

International Journal of Research in Biosciences
Vol. 6 Issue 1, pp. (26-41), January 2017
Available online at <http://www.ijrbs.in>
ISSN 2319-2844

Research Paper

Redescription and new locality record of some helminth parasites of *Clarias batrachus* in Tripura, India

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(Received June 30, 2016, Accepted September 15, 2016)

Abstract

The present study was taken to evaluate the occurrence of different helminth parasites in the freshwater catfish *Clarias batrachus*, from different parts of Tripura, India during the period of April, 2012 to March, 2015. A total of 860 fishes was collected from different sites like Agartala, Dharmanagar, Pecharthal, Damcherra, Kumarghat and Udaipur, and were dissected to recover different intestinal helminth parasites. Helminth parasites recovered from the fish were processed for whole mount preparation following standard procedure. The parasites recovered were identified as *Astiotrema reniferum* (trematode) *Lytocestus indicus*, *L. birmanicus*, *L. longicollis*, *L. attenuatus*, *L. clariae*, *L. filiformes*, *Djombangia penetrans* (cestodes) and one larval nematode belonging to the genus *Anisakis*.

Keywords: *Clarias batrachus*, helminth parasites, Tripura

Introduction

Fish is an important and cheap source of proteins, lipids, vitamins, oil and minerals^[1]. Majority of the fishes are known to be infected with diverse forms of parasites that lead to the deterioration of food value of the fishes, often leading to high mortality rates. Fishes also act as the intermediate hosts for the transmission of many zoonotic helminths. Recently, the fish-borne helminthiasis has become more widely distributed with greater economical and medical impacts than what was recognised earlier^[2].

The Clariid fish, *Clarias batrachus* is cosmopolitan in distribution and is one of the highly preferable food fishes of the state Tripura. The presence of helminth parasites is detrimental to a large extent for a fish population imposing great loss to the fish farmers^[3]. Several workers have studied the helminth fauna in the fish hosts and also described several new species from India^[4-14]. However, only limited information is available about the fish parasites of northeast India, and is mostly restricted to Meghalaya, Arunachal Pradesh and Assam^[15-19]. Except for few preliminary records, no literature is available about the helminths diversity among different edible and economically important fishes of Tripura^[20-21]. The present study deals with the occurrence of different helminth parasites in the freshwater catfish, *Clarias batrachus*, collected from the state of Tripura for a period of three years extending from April, 2012 to March, 2015.

Materials and Methods

The fish, *C. batrachus*, were collected from different locations namely, Agartala, Dharmanagar, Pecharthal, Damcherra, Kumarghat and Udaipur during April, 2012 to March, 2015. A total of 860 *C. batrachus* were examined, out of which 308 were infected with different helminth parasites. The trematode and cestode parasites were stretched over a clean slide fixed in 70% alcohol and stained with borax carmine. They were processed through a series of graded alcohol, cleared in xylene and

mounted in DPX. The nematode parasites were cleared in ascending series of graded glycerol and mounted using glycerin jelly. The parasites were identified following standard literature^[22-24].

Results and Discussion

A total of nine different helminth species were recovered during the study. These included one representative of trematode group (*Astiotrema reniferum*), seven representatives of cestode (*Lytocestus indicus*, *L. birmanicus*, *L. longicollis*, *L. attenuates*, *L. filiformes*, *L. clariae* and *Djombangia penetrans*) and one larval nematode belonging to the genus *Anisakis* (Figure 1-9). The measurements of all the forms studied herein are given in Table 1-9.

Phylum- Platyhelminthes

Class- Trematoda

Order- Plagiorchiida

Family- Plagiorchiidae

Genus- *Astiotrema*

1. *Astiotrema reniferum* (LOOSS 1898) STOSSICH 1904 (FIGURE 1) (TABLE 1)

Material: A total of 3 specimens of *A. reniferum* were collected.

