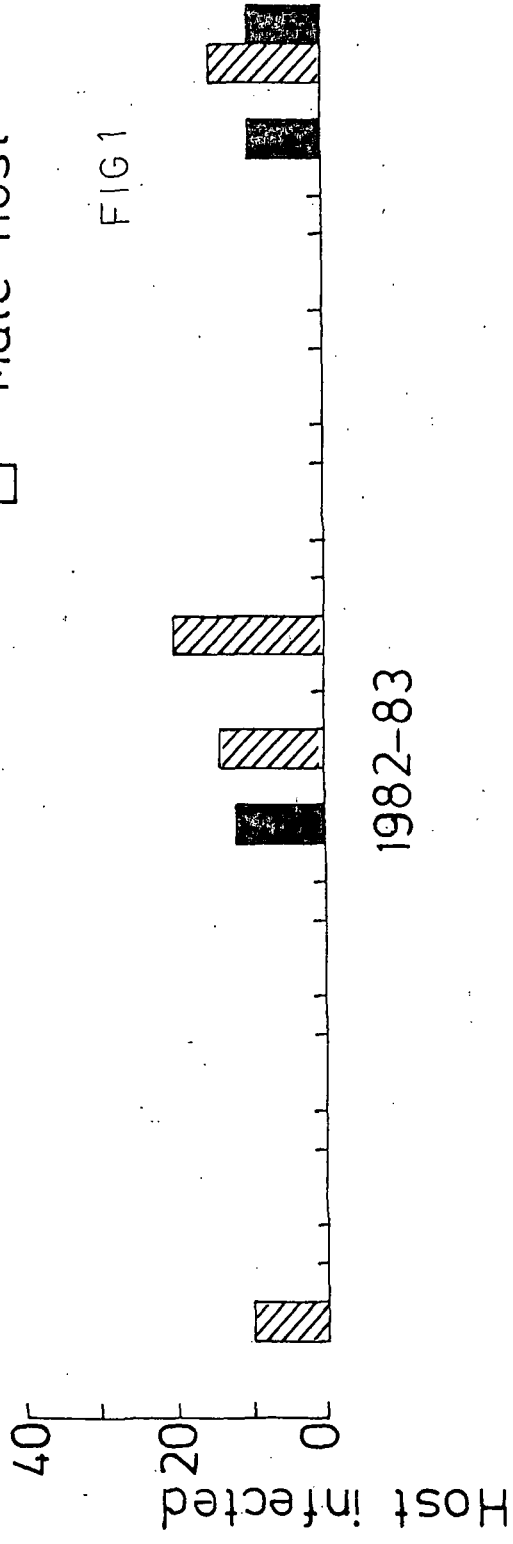
PLATE 4.4 Incidence pattern with regard to the sex of the host

Fig.1 Histogram depicting comparatively higher prevalence of infection in the male *H. fossilis* than the female fish for the year 1982-83;

Fig.2 Histogram showing a similar pattern for the year 1983-84.

PLATE 4.4

Female host
 Male host



In *C. batrachus*, the overall incidence of caryophyllid infection with regard to the sex of the host was recorded as more in males than in females (Pl.4.2; Fig.1; Pl.4.3, Fig.1). This observation has also been corroborated by the Student's t-test and is found to be significant at $P < .05$ level. A somewhat similar trend was also observed in respect of *H. fossilis* host, though the incidence of infection was considerably low in general (Pl.4.4; Fig.1-2).

Discussion

Several factors can be attributed to explain the seasonal incidence cycle of caryophyllids. Fischthal (1953), Bauer (1959a,b) and Engelbrecht (1963) considered the influence of both abiotic and biotic limnological factors responsible for the life cycles and biology of fish parasites. While, the abiotic factors like depth, hydrogen ion concentration, light, oxygen content and salinity of waters affect the distribution of parasites, there is no evidence of these factors influencing the seasonal occurrence of the helminth parasites in their piscine hosts. On the contrary, water temperature is found to be the most important factor concerned with the biology of these parasites, since low temperatures inhibit metabolism and high temperatures stimulate maturation (Arme and Walkey, 1970). Shulman (1979) also suggested water temperature to be the main influencing factor for the seasonal

dynamics of fish parasites, since it influences the parasites both directly and indirectly through the host. Indirect effect of temperature, however, is the way in which it influences the feeding behaviour of the host and resistance of the fishes to the presence of parasites. Thus Scheuring (1929) considered reduced feeding because of the seasonal drop in temperature to be the principal factor in regulating the seasonal incidence of *Caryophyllaeus*. Similarly, Reinsome (1955) reported that in Lake Kals the warming of water by hot springs enables the fish to feed throughout winter and thereby causes heavy winter infections of *C. laticeps* in *Abramis brama*.

Mackiewicz (1972) suggested the period of highest incidence for caryophyllids to be in late winter or more commonly in early spring. In the present investigation, while seven out of eight *Lylocestus* species show noticeable increase in the incidence during winter or early spring, only *L. assamensis* deviates from this pattern. However, the reason for such high incidence amongst lytocestiids during winter or early spring could be that during summer as the temperature goes up, there is increased feeding of the host, *C. batrachus* and *H. fossilis*, which have carnivorous feeding habits. This implies that during summer the fishes could have ingested a lot many intermediate hosts (perhaps

the annelids), which harbour the larval stages of these cestodes that gradually develop to maturity within their final host and appear in abundance by late winter or early spring.

Experiments performed by Kennedy (1970) on *C. laticeps* infection in *Leuciscus indus*, and also those by Awachie (1972) and Kennedy (1972) on acanthocephalan parasites of fishes showed that in each instance low water temperatures facilitated establishment and retention of the worms and higher temperatures, above 12-13°C decreased establishment and in some species increased the rate of loss of the already established parasites from the fish intestines. Thus, in the present study also, similar hypothesis can be applied for the very low incidence recorded for the lytocestiid species during summer and their higher incidence during winter. Even those species like *L. indicus*, *L. birmanicus*, *L. filiformis* and *D. penetrans*, which had year-round occurrence, showed decreased incidence during warm summer months and increased incidence during late winters or early spring. Studies of Anderson (1974, 1976) also showed that *C. laticeps* which occurred throughout the year in the population of *Abramis brama* decreased or the parasite population death rate rose with increasing water temperature, confirming thereby that rising water temperatures stimulate growth of already established parasites but increase resistance

to current invasions and also that the host reaches a point, where not only the newly acquired larvae could not establish but also the already established worms got rejected.

Kennedy (1969), however, proposed a different effect of temperature. According to him, during colder months when the ability to respond is lowest there is restriction of the recruitment, but with rise in temperature during spring there is an increase in the worm burden. Similarly, Niyogi et al. (1982b) also regarded the summer months of July to September as the recruitment period and the winter months as the rejection period of these worms. According to these authors, following the spawning season when there is a resumption of host feeding, the immune response of the host fish increases, resulting in the rejection of worms. Roberts (1978) also considered temperature to be the most important environmental stressor, affecting the amount of dissolved oxygen in water, rate of excretion of metabolites and production of antibodies. Further, reports of Robertson and Wexler (1962a,b) also suggest that during spawning time production of gonadotrophic hormone (GTH) increases with hypertrophy of the adrenal and increase in the levels of adrenocorticotrophic hormone (ACTH) and 17, hydroxycorticosteroids in blood. Boyd (1966) considered the above factors responsible for influencing phagocytic activity, depressing inflammatory

response and reticuloendothelial functions which bear a direct effect on the immune system. Therefore, Niyogi et al. (1982b) considered that it is during the spawning time that *C. batrachus* becomes susceptible to caryophyllid infection, after which the immune system of the fish becomes more effective.

In our study also, one factor responsible for the lowered incidence of *L. assamensis* n.sp. during winter months can be the effect of temperature controlled host response system. During winter, these worms were progressively lost because as the water temperature fell, the host response was probably reduced, and before the fall in temperature the autumn peak was established. Although the other species investigated herein are found to show their peak period of incidence during winter or spring, it appears that it is during spawning time of the fish which coincides with the summer season of the year that the worms gained entry into the host. The immature forms of the worms were also recovered during that period only.

The temperature thus seems to bear a causal relationship to the seasonal cycle of the parasites. Though not acting directly on the latter, the temperature indirectly influences the parasites by causing an alteration in the physiological condition of their host.

Intraspecific interactions between caryophyllids are also considered important in the dynamics of incidence and maturation cycles. Though some forms like *L. indicus*, *L. birmanicus*, *L. filiformis* and *D. penetrans* occur throughout the year, during certain periods of the year their incidence and that of the other *Lytocestus* spp. mentioned herein is decreased or increased depending on the presence of other species. Thus, when *L. assamensis* appeared in abundance during autumn, the other lytocestiid types showed a relatively lowered incidence. Mention may be made that a considerable niche length is occupied by *L. assamensis*; the other caryophyllids, if concurrently parasitizing the same host with *L. assamensis*, usually are restricted to only a portion of the upper intestinal tract. Again, during winter or spring when all other lytocestiid types exhibited increased incidence, *L. assamensis* revealed a remarkable low incidence. Similar observations were made by Calentine and Fredrickson (1965) in respect of *Monobothrium hunteri* and *Biacetabulum macrocephalum* which tended to be absent from *Catostomus commersoni* when *Glaridacris catostomus* was present. Similarly, *B. meridiandum* was excluded by *M. hunteri* in *Erimyzon oblongus* as reported by Grimes and Miller (1976). Andersen (1978) noted a significant negative correlation between the presence of *Proteocephalus percae* and *Acanthocephalus lucii* in *Perca fluviatilis*. The frequent co-occurrence of two or three

species of caryophyllids leading to reduced intensity of *Pseudocaryophyllaeus indica*, niche segregation of *Lucknowia indica*, temporal segregation of *L. indicus*, *I. raipurensis* and *L. indica*, and even competitive exclusion of *D. indica* were studied by Niyogi et al. (1982b). Their studies on co-occurrence, correlation and coefficient of partial association statistically proved a positive association of *L. indicus*, *I. raipurensis* and *L. indica* and a negative interaction between *P. indica* and *I. raipurensis*. The higher intensity of *L. indicus*, *I. raipurensis* and *L. indica* in concurrent infection suggested a reciprocal cross immunity development amongst the species, whereas the negative interaction between *I. raipurensis* and *P. indica* and competitive exclusion of *D. indica* were the result of non-reciprocal cross immunity. In the present investigation also, the co-occurrence of *L. indicus*, *L. birmanicus*, *L. filiformis* and *D. penetrans* with the other *Lytocestus* spp. can be explained in the light of cross-immunity reciprocation and the exclusion of *L. assamensis* by the presence of other *Lytocestus* species during winter or spring could be due to non-reciprocal cross immunity development.

Though the peak period of incidence for many of these *Lytocestus* species appears to occur at the same time, niche segregation and temporal segregation was evident for the different types. While *L. indicus* and *D. penetrans* occurred

in the duodenum provided with thick muscularis, *L. birmanicus* occurred in the anterior loop of the intestine. *L. filiformis* also occurred in the anterior loop but depicted spatial segregation. *L. clariae* *L. attenuatus* and *L. longicollis* were all found distributed throughout the intestine but each depicted temporal segregation.