Description: Body elliptical with rounded extremities; subterminal oral sucker, spined integument, spines sparse by distribution towards the posterior extremity; Esophagus is twisted 'S'-shaped; Intestine bifurcate between two suckers; intestinal caeca broad to narrow, more or less straight, terminate near the posterior extremity; acetabulum median, pre-equatorial, almost equal, slightly smaller than the oral sucker; tandem arrangement of the gonads; vitellaria on the lateral sides of the body; excretory pore terminal.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E)

Remarks- *Astiotrema reniferum* is a rare parasite obtained during the study. *Astiotrema reniferum* was described by Looss from turtle, *Trionyx*^[25]. Of the 21 species listed, only four are considered to be valid, viz., *A. reniferum* (Looss, 1898) Stossich, 1904, *A. implementum* Looss, 1898, *A. monticelli* Stossich, 1904 and *A. odhneri* Bhalerao, 1937^[26]. Tiwari reported two species *A. lobiorchis* and *A. mehrai* from India which was later synonymized with *A. reniferum* by Siddiqi^[27-28]. *A. reniferum* was reported from *Clarias mossambicus* in Rhodesia by Beverley-Burton and Agarwal reported it from *Heteropneustus fossilis* from India^[29-30]. Later, *A. reniferum* was redescribed by El-Naggar et al. from *C. lazera* in Egypt^[31]. The present study is the first report of the occurrence of *A. reniferum* from Tripura, India.

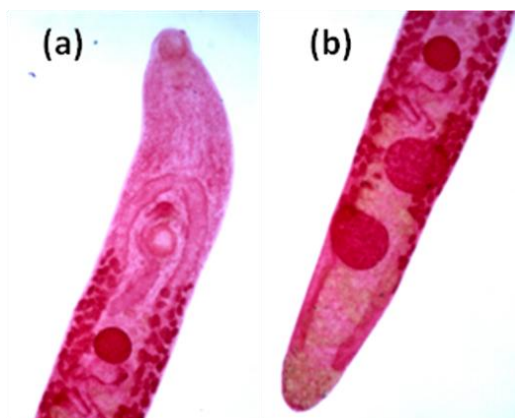


Figure 1: Light microscope images of *Astiotrema reniferum* (a) Anterior end and (b) Posterior end

Table 1: Morphometric measurement (IN MM) and characters of the species of *A. reniferum* (mean \pm standard deviation) reported from Tripura, India

Characters	<i>A. Reniferum</i>
Body length	3.2-3.4 (3.3 \pm 0.1)
Breadth/ Diameter	0.41-0.49 (0.45 \pm 0.04)
Oral sucker	0.11-0.13 (0.12 \pm 0.01)
(a) Length	0.11-0.13 (0.12 \pm 0.01)
(b) Breadth	
Pre-pharynx	0.02-0.03 (0.03 \pm 0.01)
(a) Length	0.05-0.08 (0.07 \pm 0.02)
(b) Breadth	
Pharynx	0.07-0.09 (0.08 \pm 0.01)
(a) Length	0.09-0.11 (0.10 \pm 0.01)
(b) Breadth	
Oesophagous	0.29-0.32 (0.31 \pm 0.02)
(a) Length	0.04-0.05 (0.05 \pm 0.01)
(b) Breadth	
Acetabulum	0.12-0.13 (0.13 \pm 0.01)
(a) Length	0.15-0.16 (0.16 \pm 0.01)
(b) Breadth	
Anterior testes	0.15-0.17 (0.16 \pm 0.01)
(a) Length	0.16-0.18 (0.17 \pm 0.01)
(b) Breadth	
Posterior testes	0.16-0.17 (0.16 \pm 0.02)
(a) Length	0.20-0.21 (0.21 \pm 0.02)
(b) Breadth	
Cirrus sac	0.30-0.33 (0.31 \pm 0.01)
Ovary	0.10-0.11 (0.10 \pm 0.01)
(a) Length	0.12-0.13 (0.13 \pm 0.01)
(b) Breadth	

Class- Cestoda

Order- Caryophyllidea

Family: Lytocestidae Hunter, 1927

Genus: *Lytocestus* Cohn, 1908

2. *Lytocestus indicus* (Moghe 1925) Woodland, 1926 (Figure 2) (Table 2)

Material: A total of 1167 specimens of *L. indicus* were collected.