The role that the host sex may have in determining the incidence of infection seems difficult to assess. Although examples can be cited where positive relationships are inferable, many references can also be quoted where no relationship is observed between the host sex and the incidence pattern. In a seasonal study of helminth burdens of *Salmo trutta*, Thomas (1964) observed that female fishes, of three years of age and older at spawning or during recovery from spawning, tended to have heavier infections of parasites than males but in the instance of *S. trutta* that were not spawning, the statistically significant differences showed the males to be more heavily infested than the females. Calentine (1962, 1964) observed that *Archigetes iowensis* occurred in *Cyprinus carpio*, only during the spawning period of the fishes. Lawrence (1970) found that incidence of *Glaridacris catastomi* and *Isoglaridacris bulbocirrus* between male and female *Catostomus commersoni* was not statistically significant. However, Borgstrom and Halvorsen (1968) considered the

physiological differences between male and female *Rutilus rutilus* enough to account for the absence of *Caryophyllaeides fennica* in 25 males and its presence in 12 of 118 females. Niyogi et al. (1982b) also observed higher incidence and intensity of caryophyllid infection in females compared to male *C. batrachus*. In the present findings, however, the males were more significantly infected than the female fish.

It appears, therefore, that incidence is the result of the interaction of different complex factors, like availability of infected intermediate hosts, variations in the host's feeding habits, environmental changes, host sex and interspecific interaction of the parasites themselves and possibly the changes in host resistance. Though, the population dynamics within the vertebrate host appear to be governed by host reactions or immunity stimulated by temperature, the general incidence picture appears to be more a function of temperature than any other factor (Mackiewicz, 1972). The concurrent infections and the consequent competitive exclusion or reduction in the number of one species by the other could also be considered important in this context.

Chapter V HISTOPATHOLOGY OF THE HOST

INTRODUCTION

Cestodes are well known to have certain adverse effects on their hosts (Bauer et al., 1973). Caryophyllidean cestodes also, apart from the other cestode groups, are found to be economically important pathogens. However, the pathology caused by caryophyllids have been so far extensively studied only in Europe, particularly USSR, where **Cyprinus carpio**, a common host of these cestodes is an important economic enterprise. Thus, some of the significant contributions in this area of study are those of Amlacher (1961), Hofer (1904), Plehn (1924), Schaperclaus (1954) and Sekutowicz (1934) who studied the pathogenic effect of **Caryophyllaeus laticeps** on **C. carpio**. Bauer (1958, 1959c), Bauer et al. (1969), Dogiel and Bauer (1955), Ivasik (1952), Kanaev (1956a,b) and Scherban (1965) also studied the pathogenicity caused on **C. carpio**, but due to a different species, viz. **C. fimbriceps**. Mention may be made of other workers like Akhmetova (1966), Bauer et al. (1969), Musselius et al. (1963) and Scherban (1965), who also considered the same fish to study the pathology due to **Khawia sinensis**. These authors designated the diseases caused by caryophyllidean tape worms as 'Caryophyllosis' when caused by **Caryophyllaeus** spp. and 'Khawiasis' when caused by **Khawia sinensis**. Infections of **K. iowensis** and **Archigetes iowensis** in **C. carpio** were studied by Calentine

and Ulmer (1961) and Calentine (1962), respectively.

Catastomus commersoni, another well preferred piscine host, is also frequently parasitized by caryophyllids. Therefore, Mackiewicz (1963b) studied the effect of **Monobothrium hunteri** on it. **Hunterella nodulosa** infection in **C. commersoni** was studied by Mackiewicz and McCrae (1962). These authors (1965) also studied the pathology caused in the same host due to **Biacetabulum biloculoides**. Therefore, considering the various host reactions produced due to caryophyllidean infection, Mackiewicz (1972) opined that there are three main ways by which the hosts may be affected by these worms: (1) mechanical obstruction of the intestinal tract, particularly in the small fish, (2) production of lesions or other pathological conditions, and (3) by causing a general physiological imbalance that may predispose the host to other infections.

Reports of mechanical obstruction caused are rare. However, Ivasik (1952) observed that 20 to 40 **C. fimbriceps** killed very young **Cyprinus carpio** by preventing them from utilisation of food. Scherban (1965) found that species of **K. sinensis** were also capable of killing the fish by completely obstructing the intestinal canal. Production of lesions or inflammation appears to be a rather general type of host reaction. However, studies of Mackiewicz

et al. (1972) on the comparative pathology as related to modes of attachment and scolex morphology of 15 species of caryophyllids reveal that there is an inverse relationship between scolex adaptations and pathology, whereby species with specialised holdfasts elicit little or no pathology and species lacking specialised structures produced pronounced host reaction in the form of nodules or shallow ulcers.

These findings got further support from the studies on the pathology of *Catastomus commersoni* parasitized by *Hunterella nodulosa*, *Glaridacris catastomi* and *G. laruei* by Hayunga (1979). It was shown that *H. nodulosa* which lacks specific attachment organs produces considerable pathology in the form of massive lesions, loss of tissues, distortion of muscularis, and inflammation in *C. catasomi*, whereas the other two species, viz., *G. catastomi* and *G. laruei* which are provided with attachment organs elicit minimum host reaction. Similar observations were made by Ahmed and Sanaullah (1979) also, while studying the comparative histopathology of the host fish, *Clarias batrachus*, as related to the modes of attachment in scolex morphology and the effects of *Djombangia penetrans*, *Lytocestus indicus* and *L. parvulus*. Their observations revealed *D. penetrans* to be the most pathogenic form which lacks a specialised holdfast and *L. parvulus* to be the least so with a specialised holdfast.

In India, pathological studies done so far are as scarce as they are in other parts of Asia. However, Satpute and Agarwal (1974a) studied the diverticulosis of the duodenum of *C. batrachus* due to *Djombangia indica*. Studies on the pathology caused due to infection of *L. indicus* in the duodenum of *C. batrachus* were also made by Satpute and Agarwal (1974b). and their observations were also supported by Bose and Sinha (1981). As cited by Agarwal (1985), Niyogi and Agarwal studied the pathology of *C. batrachus* due to *Introvertus raipurensis* and *Lucknowia indica* and found that these parasites cause abundant tissue destruction. Effect of *Lytocestoides fossilis* infecting *Heteropneustes fossilis* was studied by Kanth & Srivastava (1984) who considered the destruction of tissue layers a result of pathogenicity.

Studies on the physiological imbalance that is caused in the host due to caryophyllids have been made by a few workers. Jara and Szerow (1981a) observed that *Caryophyllaeus* sp. infection in carp produced a statistically significant reduction in the albumin fractions and total protein level. The same authors (1981b) also revealed that *K. sinesis* infection in carp also produces statistically significant reduction in the serum total protein level, which is related to the intensity of infection. Pathophysiological studies in India are also negligible but significant contributions in this direction have been made by a few workers.

Thus, Sircar and Sinha (1974) reported a reduction in the haemoglobin content in *C. batrachus* infected with *L. indicus*, which was also associated with a high degree of eosinophilia in addition to anisocytosis, macrocytosis and poikilocytosis of the RBC.

Kadav and Agarwal (1982) compared the levels of serum amino acids of *C. batrachus* between males and females related to the intensity of infection and the studies showed depleting cysteine and methionine, histidine, tyrosine, valine and phenylalanine in males; and cystine, methionine, aspartic acid, tyrosine and phenylalanine in females. Kadav and Agarwal (1983a) reported in *C. batrachus* a depletion in haemoglobin percentage, packed cell volume, and RBC count throughout the year, and it was related to the intensity of infection. The blood sugar and liver and muscle glycogen were found as lowered in caryophyllid infected fish when compared to the uninfected ones (Kadav and Agarwal, 1983b). Rasheed Unnisa (1983) showed that the levels of pyruvate and lactate in infected *C. batrachus* were 23 and 34% higher, respectively, than uninfected hosts.

Mackiewicz (1981b) observed that in the south of Himalayas though the dominant hosts for the distribution of caryophyllids are the siluriform and cypriniform fishes, *Clarias batrachus* and *Heteropneustes fossilis* are the chief

hosts. In the present study also, since *C. batrachus* is found to be frequently parasitized by the caryophyllids, it seems desirable to investigate into the histopathological changes appearing in the intestinal wall of the host as a consequence of its inhabitation by the most commonly occurring species, namely, *Djombangia penetrans* and *Lytocestus indicus*. Besides, the effect of multiple infection, i.e., several caryophyllidean species occurring concurrently with the forementioned species, has also been studied.

Materials and Methods

Intestinal tissue with parasite in situ, was fixed in Bouin's fixative and processed for routine histological investigations. Paraffin sections, cut at a thickness of 6-7 μ , were stained using Mallory-Haeidenhein's triple staining technique. Intestinal tissue from uninfected host fish was taken as normal for the purpose of comparison.

Observations

The normal intestinal tissue of the host *C. batrachus* is composed of mucosa, sub-mucosa, muscularis and serosa as studied by Khanna (1980). Histologically, there appears to be no difference in the proximal and distal parts of the intestine. The intestine is thin walled with its mucous membrane thrown into deep folds called villi. These villi are numerous

in the proximal part and are fused with one another distally. The mucosa is composed of columnar epithelium consisting of absorptive and mucus secreting cells. The sub-mucosa is highly vascularised and extends into the villi as lamina propria. The inner circular muscle fibres and the outer longitudinal muscle fibres together comprise the muscularis and the outer most layer or the coat which is richly supplied with blood capillaries from the serosa (Pl.5.1; Fig.1).

With the occurrence of *D. penetrans*, *L. indicus* as well as of the caryophyllidean mix that lie free in the lumen, the intestinal wall revealed different pathological manifestations in respect of the type of infecting worm.