Description: Body broad and flat, with traces of external segmentation; Scolex short, bluntly rounded and unarmed, markedly narrower than body and provided with longitudinal furrows in some specimens; neck very short and distinct; testes numerous, occupying medullary region of the body, ovoid in shape, larger than vitelline follicles and extending from base of neck to the cirrus sac region posteriorly; cirrus sac is prominent, opening separately below the utero-vaginal pore; ovary bi-lobed, wing like in shape, follicular, the two lobes of ovary joined to each other by an ovarian isthmus; vagina distinct, joining terminal end of the uterus to open unitedly to the exterior at the utero-vaginal pore; vitelline follicles cortical, in a ring around testes, no post-ovarian vitelline follicles present; eggs oval in shape, smooth, embryonated; excretory pore terminal.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E), Damcherra (23.72124°N and 91.74458°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: The species, *L. indicus*, was first described as *Caryophyllaeus indicus* from the common Indian Siluroid fish, *Clarias batrachus*^[32]. The species was shifted from *Caryophyllaeus* to *Lytocestus* after clearing the doubt regarding the presence of post ovarian vitelline follicles and maintained that

they were in reality ovarian follicles^[33]. The present observations are in conformity with those of Moghe^[32], except for minor deviations in size of the body and its organs.

Lytocestus indicus was earlier reported from the same host in Guwahati (Assam)^[15]. The present study is the first report of the occurrence of *L. indicus* from Tripura, India.

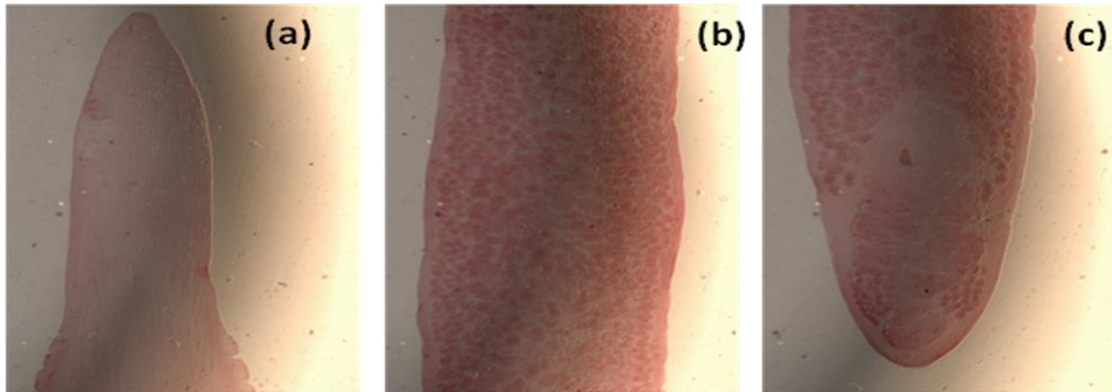


Table 2: Morphometric measurement (in mm) and characters of the species *Lytocestus indicus* (mean ± Standard Deviation) reported from Tripura, India

Characters	<i>L. indicus</i>
Body length	6.3-6.6 (6.48±0.10)
Max. Breadth	1.75-1.95 (1.86±0.07)
Length of scolex	0.39-0.51 (0.46±0.05)
Length of neck	0.53-0.56 (0.55±0.01)
Testicular follicles	
(a) Length	0.03-0.13 (0.09±0.04)
(b) Breadth	0.03-0.11 (0.07±0.04)
Ovarian lobes	
(a) Length	0.02-0.03 (0.03±0.01)
(b) Breadth	0.02-0.03 (0.03±0.01)
Pre-testis distance	1.90-1.98 (1.94±0.03)
Pre vitellaria distance	1.36-1.41 (1.40±0.02)
Distance between anterior extent of testes and vitellaria	0.55-0.57 (0.56±0.01)
Position of genital pore from posterior extremity	0.86-0.89 (0.88±0.01)
Eggs	
(a) Length	-
(b) Breadth	
Vitelline follicles	
(a) Length	0.02-0.05 (0.03±0.01)
(b) Breadth	0.02-0.04 (0.03±0.01)

3. *Lytocestus birmanicus* Lynsdale 1956 (Figure 3) (Table 3)

Material: A total of 596 specimens of *L. birmanicus* were collected.

Description: Body flattened, elongated, posterior end broader than the anterior with traces of external segmentation; scolex lanceolate, smooth and narrows to form neck that gradually widens into posterior part of body; testes numerous, medullary in disposition, spherical or oval in shape, extending a short distance from behind anterior vitellaria to cirrus sac posteriorly; cirrus sac oval, lined by a thick muscular wall, opening slightly anterior to utero-vaginal pore; ovary bi-lobed, follicular, cortical and extends posteriorly behind Mehlis gland, united by a median isthmus; Mehlis gland prominent behind ovarian isthmus; uterine coils glandular, extending up to level of cirrus sac; vagina a straight tube, joins uterus at proximal part to open unitedly at utero-vaginal pore. Vitellaria transversely elongated, cortical, arranged in an annular manner in space between two longitudinal muscle layers, extend as far as utero-vaginal aperture; vitelline follicles appear concentrated in two lateral bands on either side

of the body but some are also scattered in median field, no post-ovarian vitelline follicles present. Eggs smooth, oval in shape.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E), Damcherra (23.72124°N and 91.74458°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: *L. birmanicus* was first described from the intestine of *Clarias batrachus* in Rangoon, Burma^[34]. The present observations are in conformity with those of Lynsdale^[34] except for minor deviation with regards to measurements of the body. The species was earlier reported from the same host but from a different location, i.e. Guwahati (Assam)^[15].

Occurrence of *L. birmanicus* from different parts of Tripura, India are new locality records for the species.

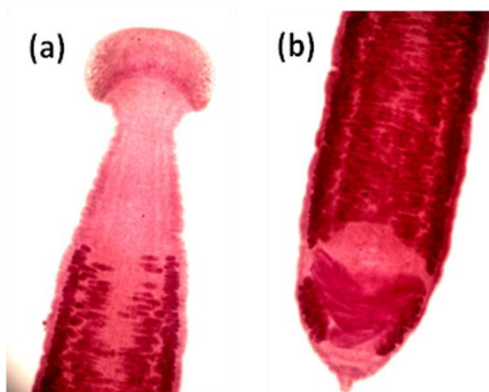


Figure 3: Light microscope images of *Lytocestus birmanicus* (a) Anterior end and (b) Posterior end

Table 3: Morphometric measurement (in mm) and characters of the species *Lytocestus birmanicus* (mean ± Standard Deviation) reported from Tripura, India

Characters	<i>L. birmanicus</i>
Body length	5.01-5.03 (5.02±0.01)
Max. Breadth	1.05-1.20 (1.14±0.05)
Length of scolex	0.50-0.55 (0.54±0.02)
Length of neck	1.30-1.37 (1.351±0.03)
Testicular follicles	
(a) Length	0.06-0.14 (0.107±0.02)
(b) Breadth	0.03-0.04 (0.033±0.01)
Ovarian lobes	
(a) Length	0.03-0.04 (0.034±0.01)
(b) Breadth	0.02-0.03 (0.024±0.01)
Pre-testis distance	1.990-1.92 (1.909±0.01)
Pre vitellaria distance	2.00-2.03 (2.022±0.01)
Distance between anterior extent of testes and vitellaria	0.10-0.11 (0.106±0.01)
Position of genital pore from posterior extremity	0.09-0.10 (0.096±0.01)
Eggs	
(a) Length	0.02-0.03 (0.024±0.01)
(b) Breadth	0.01-0.02 (0.014±0.01)
Vitelline follicles	
(a) Length	0.05-0.07 (0.061±0.01)
(b) Breadth	0.04-0.06 0.051±0.01)