Lytocestus indicus

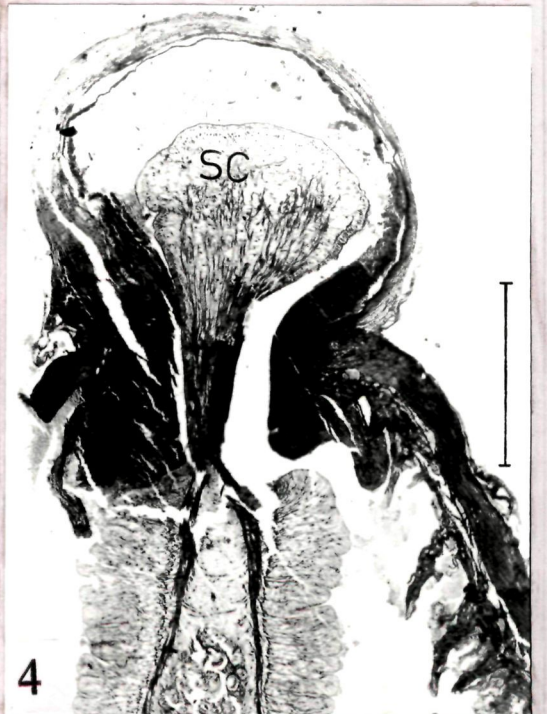
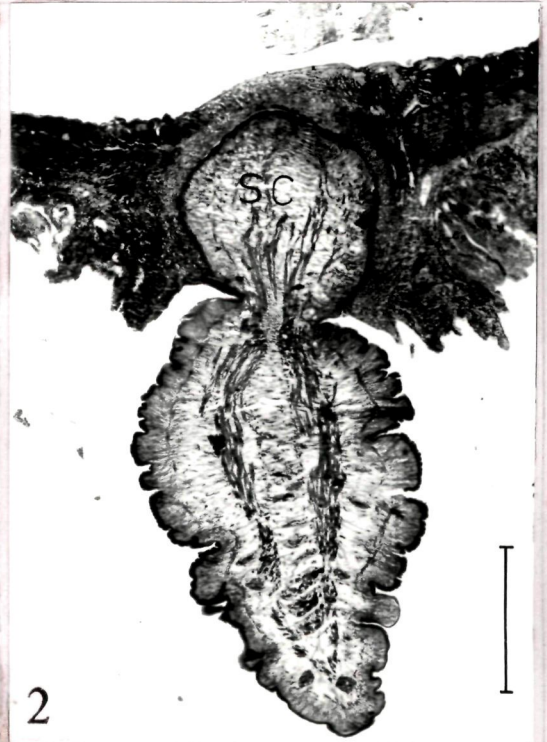
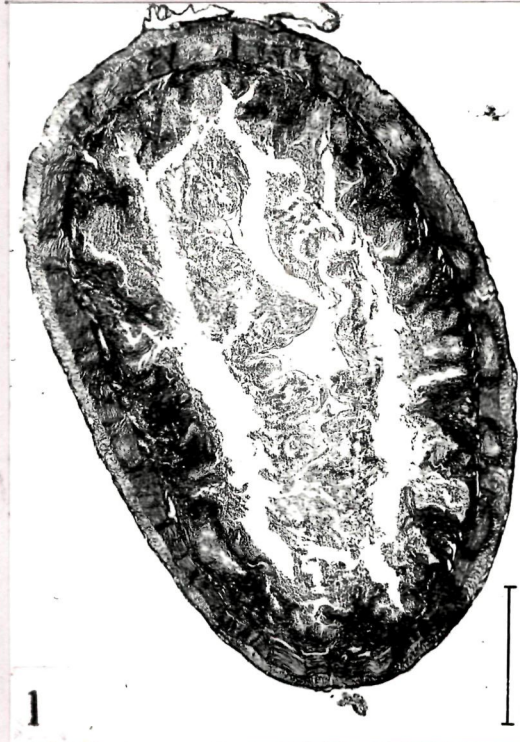
Usually globular or irregular in shape the undifferentiated scolex of *L. indicus* penetrates deep into the muscularis layer (Pl.5.1; Fig.2). The scolex is devoid of bothria and introvert, but shallow longitudinal furrows or grooves are seen which vary in number from specimen to specimen and do not extend much posteriorly in the holdfast. Well developed parenchymal muscles, especially the longitudinal, and gland cells are also present in the scolex. Histochemically, the nature of these gland cells was observed to be proteinaceous.

It was observed that in spite of the worm burrowing

PLATE 5.1 Histopathology of the host tissue (Photomicrographs)

- Fig.1** Normal intestinal tissue of *Clarias batrachus* showing the different layers (scale bar = 0.5 mm);
- Fig.2** *Lytocestus indicus* anchored to the intestine, illustrating penetration up to the muscularis layer (scale bar = 0.5 mm);
- Fig.3** The same at higher resolution showing hyperplasia of the muscularis and a thin interface layer (scale bar = 0.15 mm);
- Fig.4** Section through a nodule harbouring the scolex of *D. penetrans*. Note the muscularis thickening adjacent to the tunnel (scale bar = 0.5 mm).

PLATE 5.1



deep up to the muscularis no nodule formation takes place. At the site of scolex attachment to the intestinal wall of the host, mechanical displacement and compression of tissue layers such as mucosa, submucosa and the muscularis were noticed. Due to the excessive pressure exerted by the scolex, in severe cases of pathogenicity the submucosa became hyperplastic (Pl.5.1; Fig.3). Though no leukocytic infiltration was observed, in some cases a thin mucoid interface layer was seen between the host tissue and the scolex end of the worm. Loosening of muscle fibres was also evident.

Apart from causing such shallow ulcers in the intestinal tissue, the density of infection with 8 to 10 worms per sq cm. area seemed to bear some overcrowding effect. Observations revealed that such overcrowding of the worms led to the blockage of the space in the intestinal lumen leaving thereby a very narrow space for the chyme to pass through.

Djombangia penetrans

A sucker at the tip of the scolex of *D. penetrans* aids the worm in its firm attachment to the host's tissue. Frequently nodular swellings were observed on the outer intestinal wall, which seemed to be the result of penetration of the parasite into the serosa. Formation of nodules led to production of tunnels through the intestinal wall in which

the neck portion of the worm remains embedded. While the scolex is encapsuled in the lumen of the nodule, the body remains free in the lumen of the intestine. As a result of compression of the mucosal folds, submucosa and muscularis, plaques were formed at the site of attachment. The compressed tissue layers appeared thickened on the two sides of the tunnel formed, in which the neck remains lodged. However, the nodular lining did not show such thickening. Each nodule contained only one worm, but debris and necrotic tissue were seen to surround the scolex of the worm. Besides, an interface layer separated the host's tissue from the holdfast of the worm (Pl.5.1; Fig.4).

Like *L. indicus*, *D. penetrans* also occupies a considerable width of the intestinal lumen. Often the density of the worm ranged from 4 to 8 worms per sq. cm. area and therefore, accumulation of such a large number of worms in one region led to considerable reduction in the luminal space by mechanical blockage of the passage.

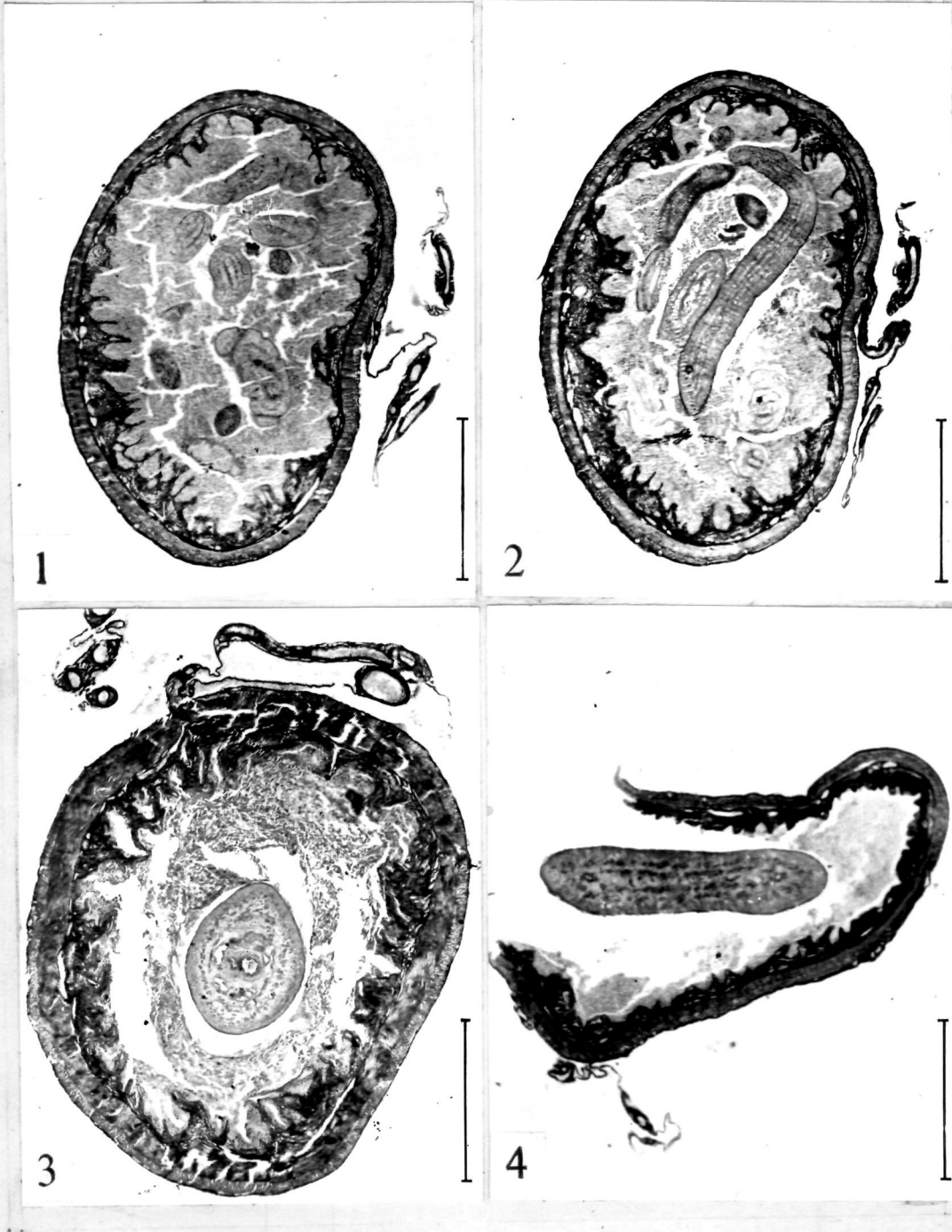
Multiple infections of caryophyllids

In *C. batrachus* multiple infection of caryophyllids commonly occurs during winters, when most of the worm species reach their peak period of incidence. It was observed that generally the multiple infection comprised as many as 3 to 4 different species and a total of 10 to 12 worms concurrently

PLATE 5.2 Histopathology of the host tissue (Photomicrographs)

- Fig.1** Ruptured mucosal layers due to multiple infection (scale bar = 0.5mm);
- Fig.2** Disruption of villi due to multiple infection (scale bar = 0.5 mm);
- Fig.3** Loosening of muscle layers due to caryphyllid infection (scale bar = 0.5 mm);
- Fig.4** Shortening of villi due to infection of *L. assamensis* n.sp. (scale bar = 0.5 mm).

PLATE 5.2



parasitizing a single host. However, in such cases each individual species occupied a very less surface area to remain attached to the host tissue but most of these worms having a considerable length size occupied a considerable niche length.

Observations revealed that pathogenicity is not severe because no ulceration or nodular formation took place in the host's intestinal wall, but the intestinal villi or the mucosal folds got compressed (Pl.5.2; Fig.1-3). However, the density of worms appeared to have a direct effect on the host by causing mechanical obstruction of the gut.

L. assamensis n.sp. occupied a considerable length of the host's intestine. It was observed that multiple infections do not occur along with the occurrence of *L. assamensis*, but the pathogenicity caused by it is equivalent to that caused due to multiple infection. Compression of villi took place throughout the length of the intestine which is occupied by *L. assamensis* (Pl. 5.2; Fig.4).

Discussion

The distribution of *L. indicus*, *D. penetrans* and the species constituting the multiple infection within the host's intestine shows a definite habitat preference depending on the scolex type that they possess. While *L. indicus* and

D. penetrans inhabit the duodenum which has thick muscularis, species such as *L. birmanicus*, *L. longicollis*, *L. filiformis*, *L. clariae* n.sp. and *L. attenuatus* n.sp. considered for multiple infection are always found in the anterior and posterior loop of the intestine, i.e., spread throughout the intestinal length.

The effects on the host seem to be related to the mode of attachment of the scolex. Thus, the scoleces of *L. indicus* and *D. penetrans* which penetrate the deep tissue layers caused serious pathogenicity. This view is also supported by the studies of Mackiewicz et al. (1972) on the comparative pathology as related to modes of attachment and scolex morphology of 15 species of caryophyllids. Bauer et al. (1973) also consider that the scolex types have a direct bearing on the resultant effects produced in the host.

L. indicus produced shallow ulcers within the host's tissue by causing destruction of villi, mucosa and submucosa. These observations are in conformity with those made by Satpute and Agarwal (1974b), Ahmed and Sanaullah (1979), Bose and Sinha (1981) and Niyogi and Agarwal (in Agarwal, 1985). However, in the present investigation, *L. indicus* was never found to reach the serosa and no leukocytic infiltration was observed as mentioned by Satpute and Agarwal (1974b). Pinocytic vesicles in the holdfast end of the worm as observed by Niyogi and

Agarwal, were also found lacking, but folded margins of the scolex end were distinct in the present investigation. Since no degenerated tissues were seen within the folds of the scolex end, they (folds) could not be considered as pinocytic vesicles. In severe cases of pathogenicity, submucosal layer was found to become hyperplastic and at times a thin mucoid interface layer was also observed.

Possibly this pronounced tissue reaction could be due to the presence of gland cells present in the holdfast end of *L. indicus*. Such gland cells, viz., "Frontal-drussen and faserzellenstrange" are reported to be present in the scolex and neck regions of many caryophyllids by earlier workers like Will (1893), Mrazek (1901) and Jenizewska (1954) and that these frontal glands assist in attachment was suggested by Hunter (1930) and Szidat (1937). In the opinion of Wisniewski (1930) these gland cells that are homologous to those of pseudophyllidean procercooids or of *Amphilina* (Cestodaria) are probably responsible for secreting lytic enzymes to enable the organism to penetrate the gut wall of the fish host. In *L. indicus* histochemically the secretion of the gland cells was found to be proteinaceous in nature. Therefore, the deep burrowing by *L. indicus* can be attributed to the activity of the proteinaceous gland cells in the scolex. The highly developed musculature in the holdfast end may also be responsible for the exertion of high pressure into

the tissue layers, whereby it gets compressed. However, the mechanical obstruction is caused due to the occurrence of the parasites in clusters.

The pathogenicity caused by *D. penetrans* can be compared to that of *Hunterella nodulosa*, *Biacetabulum biloculoides*, *Monobothrium ulmeri* and *M. ingens* which also produce pronounced host reaction in the form of nodules (Mackiewicz et al., 1972). However, differences are observed in nodules formed by *D. penetrans* when compared to these species.

Like *D. penetrans*, *H. nodulosa* which lacks a specialized holdfast, produces a large nodular thickening with a pronounced active chronic inflammatory reaction. However, the nodular lining of *D. penetrans* appear to be necrotic with debris in the pit, whereas that of *H. nodulosa* is not necrotic and there is no debris in the pit. Further, the nodule of *D. penetrans* is single chambered, harbouring only one worm but the nodules of *H. nodulosa* sometimes have more than one chamber containing as many as 127 worms in various stages of development (Mackiewicz and McCrae, 1962).

The nodule formed by *B. biloculoides* is smaller than that formed by *H. nodulosa* but larger than that by *D. penetrans* as it contains 1 to 6 worms per nodule. Debris and necrotic tissue surrounding the scolex as seen in *D. penetrans* are also found in *B. biloculoides*, but unlike the

former muscular thickning at the site of attachment is not observed.

Nodules formed by *M. ingens* and *M. ulmeri* are also similar to the one formed by *D. penetrans* and *B. biloculoides* in containing necrotic debris and in having an interface layer but the size of the nodule produced by *D. penetrans* appears to be the smallest by far in harbouring just a single worm. In case of *M. ingens* also, each nodule contained fewer than seven cestodes.

Polygonchobothrium clarias, a pseudophyllidean cestode, produces pronounced nodules in the gall bladder of *Clarias mossambicus* (Wabuke-Bunoti, 1980). Histologically, this nodule is composed of three layers — the continuous outer epithelial layer, a granulomatous subepithelial layer and the innermost fibrous connective tissue layer. The nodule of *D. penetrans* also appears to be similar histologically, but the innermost and subepithelial layers are not found to be highly vascularised.

The host reaction in all these infections is manifested in the production of a nodule irrespective of the differences in the scolex morphology: *B. biloculoides*, *M. ulmeri*, *M. ingens* and *P. clarias*, all possess specialised attachment organs — *B. biloculoides* and *M. ulmeri* with a terminal introvert, *M. ingens* with both an introvert and shallow bothria and *P. clarias* with only bothria; *D. penetrans*,



however, possesses a sucker at the tip of the holdfast with a large number of gland cells spread over the scolex. The mode of attachment being penetrative all these species probably have a similar scolex adaptation to the intestinal surface, hence produce a similar pathological manifestation.

Originally, the pathology associated with these worms was thought to be caused by proteolytic secretions of the frontal glands (Mackiewicz et al., 1972). However, electron microscopic studies of Hayunga (1977) on *H. nodulosa* showed no evidence of proteolysis or necrosis of host tissue. In his opinion, probably the eosinophilic matrix was primarily an adhesive layer which acted as a strong irritant, and the latter combined with a strong muscular contraction of the worm caused ulceration and loss of epithelium. However, the occurrence of necrotic debris in the present study is suggestive of the presence of proteolytic enzymes in the holdfast end.

Another remarkable difference between the host reaction produced by *D. penetrans* and other nodule-forming species is that in none of the latter cases there occurs a muscular thickening on the sides of the penetration site. As a result of penetration or burrowing deep up to the serosa by *D. penetrans* the tissue layers across which it penetrates get thickened. Similar observations were made by Satpute and Agarwal (1974a) and Ahmed and Sanaullah (1979) in *Djombangia*

infections in *C. batrachus*. The nodule formation, which seems an inflammatory response of the host, provides a sheltered habitat and firm anchorage to the worm. This host response is an example of exploitation by the parasite of its host defence mechanism to its own advantage (Hayunga, 1977).

The pathology manifested in the form of shortening of intestinal villi or compression of the mucosal folds due to multiple infection and *L. assamensis* n.sp. is probably a consequence of pressure necrosis. Excepting for the alterations in the absorptional area of the intestine, and mechanical blockage of the luminal space of the intestine, no serious host reaction is produced due to multiple infection and *L. assamensis*. However, multiple infections of caryophyllids, are of common occurrence. Thus, of 162 infected *C. commersoni* Mackiewicz et al. (1972) observed 29% of them to have multiple infection. An infection comprising as many as 200 *L. indicus*, 1500 *Introvertus raipurensis* and 2000 *Lucknowia indica* in a single host fish *C. batrachus*, seemed to have no detrimental effect (Agarwal, 1985).

The present study also suggests that the mode of attachment is primarily responsible for triggering a significant host reaction. While a single worm of *D. penetrans* or *L. indicus* results in nodule formation and shallow ulcers respectively, multiple infection results only in the atrophy

of the intestinal villi. In spite of the larger number, size and variety of worms in case of multiple infection, the host reaction elicited is minimum. The reason for this seems to be the fact that mode of attachment of the worms in such a case is very weak.

SUMMARY

1. Exploration of the edible cat-fishes, *Clarias batrachus* and *Heteropneustes fossilis*, for their caryophyllidean fauna revealed that, nine different types of caryophyllids parasitize these piscine hosts of north-east India. While eight of them represent the subfamily Lytocestinae and belong to the genus *Lytocestus*, only one represents the subfamily Djombanginae and belongs to the genus *Djombangia*. Out of the eight *Lytocestus* spp., four, namely *L. indicus*, *L. birmanicus*, *L. filiformis* and *L. longicollis* are already known forms representing new locality record and the remaining four appear new to science. These have been named as *L. clariae* n.sp.; *L. attenuatus* n.sp.; *L. assamensis* n.sp. and *L. heteropneustii* n.sp. The validity of the new species has been discussed.

The spectrum of the parasitic infection was more diversified in *C. batrachus* in comparison to *H. fossilis* which also shares the same benthic habitat, since only a single species of *Lytocestus* was obtained from the latter, throughout the period of caryophyllidean faunistic survey.

2. Scanning electron microscopic studies on the surface fine topography of *L. indicus* and *D. penetrans*, the two most commonly occurring types revealed a dense and uniform covering of microtriches throughout the surface of the body, without showing any regional differentiation with regard to their morphology.
3. Histochemical studies on *L. indicus* and *D. penetrans*, the two most apparently pathogenic species, indicates towards a similarity in the distributional pattern of the different metabolites between the two species.

While in both the carbohydrates, proteins and lipids showed a generalised pattern of distribution in the tegument, parenchyma, reproductive organs as well as the scolex gland cells, differences occurred with regard to glycogen and lipid in the eggs of two species. The vitelline cell nuclei in the egg of *L. indicus* contained a higher concentration of glycogen than lipid, but those in case of *D. penetrans* had lesser concentration of glycogen and higher of lipid. Besides, another distinguishing feature between the two species is that, in the vitelline lobes of *L. indicus* usually 3 to 4 patches of glycogen masses were observed whereas in *D. penetrans* only few cells of the vitelline lobe became vacuolated and stained positively for glycogen.

DNA concentration appeared to be higher in the eggs of *D. penetrans* as compared to *L. indicus*.

4. Incidence pattern for the different caryophyllid types recovered appeared to be species specific. Even for the most frequently occurring types like *L. indicus*, *L. birmanicus*, *L. filiformis* and *D. penetrans*, seasonal fluctuation was distinct. While there occurred low incidence of infection during the summer months, the peak period of incidence obtained by the different species was during late winter or early spring months. Only a single species *L. assamensis* n.sp. depicted its peak during autumn.

The prevalence of infection was more in the male hosts than in the female host fishes. However, though host reactions or immunity stimulated by temperature appears to be responsible for the population dynamics within the vertebrate host, the general incidence picture is more a function of temperature than any other factor.

5. Based on histopathological changes *D. penetrans* was found to be the most highly pathogenic type, producing large nodules in the intestinal wall as a result of complete penetration. Like *D. penetrans*, *L. indicus* is also the burrowing type, but its penetration was

not as deep (i.e., up to the serosa), as that of *D. penetrans*, but was restricted only up to the muscularis layer. Hence, *L. indicus* is comparatively less pathogenic causing ulceration of the affected tissue. In severe cases of pathogenicity however, hyperplasia of the muscularis was observed.

Pathogenicity due to multiple infection comprising several species of caryophyllids was minimum, since only the denudation of the mucosal folds was observed. Similar host reaction was also produced due to *L. assamensis* n.sp. infection, which appeared singly and not along with multiple infection and occupied a considerable length of the intestine.

Thus extent of damage was maximum due to a single ~~Worms~~ of *D. penetrans* and minimum due to multiple invasion of the caryophyllids.

REFERENCES

- Agarwal, S.M. 1985. Caryophyllaeids and Caryophyllidiasis in India. **Indian Reviews of Life Sciences**, 5: 139-161.
- Ahmed, A.T.A. and Sanaullah, M. 1979. Pathological observations on the intestinal lesions induced by caryophyllid cestodes in **Clarias batrachus** (L.) (Siluriformes: Clariidae). **Fish Pathology**, 14: 1-7.
- Akhmetova, B. 1966. Epizootiology of kariozoa of carp on the Alma-Ata fish farms. **Scientific and Production conference on the control of diseases of fish in Kazakhstan and Republics of Central Asia**, Alma-Ata, March, 1966, 15-19.
- Amalacher, E. 1961. **Traschenbuch der Fischkrankheiten**. Gustav Fischer Verlag, Jena, 286pp.
- Andersen, K. 1975. Comparison of surface topography of three species of **Diphyllbothrium** (Cestoda, Pseudophyllidea) by scanning electron microscopy. **International Journal for Parasitology**, 5: 293-300.
- _____ 1978. The helminths in the gut of perch (**Perca fluviatilis** L.) in a small oligotrophic lake in Southern Norway. **Zeitschrift für Parasitenkunde**, 56: 17-27.
- Anderson, R.M. 1974. Population dynamics of the cestode **Caryophyllaeus laticeps** (Pallas, 1781) in the bream (**Abramis brama** L.) **Journal of Animal Ecology**, 43: 305-321.
- _____ 1976. Seasonal variation in the population dynamics of **Caryophyllaeus laticeps**. **Parasitology**, 72: 281-305.
- Arme, C. and Walkey, M. 1970. The physiology of fish parasites. In **Aspects of Fish Parasitology**. (A.E.R. Taylor and R. Muller, eds.), pp. 79-101. Blackwell, Oxford.
- Arndt, W. 1922. Ueber Lipoide and Lipoidstoff wechsel bei Evertebraten. **Verhandl. Beutsch. Zool. Gosellsch.** 76-78.
- Awachie, J.B.E. 1972. Experimental studies on some host parasite relationships of the Acanthocephala. Effects of primary heavy infection and superimposed infection of **Salmo trutta** L. by **Echinorhynchus truttae** Schrank, 1788. **Acta Parasitologica Polonica**, 20: 375-382.

- Barrett, J. 1981. **Biochemistry of Parasitic helminths**. The Scientific and Medical Division McMillan Publisher Ltd. London and Basingstoke. 308pp.
- Bauer, O.N. 1958. Parasitic diseases of cultured fishes and methods of their prevention and treatment, pp. 265-298. In Dogiel, V.A. Petrusheveski G.K. and Polyanski, Yu. I., **Parasitology of Fishes** (English translation, 1961). Translated by Z. Kabata, Oliver and Boyd Ltd. London.
- _____ 1959a. Ecology of the parasites of fresh water fish. Interrelationships between the parasite and its habitat. **Izvestiya Gosudarstvennogo Nauchno Issledovatel'skogo Instituta Ozernaogo i Rechnogo Rybnogo khozyaistva**, 49: 5-206.
- _____ 1959b. The influence of environmental factors on reproduction of fish parasites. **Voprosy Ekologii**, 3: 132-141.
- _____ 1959c. The ecology of parasites of fresh-water fish. **Izvestiya Gosudarstvennogo Nauchno-Issledovatel'skogo Instituta Ozernago i Rechnogo Rybnogo khozyaistva**, 49: 5-185. (In parasites of fresh-water fish and the biological basis for their control. **Bulletin of the State Scientific Research, Institute of Lake and River Fisheries**. 49: 3-215 [1962]. Israel Programme for Scientific Translations, Jerusalem).
- _____, Musselius, V.A. and Strelkov, Yu. A. 1969. **Diseases of Pond Fishes**. Kolos, Moscow, 335pp.
- _____ 1973. **Diseases of Pond Fishes**. Israel Programme for Scientific Translations, Jerusalem, 220pp.
- Baugh, S.C. and Singh, J.P. 1979. Glycogen distribution in **Raillietina echinobothriidia** (Magnin, 1880). **Rev. Ibe. Parasitol.** 39: 223-232.
- Beneden, P.J. van, and M. Olsson. 1893. **Kgl. Sevenska Vetenskapsakad. Handl.** 25: 1-41. (cited in **The Zoology of Tapeworms**, Wardle, McLeod and Radinovsky, 1974).
- Berger, J. and Mettrick, D.F. 1971. Microtrical polymorphism among hymenolepid tapeworms as seen by scanning electron microscopy. **Transactions of the American Microscopical Society**, 90: 393-403.
- Borgstrom, R. and Halvorsen, O. 1968. Studies of the helminth fauna of Norway. XI. **Caryophyllaeides fennica** (Schneider) (Cestoda: Caryophyllidae) in lake Bogstad. **Nyht. Magasin for Zoologie**. 16: 20-23. ✓

- Bose, K.C. and Sinha, A.K. 1981. Histopathology of *Clarias batrachus* (Linn.) infected by *Lytocestus indicus* (Moghe). **Science and Culture**, 47: 186-187. ✓
- Bovien, P. 1926. Caryophyllaeidae from Java. **Videnskabelige Meddeleser fra Dansk naturhistorisk Forening L. kobenhavn**, 82: 157-181.
- Boyd, W.C. 1966. **Fundamentals of Immunology**. 4th edition. Interscience Publishers, London.
- Brandt, T. Von. 1933. Untersuchungen ueber den stoff bestand einiger cestoden und den Stoff weckselvon *Moniezia expansa* **Ztschr. Vergl. Physiol.** 118: 562-596.
- _____ 1952. **Chemical Physiology of Endoparasitic Animals**. Academic Press, Inc. New York.
- Bråten, T. 1968a. An electron microscopic study of the tegument and associated structures of the proceroid of *Diphyllobothrium latum* (L). **Zeitschrift für Parasitenkunde**, 30: 95-103.
- _____ 1968b. The fine structure of the tegument of *Diphyllobothrium latum* (L) A comparison of the plerocercoid and adult stages. **Zeitschrift für Parasitenkunde**, 30: 104-112.
- Brault, A. and Loeper, M. 1904a. Le glycogene dans la membrane germinale des kystes Hydatigues. **J. Physiol. et Path. Gen.** 6: 295-301.
- _____ 1904b. Le glycogene dans le development de certains parasites (Cestodes et nematodes) **J. Physiol. et Path Gen.** 6: 503-512.
- Braun, M. 1900. Cestodes in Bronn's Klassen und Ordnungen des tierreichs V.
- Busch, P.W.C.M. 1905a. Sur lac localisation du glycolgene chez quelques parasites intestinaux. **Arch. Internat. Physiol.** 3: 49-51.
- _____ 1905b. Over de localisatie van het glycogen bij einige darm-parasieten. **Onderzoek. Ged. Physiol. Lab. Utrecht.** 6: 72-89.
- Burton, J. and Bogitsh. 1963. Histochemical Studies on *Hymenolepis microstoma* (Cestoda: Hymenolepidae). **Journal of Parasitology**, 49: 989-997. ✓
- Calentine, R. 1962. *Archigetes iowensis* sp.n. (Cestoda: Caryophyllaeidae) from *Cyprinus carpio* L. and *Limnodrilus hoffmeisteri* Claparide. **Journal of Parasitology**, 48: 513-524. ✓

- Calentine, R. 1964. The life cycle of *Archigetes iowensis* (Cestoda: Caryophyllaeidae) **Journal of Parasitology**, 50: 454-458.
- _____ and Fredrickson, L. 1965. Periodicity of Caryophyllaeid cestodes in the white sucker, *Catostomus commersoni* (Lacepede). **Iowa State Journal of Science**, 39: 243-250.
- _____ and Ulmer, M. 1961. *Khawia iowensis* n.sp. (Cestoda: Caryophyllaeidae) from *Cyprinus carpio* L. in Iowa. **Journal of Parasitology**, 47: 795-805.
- Calow, P. and Jennings, J.B. 1974. Calorific values in the phylum Platyhelminthes. The relationship between potential energy, mode of life and the evolution of entoparasitism. **Biological Bulletin**, 147: 81-94.
- Chandler, A.C. 1930. Some polyradiate specimens of *Taenia pisiformis* with a completely double strobila. **Transactions American Microscopical Society**, 1: 168-171.
- Charles, G.H. and Orr, T.S.C. 1968. Comparative fine structure of outer tegument of *Ligula intestinalis* and *Schistocephalus solidus*. **Experimental Parasitology**, 22: 137-149.
- Chowdhury, A.B., Dasgupta, B. and Roy, H.N. 1962. On the nature and structure of the calcareous corpuscles in *Taenia saginata*. **Parasitology**, 52: 153-157.
- Christina Ohman-James. 1968. Histochemical Studies of the cestode *Diphyllobothrium dendriticum* Nitzsch., 1824. **Zeitschrift für Parasitenkunde**, 30: 40-56.
- Clapham, P.A. 1939. Some polyradiate specimens of *Taenia pisiformis* and *Dipylidium caninum* with a bibliography of the abnormalities occurring among cestodes. **Journal of Helminthology**, 17: 163-176.
- Cohn, L. 1908. Die Anatomie eines neuen Fischcestoden. **Centralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung I. Originale**, 46: 134-139.
- Coutelen, F. 1931. Presence, Chez les hydatides echinococciques, de cellules libres a glycogens et a graisses. Leur role biologique possible. **Annals of Parasitology**, 9: 97-100.

- Dogiel, V.A., and Bauer, O.V. 1955. Measures against parasitic diseases of fishes in pond cultures. **Academy of Science USSR, Scientific Popular Series**, 87pp.
- Domenici, L. and Gremigni, V. 1974. Electron microscopical and Cytochemical study of vitelline cells in the fresh-water triclad *Dugesia lugubris* s.l. II. Origin and distribution of reserve materials. **Cell Tissue Research**, 152: 219-228.
- Dubinina, M.N. 1949. Influence on the parasite fauna of fish of their overwintering in the overwintering branches of the Volga delta. **Parazitologicheskii Shornik**, 11: 104-125.
- Dwight, M.R. and Arai, H.P. 1973. Population dynamics of *Hunterella nodulosa* (Cestoidea: Caryophyllidea) in Alberta. **Canadian Journal of Zoology**, 51: 787-792.
- Engelbrecht, H. 1963. Der Einfluss der Umwelt auf die Entwicklung parasitärer Wurmer. **Ceskolovenskia Parasitologie**, 10: 74-80. (Translation Fisheries Research Board of Canada Translation No.456).
- Featherston, D.W. 1975. *Taenia hydatigena*. IV. Ultrastructure study of the tegument. **Zeitschrift für Parasitenkunde**, 38: 214-232.
- Fischthal, J.H. 1953. Parasites of north-west Wisconsin fishes. IV. Summary and limnological relationships. **Transactions of the Wisconsin Academy of Sciences, Arts and Letters**, 42: 82-108.
- Fotedar, D.N. 1958. On a new caryophyllaeid cestode, *Adenoscolex oreini* gen. et sp. nov. from fresh-water fish in Kashmir, and a note on some related genera. **Journal of Helminthology**, 32: 1-16. ✓
- Fuhrmann, O. 1931. Ordnung der Unterklasse der Cestoda: Pseudophyllidea, 289-334. (W. kukenthal and T. Krumbach, eds), pp.141-416. (1928-1953). In **Handbuch der Zoologie**.
- Fuhrmann, O. and Baer, J.G. 1925. Zoological results of the third Tanganyika expedition conducted by Dr. W.A. Cunningham, 1904-1905. Report on the Cestode. **Proceedings of the Zoological Society of London**, 79-100. ✓
- Furtado, J.I. 1963. A new caryophyllaeid cestode, *Lytocestus parvulus* sp. nov. from a Malayan catfish. **Annals and Magazine of Natural History (Ser B)**, 6: 93-106. ✓

- Furtado, J.I., and Tan, K.L. 1973. Incidence of some helminth parasites in the Malaysian catfish *Clarias batrachus* (Linnaeus). *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 18: 1674-1685.
- Gaur, A.S., and Agarwal, S.M. 1981. Non specific phospho-monoesterases in three species of caryophyllids from *Clarias batrachus* (Linn.). *Proceedings of the Indian Academy of Parasitology*, 2: 71-75.
- Ginetsinskaya, T.A., and Upenskaya, Z.I. 1965. The characteristics of glycogen and fat stores in the tissues of some fish helminths, regarding their location in the body of the host. *Helminthologia*, 6: 319-333.
- _____, Palm, U., Bessedina, V.V., and Timopheeva, T.A. 1971. Accumulation of reserve substances in the yolk glands of platyhelminthes. *Parazitologiya*, 5: 147-154.
- Grammeltvedt, A. 1973. Differentiation of the tegument and associated structures in *Diphyllobothrium dendriticum* Nitzsch (1824) (Cestoda: Pseudophyllidae) An electron microscopical study. *International Journal for Parasitology*, 3: 321-327.
- Grimes, L.R. and Miller, G.C. 1976. The seasonal periodicity of three species of caryophyllaeid cestodes in the creek chubsucker, *Erimyzon oblongus* (Mitchill) in North Carolina. *Journal of Parasitology*, 62: 434-441.
- Gupta, S.P. 1961. Caryophyllaeids (Cestoda) from fresh-water fishes of India. *Proceedings of the Helminthological Society*, 28: 38-50.
- Gupta, N.A. and Kapoor, M. 1979. Histochemical studies on *Cotugnia digonopora* (Pasquale, 1890) in *Gallus gallus domesticus*. *Rivista Di Parasitologia*, Vol. XL. N. 1/2: 63-79.
- Gupta, V. and Parmar, S. 1984. On a new caryophyllaeid *Pseudocaryophyllaeus mackiewiczzi* sp. nov. from the intestine of a freshwater fish, *Heteropneustes fossilis* (Ham.) from Gorakhpur, U.P. *Indian Journal of Helminthology* (1982, publ. 1984), 34: 136-138.
- _____, Singh, S.R. 1984. On a n.sp. *Pseudocaryophyllaeus ritai* sp. nov. (Family Caryophyllaeidae) from the intestine of a fresh-water fish *Rita rita* from river Gomati at Lucknow, U.P. *Indian Journal of Helminthology*, 35: 11-14.

- Gupta, V., and Sinha, N. 1984. On three new species of caryophyllaeids from fresh-water fishes of Lucknow. **Indian Journal of Helminthology**, **36**: 73-80.
- Halton, D.w., Stranock, S.D. and Hardcastle, A. 1974. Vitelline cell development in monogenean parasites. **Zeitschrift fur Parasitenkunde**, **45**: 45-61.
- Hayunga, E.G. 1977. Comparative histology of the scoleces of three caryophyllid tapeworms: relationship to pathology and site selection in the host intestine. **Thesis**, State University of New York at Albany, New York. ✓
- _____ 1979. Observations on the intestinal pathology caused by three caryophyllid tapeworms of the white sucker **Catostomus commersoni** Laecephede. **Journal of Fish Diseases**, **2**: 239-249.
- _____ and Mackiewicz, J.S. 1975. An electron microscope study of the tegument of **Hunterella nodulosa** Mackiewicz and McCrae 1962 (Cestoidea: Caryophyllidae). **International Journal for Parasitology**, **5**: 309-19.
- Hedrick, R.M. 1958. Comparative histochemical studies on cestodes. II. The distribution of fat substances in **Hymenolepis diminuta** and **Raillietina cesticillus**. **Journal of Parasitology**, **44**: 75-84.
- _____ and Daugherty, 1957. Comparative histochemical studies on cestodes. I. The distribution of glycogen in **Hymenolepis diminuta** and **Raillietina cesticillus**. **Journal of Parasitology**, **43**: 497-504.
- Himes, M.N. and Polister, A.W. 1962. Symposium: Synthetic process in the cell nucleus. V. Glycogen accumulation in the nucleus. **Journal of Histochemistry and Cytochemistry**, **10**: 175-185.
- Hofer, B. 1904. **Handbuch der Fischkrankheiten**. Verlag der Allg. Fischerei-Zeitung. Munchen, 359pp.
- Hunter, G.W. III. 1927. Notes on the Caryophyllaeidae of North America. **Journal of Parasitology**, **14**: 16-26. ✓
- _____ 1929. New Caryophyllaeidae from North America. **Science (N.S.)**, **69**: 645-646.
- _____ 1930. Studies on the Caryophyllaeidae of North America. **Illinois Biological Monographs**, **11**:(1927) 186pp.

- Ivasik, V. 1952. Some observations on pathogenicity of *Caryophyllaeus fimbriceps* to carp. *Trudy Nauchno-Issledevotel'skogo Instituta Prudovogo i Ozerno-rechnogo Rybnogo Khozyastva, Kiev*, No.8: 127-130.
- Janiszewska, J. 1954. Caryophyllaeidae europejskie ze szczegolnym uwzglednieniem Polski. *Travaux de la Societe des Sciences et des Lettres de Wrocklaw* (Seria B. Nr.66), 73pp.
- Jara, Z. and Szerow, D. 1981a. Electrophoretic examination of the serum of carp (*Cyprinus carpio*) infected with the cestode *Caryophyllaeus* sp. *Wiadomosci Parazytologiczne*. 27: 713-716.
- _____ 1981b. The level of total protein and its fractions in the serum of carp (*Cyprinus carpio*) infected with the cestode *Khawia sinensis* *Wiadomosci Parazytologiczne*. 27: 705-711.
- Jennings, J.B. 1973. Symbiosis in the Turbellarian and their implications in studies on the evolution of parasitism. In *Symbiosis in the Sea*. (W.B. Vernberg, ed.) pp. 127-160. University of South Carolina Press, Columbia.
- _____ and Calow, P.1975. The relationship between high fecundity and the evolution of entoparasitism. *Oecologia, Berl.* 21: 109-115.
- Jha, R.K. and Smyth, J.D. 1971. Ultrastructure of the rostellar tegument of *Echinococcus granulosus* with special reference to biogenesis of mitochondria. *International Journal for Parasitology*, 41: 169-177.
- Johri, G.N. 1959. On a remarkable new caryophyllaeid cestode, *Hunterella mystei* gen. et sp. nov. from a fresh-water fish in Delhi State. *Zeitschrift für Parasitenkunde*, 19: 368-374.
- Jones, A.W. and Mackiewicz, J.S. 1969. Naturally occurring triploidy and parthenogenesis in *Atractolytocestus huronensis* Anthony (Cestodea: Caryophyllidea) from *Cyprinus carpio* L. in North America. *Journal of Parasitology*, 55: 1105-1118.
- Joyeux, Ch. and Baer, J.G. 1961. Class des Cestodes, 347-560. In Grasse, P.P. (ed) *Traite de Zoologie* Tome IV Premier fascide, Masson et Cie, Paris: 944pp.
- Kadav, M. and Agarwal, S.M. 1982. Amino acid picture (qualitative and quantitative) of host serum of uninfected and infected *Clarias batrachus* parasitized with caryophyllids. *Indian Journal of Helminthology*, 33: 78-86.

- Kadav, M. and Agarwal, S.M. 1983a. Parasitic effects on haematology of *Clarias batrachus* infected with caryophyllids. **Indian Journal of Helminthology**, **33**: 137-143.
- _____ 1983b. Parasitic effects on carbohydrate metabolism of *Clarias batrachus* parasitized by caryophyllids. **Indian Journal of Helminthology**, **33**: 153-154.
- Kanaev, A.I. 1956a. On the treatment of carp infected by *Caryophyllaeus*. **Rybnoe Khezyaistvo Unnutrennikh Vodoemov Latviiskoi SRR**, **4**: 50-52.
- _____ 1956b. Caryophylliasis in carp and methods of controlling it. **Avtoferat, Mosrybvtuz**, 137-149.
- Kanth, L.K. and Srivastava, L.P. 1984. Host parasite relations in monozoic tapeworm *Lytocestoides fossilis* infection of freshwater fish *Heteropneustes fossilis* (Bloch). **Current Science**, **53**: 607-608.
- Kennedy, C.R. 1968. Population biology of the cestode *Caryophyllaeus laticeps* (Pallas 1781) in dace, *Leuciscus leuciscus* of the River Avon. **Journal of Parasitology**, **54**: 538-543. ✓
- _____ 1969. Seasonal incidence and development of the cestode *Caryophyllaeus laticeps* (Pallas) in the River Avon. **Parasitology**, **59**: 783-794.
- _____ 1970. The population biology of helminths of British freshwater fish, p. 145-159. In Taylor, A.E.R., and Muller, R. [Eds.], **Aspects of fish parasitology, Symposia of the British Society for Parasitology 8**. Blackwell Scientific Publications, Oxford, 167pp.
- _____ 1972. The effect of temperature and other factors upon the establishment and survivals of *Pomphorhynchus laevis* (Acanthocephala) in gold fish *Carassius auratus*. **Parasitology**, **65**: 283-294.
- Khanna, S.S. 1980. **Introduction to Fishes**. Central Book Depot. Allahabad. viii, 492pp.
- Kilejian, A; Schinazi, L.A. and Schwabe, C.W. 1961. Host parasite relationships in Echinococcosis. V. Histochemical observations of *Echinococcus granulosus*. **Journal of Parasitology**, **47**: 181-188.
- Kulakovskaya, O.P. 1962. The Seasonal changes in representative of the family Caryophyllaeidae (cestoda) under conditions existing in western Ukrainian Region, USSR. **Scientific memoirs of Science — Biological Museum of the Ukrainian Academy of Science**, **10**:88-93.

- Kulakovskaya, O.P. 1964. Life cycle of Caryophyllaeidae (Cestoda) in the conditions of Western Ukraine. **Ceskoslovenska Parasitologie**, **11**: 177-185.
- _____ and Demshin, N.I. 1978. Origin and phylogenetic relationships of caryophyllideans. In **Problemy Gidro Parazitologii** (ed. A. Markevic) pp. 95-104. Kiev. 'Naukova Dumka'.
- Lawrence, J.R. 1970. Effects of season, host-age and sex on endohelminths of *Catastomus commersoni*. **Journal of Parasitology**, **56**: 567-571.
- Lee, D.L. 1972. The structure of the helminth cuticle. **Advances in Parasitology**, **4**: 186-206.
- Leuckart, R. 1878. *Archigetes sieboldi*, eine geschlechtsreife Cestodenart mit geschichte der Bandwürmer. **Zeitschrift für Wissenschaftliche Zoologie**, **30**: 595-606.
- Luhe, M.F. 1910. **Parasitische Plattwürmer II: Cestodes**. Die Süßwasserfauna Deutschlands, (Dr. Brauer, ed.), Heft 18. Gustav Fischer, Jena, 153pp.
- Lumsden, R.D. 1966. Cytological studies on the absorptive surface of cestodes. I. The fine structure of the strobilar integument. **Zeitschrift für Parasitenkunde**, **27**: 355-382.
- Lynsdale, J.A. 1956. On two n.sp. of *Lytocestus* from Burma and the Sudan respectively. **Journal of Helminthology**, **30**: 87-96.
- Lyons, K.M. 1977. Epidemics of parasitic platyhelminths. **Symposium of the Zoological Society of London**, **39**: 97-144.
- Mackiewicz, J.S. 1962. Systematic position of *Caryophyllaeus fuhrmanni* Sridat, 1937 and *Lytocestus alestesi* Lynsdale 1956 (Cestoidea: Caryophyllaeidae) **Revue Suisse de Zoologie**, **69**: 729-735.
- _____ 1963a. Subfamily status of Bovieninae Fuhrmann, 1931 (Cestoidea: Caryophyllaeidae). **Zeitschrift für Parasitenkunde**, **23**: 92-98.
- _____ 1963b. *Monobothrium hunteri* sp.n. (Cestoidea: Caryophyllaeidae) from *Catastomus commersoni* (Lacepede) (Pisces: Catastomidae) in North America. **Journal of Parasitology**, **49**: 723-730.
- _____ 1965a. *Isoglaridacris bulbocirrus* gen. et sp. n. (Cestoidea: Caryophyllaeidae) from *Catastomus commersoni* in North America. **Journal of Parasitology**, **51**: 554-564.

- Mackiewicz, J.S. 1965b. Redescription and distribution of *Glaridacris catastomi* Cooper, 1920 (Cestoidea: Caryophyllaeidea) **Journal of Parasitology**, **51**: 554-560.
- _____ 1968a. *Isoglaridacris hexacotyle* comb. n. (Cestoidea, Caryophyllidae) from Catastomid fishes in Southwestern North America. **Proceedings of the Helminthological Society of Washington**, **35**: 193-196.
- _____ 1968b. Vitellogenesis and egg — shell formation in *Caryophyllaeus laticeps* (Pallas) and *Caryophyllaeides fennica* (Schneider) (Cestoidea: Caryophyllaeidea). **Zeitschrift für Parasitenkunde**, **30**: 18-32.
- _____ 1972. Caryophyllidae (Cestoidea): a review. **Experimental Parasitology**, **31**: 417-512. ✓
- _____ 1978. Duplication of reproductive system in monozoic cestodes (Caryophyllidae). **Proceedings of the Helminthological Society, Washington**, **45**: 28-33.
- _____ 1981a. Caryophyllidae (Cestoidea): Evolution and Classification. In **Advances in Parasitology**, **19**: 139-199. Academic Press. London, New York, San Francisco, Toronto, Sydney. 225pp.
- _____ 1981b. Synoptic Review of the Caryophyllidae (Cestoidea) of India, Pakistan and Nepal. **Himalayan Journal of Science**, **1**: 1-14.
- _____ 1982. Caryophyllidae (Cestoidea): Perspectives. **Parasitology**, **84**: 397-417.
- _____ and Blair, D. 1979. *Balanotaeniidae* fam. n. and *Balanotaenia newguinensis* (Cestoidea: Caryophyllidae) from *Tandanus* (Siluriformis: Plotosidae) in New Guinea. **Journal of Helminthology**, **52**: 199-204.
- _____ Cosgrove, G.E. and Gude, W.D. 1972. Relationship of Pathology to scolex morphology among caryophyllid cestodes. **Zeitschrift für Parasitenkunde**, **39**: 233-246.
- _____ and McCrae, R. 1962. *Hunterella nodulosa* gen. n. sp. n. (Cestoidea: Caryophyllaeidea) from *Catastomus commersoni* (Lacepede) in North America (Pisces: Catastomidae). **Journal of Parasitology**, **48**: 798-806.

- Mackiewicz, J.S. and McCrae, R. 1965. *Biacetabulum biloculoides* n.sp. (Cestodea: Caryophyllaeidae) from *Catostomus commersoni* (Lacepede) in North America. *Proceedings of the Helminthological Society, Washington*, **32**: 225-228.
- _____ and Murhar, B.M. 1972. Re-description of *Bovienia serialis* (Bovien, 1926) (Cestodea: Caryophyllidae) from the catfish *Clarias batrachus* (L.) in India. *Journal of Helminthology*, **46**: 309-405.
- Mehra, H.R. 1930. On a new species of *Caryophyllaeus* O.F. Muller from Kashmir with remarks on *Lytocestus indicus* (Moghe, 1925). *Proceedings of the 17th Indian Science Congress Association*, 247pp.
- Moczon, T. 1975. Glycogen distribution and accumulation of radioactive compounds in tissues of mature specimens of *Hymenolepis diminuta* after incubation in glucose- $^{14}\text{C}_{16}$. *Acta Parasitologica Polonica*, **23**: 135-146.
- Moghe, M.A. 1925. *Caryophyllaeus indicus* n.sp. (Trematoda) from the catfish *Clarias batrachus* (Bl.). *Parasitology*, **17**: 232-235.
- _____ 1931. A supplementary description of *Lytocestus indicus* Moghe (Syn. *Caryophyllaeus indicus*, Moghe, 1925, Cestoda). *Parasitology*, **23**: 84-87. ✓
- Mokhtar Moamouri, F. and Swiderski, Z. 1976. Vitellogenesis chez *Echeneibothrium beauchampi* Euzet. 1959 (Cestoda: Tetracophyllidae, Phyllobothriidae). *Zeitschrift für Parasitenkunde*, **50**: 293-302.
- Monne, L. 1959. On the external cuticles of various helminths and their role in the host-parasite relationship. *Arch. Zool.* **12**: 343-358.
- Monticelli, G.S. 1892. Appunti sui cestodaria. *Atti della Reale Accademia della Scienze Fisiche e Matematiche*, Napoli, 5 (Serie 2, No. 6), 11pp.
- Morseth, D.J. 1966. The fine structure of the tegument of adult *Echinococcus granulosus*, *Taenia hydatigena* and *Taenia pisiformis*. *The Journal of Parasitology*, **52**: 1074-1085.
- Mrazek, A. 1901. Über die Larvae von *Caryophyllaeus mutabilis* Rud. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Erste Abteilung, Originale*, **29**: 485-491.

- Mrazek, A. 1908. Ueber eine neue Art der Gattung *Archigetes*. **Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, Erste Abteilung, Originale**, 46: 719-723.
- Murhar, B.M. 1963. *Crescentovitus biloculus* gen. nov., sp. nov., a fish cestode (Caryophyllaeidae) from Nagpur, India. **Parasitology**, 53: 413-418.
- Musselius, V., Ivanova, N.; Laptev, V; and Apazidi, L. 1963. Concerning clove-worms in carp. **Ribovodstvo i Ribolovsyvo**, 25-27.
- Muthukrishnan, S. 1974. Studies on the integument of cestodes: I. Histology and Histochemistry of the tegument of the adult cestode *Taenia hydatigena*. **Acta Histochem.** 50: 174-180.
- _____ 1975a. Studies on the integument of cestodes: II. The nature of protein component in the integument of gravid proglottids of *Taenia hydatigena*. **Acta Histochem.** 53: 175-181.
- _____ 1975b. Studies on the integument of cestodes: V. Histochemical observations on the integument of the neck region of *Taenia hydatigena*. **Acta Histochem.** 53: 234-238.
- _____ 1975c. Studies on the integument of cestodes: VI. Histochemical studies on the fate of structural polysaccharide in the integumental gravid proglottids of *Taenia hydatigena*. **Acta Histochem.** 54:1-4.
- Niyogi, A; Gupta, A.K. and Agarwal, S.M. 1982a. Morphology of *Lucknowia indica* sp. nov. (Lytocestidae: Caryophyllidea). **Proceedings of Indian Academy of Parasitology**, 3: 17-22.
- _____ 1982b. Population dynamics of caryophyllids from *Clarias batrachus* at Raipur. **Geobios new Reports** 1: 81-93.
- Nyebelín, O. 1922. Anatomish systematische studien uber Pseudophyllidean. **Goteborgs kungl Vetenskaps-och Vitterhets-Samhalles Handlings, Fjarde foljden**, 26: 228.
- Ortner-Schonbach, P. 1913. Zur Morphologie des Glykogens bei Trematoden und Cestoden. **Archiv fur Zellforschung** 11: 413-449.
- Pandey, K.C. 1973. Studies on some cestodes from fishes, bird and mammal. **Indian Journal of Zoology**, 15: 221-226.
- Parshad, V.R., Guraya, S.S. and Parshad, R.K. 1981. Biochemical and Histochemical observations on the lipids of *Raillietina cesticillus* (Davaenidae: Cestoda). **Indian Journal of Parasitology**, 5: 229-232.

- Patrick, M. Muzall. 1980. Comparison of the parasite communities of the white sucker (*Catostomus commersoni*) from two rivers in New Hampshire. **Journal of Parasitology**, 68: 300-306.
- Pearse, A.G.E. 1968. **Histochemistry: Theoretical and Applied**. I. London: Jand A. Churchill. 759pp.
- Pinter, T. 1922. Die vermittelte Bedeutung der Helminthenwanderungen. **Sitzungsber. Akad. Wissensch. Wien., Math-Naturw. Kl. Abt. I.** 131: 129-130.
- Plehn, M. 1924. "Praktikum der Fischkrankheiten". **Handbuch der Binnenfischerei Mitteleuropas**, Vol. I., E. Schweizerbart'sche, Verlagsbuchhandlung, Stuttgart, 479pp.
- Rama Devi, P. 1973. *Lytocestus longicollis* sp. nov. (Cestoidea: Caryophyllidea) from the catfish *Clarias batrachus* (L) in India. **Journal of Helminthology**, 47: 415-420.
- Rasheed Unnisa, 1983. Estimation of Pyruvate, lactate and inorganic phosphate content in *Lytocestus indicus* and its effect on infected host. **Proceedings of the Indian Academy of Parasitology**, 41: 59-61.
- _____ and Simha, S.S. 1982. Non-specific phosphomonoesterase in *Lytocestus indicus* and its host, *Clarias batrachus*. **Indian Journal of Parasitology**, 6: 135-136.
- Reinsome, A.D. 1955. The fish parasito-fauna of industrial lakes in the Latvian S.S.R. Avtoreferat. **Latviiskii Gosud arstvennyi Universitet**. 19pp (Cited by Bauer, 1959).
- Richards, K.S. and Arme, C. 1981a. The ultrastructure of the scolex-neck syncytium, neckcells and frontal gland cells of *Caryophyllaeus laticeps* (Caryophyllidea: Cestoda). **Parasitology**, 83: 477-487.
- _____ 1981b. Observations on the microtriches and stages in their development and emergence in *Caryophyllaeus laticeps* (Caryophyllidea: Cestoda). **International Journal for Parasitology**, 11: 369-376.
- _____ 1982a. Microarchitecture of structured bodies in the tegument of *Caryophyllaeus laticeps* (Caryophyllidea: Cestoda). **Journal of Parasitology**, 68: 425-432.

- Richards, K.S. and Arme, C. 1982b. Sensory receptors in the scolex-neck region of *Caryophyllaeus laticeps* (Caryophyllidea: Cestoda). **Journal of Parasitology**, **68**: 416-425.
- Roberts, R.J. 1978. The pathophysiology and systematic pathology of teleosts. In: **Fish Pathology**. (ed.) R.J. Roberts. Bailliere, Tindall, London, pp. 55-91.
- Robertson, O.H. and Wexler, B.C. 1962a. Histological changes in the pituitary gland of rainbow trout (*Salmo gairdneri*) accompanying sexual maturation and spawning. **Journal of Morphology**, **110**: 157-169.
- _____ 1962b. Histological changes (Genus *Onchorhynchus*) accompanying sexual maturation and spawning. **Journal of Morphology**, **110**: 171-185.
- Sahay, S.N. and Sahay, U. 1977. On a new caryophyllid cestode, *Djombangia caballeroi* sp. nov. from fresh-water fish *Heteropneustes fossilis* in Chotanagpur with an amendment of the generic character. **Excerta Parasitologica en Memoria del Doctor Eduardo Cabellero y Ceballero, Maxico. Universidad Nacional Autamoma de Maxico, Instituto do Biologia, Publicaciones Especiales**, **4**: 371-376.
- Satpute, L.R. and Agarwal, S.N. 1974a. Diverticulosis of the fish duodenum infested with cestodes. **Indian Journal of Experimental Biology**, **12**: 373-375.
- _____ 1974b. Seasonal infection of *Clarias batrachus* (Linn.) by *Lytocestus indicus* Moghe, and parasitic effects on its haematology. **Indian Journal of Experimental Biology**, **12**: 584-586. ✓
- _____ 1980a. Morphology and Systematics of *Djombangia indica* Satpute and Agarwal, 1974 causing diverticulosis of duodenum of *Clarias batrachus*. **Proceedings of the Indian Academy of Parasitology**, **1**: 13-16.
- _____ 1980b. *Introvertus raipurensis* gen nov. A. fish cestode (Cestoda: Caryophyllidea: Lytocestidae) from Raipur, India. **Proceedings of Indian Academy of Parasitology**, **1**: 17-19. ✓
- _____ 1980c. Studies on seasonal infection of *Clarias batrachus* (Linn.) by *Lytocestus indicus* Moghe, 1925. **Indian Journal of Helminthology**, **32**: 9-17. ✓

- Schaperclaus, W. 1954. **Fischkrankheiten**. 3rd ed. 708pp. Akademic Verlag, Berlin.
- Schauinsland, H. 1885. Die embryonale Entwicklung der Bothriocephalen. **Jenaische Zeitschrift für Medizin und Naturwissenschaft**, **19**: 520-572.
- Scherban, N.P. 1965. Tsestodozy karpov. (Cestodoses of Carp). Kiev, Izdatel'stvo **Urozhae**.
- Schiefferdecker, P. 1874. Beitrage zur knntniss des feinerens Baues der Taniem. **Jonwische Ztschr, Naturw.** **8**: 458-487.
- Scheuring, L. 1929. Beobachtungen zur Biologie des genus **Triaenophorus** und Betrachtungen uber das jahreszeitliche Auftreten von Bandwurmen. **Zeitschrift für Parasitenkunde**, **2**: 157-177.
- Sekutowicz, S.I. 1934. Untersuchungen zur Entwicklung und Biologie von **Caryophyllaeus laticeps** (Pall.) **Memoires de l' Academic Polonaise des Sciences et des lettres Serie B, Science Naturelles.** **6**: 1-26.
- Shinde, G.B. 1970. A new species and two new varieties of the genus **Lytocestoides** Baylis, 1928 in fresh-water fishes in Maharashtra, India. **Marathwada University Journal of Science**, **9**: 173-178.
- _____ and Deshmukh, R.A. 1975. On a new species of **Lytocestoides**, Baylis (1928) from a fresh-water fish in Maharashtra India. **Nat. Sci. J. Marath. Univ.** **14**: 233-236.
- Shulman, R.E. 1979. Dependence of the seasonal dynamics of fish parasite on some environmental factors. In **Ekologicheskay i Eksperimental naya Parazitologiya, Vypusk 2**, (Yu I. Lopyanskogo, ed.) pp.117-136. Izdatel'stv Leningradskogo Universiteta, Leningrad.
- Simha, S.S. and Rasheed, U. 1981. An anomaly in caryophyllid cestode, **Lytocestus indicus** Moghe, 1931. **Indian Journal of Parasitology**, **5**: 197-198.
- Singh, S.S. 1975. On **Lytocestus fossilis** n.sp. (Cestoda: Lytocestidae from **Heteropneustes fossilis** from Nepal. In **Dr B.S. Chauhan Commemoration Volume, 1975**. (eds Tiwari K.K. and Srivastava, C.B. Orissa, India. **Zoological Society of India.** 79-82.
- Sircar, Mira and Sinha, D.P. 1974. Haematological investigations on pigeons and **Clarias batrachus** carrying cestode infection. **Annals of Zoology (Agra)**, **10**: 1-11.

- Smyth, J.D. 1947. Studies on Tapeworm Physiology. III. Aseptic Cultivation of larval Diphylobthriidae in vitro. **Journal of Experimental Biology**, **24**: 374-386.
- _____ 1949. Studies on Tapeworm Physiology. IV. Further observation on the development of *Ligula intestinalis* in vitro. **Journal of Experimental Biology**, **26**: 1-14.
- _____ 1956. Studies on Tapeworm Physiology. IX. A histochemical study of egg-shell formation in *Schistocephalus solidus* (Pseudophyllidae). **Experimental Parasitology**, **5**: 519-540.
- _____ 1969. **The Physiology of Cestodes**. University Review in Biology. Oliver and Boyd. 279pp.
- _____ 1972. In Functional Aspects of Parasite surfaces, **Symposia for the British Society for Parasitology** (Edited by Taylor, A.E. and Muller, R.), **10**: 41-70.
- Swiderski, Z.; Eklun-Natey, R.; Subilia, L. and Huggel, H. 1978. Fine structure of the vitelline cells in the cestode *Proteocephalus longicollis* (Proteocephalidea). **Proceedings of the Ninth International Congress on Electron Microscopy**, Toronto. II. 442-443.
- _____; Huggel, H. and Schonenberger, N. 1970. Comparative fine structure of vitelline cells in cyclophyllidean cestodes. **Septieme Congres International de Microscopie Electronique Grenoble**, 825-826.
- _____ and Mackiewicz, J.S. 1976. Electron microscope study of vitellogenesis in *Glaridacris catastomi* (Cestoidea: Caryophyllidea). **International Journal for Parasitology**, **6**: 61-73.
- _____ and Mokhtar, F. 1974. Etude de la vitellogenese de *Bothriocephalus clavibothrium* Ariola, 1899. (Cestoda Pseudophyllidea). **Zeitschrift für Parasitenkunde**, **43**: 135-149.
- Szidat, L. 1937. *Archigetes* R. Leuckart, 1878. Die progenetische Larvae einer für Europa neuen Caryophyllaeiden-Gattung *Biacetabulum* Hunter, 1927. **Zoologischer Anzeiger**, **119**: 166-172.
- Thomas, J.d. 1964. A comparison between the helminth burdens of male and female brown trout *Salmo trutta* L. from a natural population in the river Teify, West Wales. **Parasitology**, **54**: 263-272.

- Tkachuck, R.; Saz, H.; Weinstein, P. Finnengan, K. and Mueller, J. 1977. The presence and possible function of methylmalonyl CoA mutase and propionyl CoA carboxylase in *Spirometra mansenoides*. **Journal of Parasitology**, **63**: 769-774.
- Verma, S.L. 1971. Helminth parasites of fresh-water fishes. Part I. On two Caryophyllaeids from fresh-water fishes of Lucknow. **Indian Journal of Helminthology**, **23**: 71-80.
- Vykhrestyuk, N.P.; Yarygina, G.V. and Ilgasov, I.N. 1981. Lipids of the cestodes *Raillietina tetragona* and *Raillietina echinobothrida* from the intestine of chickens. **Parasitologia**, **15**: 525-583.
- Wabuke-Bunoti, M.A.N. 1980. The prevalence and Pathology of the cestode *Polyonchobothrium clarias* (Woodland, 1925) in the teleost, *Clarias mossambicus* (Peters). **Journal of Fish Diseases**, **3**: 223-230.
- Waitz, J.A. 1963. Histochemical studies of the Cestode *Hydatigera taeniaeformis* Batsch, 1786. **Journal of Parasitology**, **49**: 73-80.
- _____, Allan and James, L. Schardein. 1964. Histochemical studies of four cyclophyllidean cestodes. **Journal of Parasitology**, **5**: 271-277.
- Wardle, R.A. 1937. The Physiology of the sheep tapeworms, *Moniezia expansa* Blanchard. **Canadian J. Research D**. **15**: 117-126.
- _____, and MeLeod, J.A. 1952. **The Zoology of Tapeworms**. University of Minnesota Press. Minneapolis, 780pp.
- _____, MeLeod, J.A. and Radinovsky, S. 1974. **Advances in the Zoology of Tapeworms. 1950-1970**. University of Minnesota Press, Minneapolis, 274pp.
- Will, H. 1893. Anatomie von *Caryophyllaeus mutabilis* Rud. Ein Beitrag zur Kenntnis der Cestoden. **Zeitschrift für Wissenschaftliche, Zoologie**, **56**: 1-39.
- Williams, D.D. and Ulmer, M.J. 1970. Caryophyllaeid cestodes from species of *Carpiodes* (Teleostei: Catastomidae). **Proceedings of the Iowa Academy of Science**, **77**: 185-195.
- Wisniewski, L.W. 1930. Das Genus *Archigetes* R. Leuck. Eine Studie zur Anatomie, Histogenese, Systematik und Biologie. **Memoires de L'Academie Polonaise des Sciences et des Lettres, Classe des Sciences Mathematiques et Naturelles, Serie B. Sciences Naturelles**, **2**: 160pp.

- Woodland, W.N.F. 1923. On some remarkable new forms of Caryophyllaeidae from the Anglo-Egyptian Sudan, and a revision of the families of the Cestodaria. **Quarterly Journal of Microscopical Science**, (New Series), **67**: 435-472.
- _____ 1926. On the genera and possible affinities of the Caryophyllaeidae: A reply to Drs. O. Fuhrmann and J.G. Baer. **Proceedings of the Zoological Society of London**, 49-69.
- _____ 1937. Some cestodes from Sierra Leone. I. On *Wenyonia longicauda* sp. n. and *Proteocephalus bivittellatus* sp. n. **Proceedings of the Zoological Society of London**, (1936), 931-937.
- Winder, W. 1939. Das Jahreszeitliche Auftreten des Bandwurmes *Caryophyllaeus laticeps* Pall. im Darm des Karpfens (*Cyprinus carpio* L.) **Zeitschrift für Parasitenkunde**, **10**: 704-713.
- Yamaguti, S. 1959. **Systema Helminthum**. Vol. II. The Cestodes of Vertebrates. Inter-Science Publishers, Inc. New York. 860pp.
- Yamane, Y. 1968. On the fine structure of *Diphyllobothrium erinacei* with special reference to the tegument. **Yonago Acta Medica**, **12**: 169-181.
- _____: Maejima, J. and Yazaki, S. 1975. SEM observations on the tegumental surface structure of diphyllobothriid cestodes. **Yonago Acta Medica**, **19**: 197-206.
- Yamao, Y. 1952a. Histochemical studies in endoparasites. VII. Distribution of glyceromonophosphatases in the tissues of the cestodes, *Anoplocephala perfoliata* A. magna, *Moniezia benedeni*, *M. expansa* and *Taenia taeniaeformis*. **Zool. Magazine (Jap.)** **61**: 254-260.
- _____ 1952b. Histochemical Studies on endoparasites. IX. On the distribution of glycogen. **Zool. Mag. Tokyo**. **61**: 317-322. (Jap. text; Eng. summary).

No. 102010
 No. by R. N. 25-10-8
 Class by
 Sub. Heading by
 Date by
 Transcribed by