4. *Lytocestus longicollis* Rama Devi 1973 (Figure 4) (Table 4)

Material: A total of 572 specimens of *L. longicollis* were collected.

Description: Body ribbon-like, long and slender; scolex unarmed, undifferentiated and may vary from spatulate or oblong in relaxed specimens to swollen and pear-shaped in contracted ones; neck long, slender occupying one-third of body length; testes numerous, occupying medullary region of the body, spherical in shape, extending from anterior narrow end of the body to cirrus sac posteriorly; cirrus sac oval, lined by a thin muscular wall, enclosing long ductus ejaculatorius that opens separately from utero-vaginal pore; ovary bi-lobed, follicular, H-shaped, connected by band-like ovarian isthmus, cortical in disposition; Mehlis' gland situated posterior to isthmus in between two ovarian lobes, uterine coils glandular, extending from behind ovarian isthmus to level of cirrus pore beyond anterior horns of ovary; vagina straight or slight convoluted, opening unitedly with uterus as utero-vaginal pore, receptaculum seminis a conspicuous sac, oval in shape, lying anterior to ovarian isthmus; vitellaria cortical, in a ring around testes, lobular, smaller than testes, extending from a little anterior to testes up to cirrus sac, no post-ovarian vitelline follicles present; eggs oval in shape, smooth, operculate; excretory pore terminal.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E), Damcherra (23.72124°N and 91.74458°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: *L. longicollis* was originally described from *Clarias batrachus* in Visakhapatnam district of Andhra Pradesh^[35]. This species was distinguished from rest of the Lytocestid types in having a receptaculum seminis, which is absent in other species. Earlier the species was reported from Guwahati (Assam) in Northeast India^[15].

The present study is the first report of the occurrence of *L. longicollis* from different parts of Tripura, India.

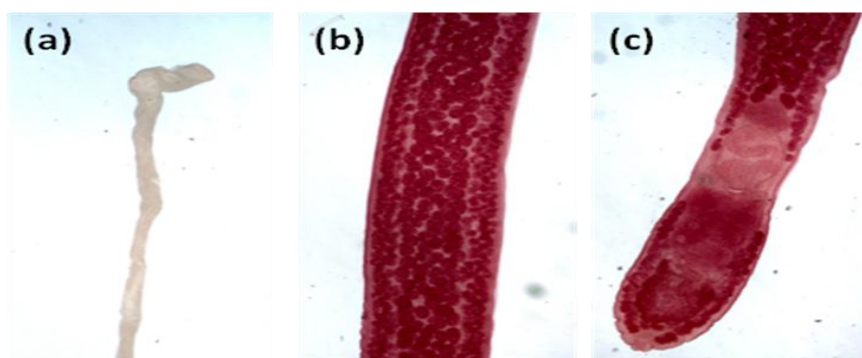


Figure 4: Light microscope images of *Lytocestus longicollis* (a) Anterior end, (b) Middle region and (c) Posterior end

Table 4: Morphometric measurement (in mm) and characters of the species *Lytocestus longicollis* (Mean \pm Standard Deviation) reported from Tripura, India

Characters	<i>L. longicollis</i>
Body length	14.76-15.98 (15.638 \pm 0.42)
Max. Breadth	0.38-0.45 (0.411 \pm 0.03)
Length of scolex	0.20-0.25 (0.233 \pm 0.02)
Length of neck	7.91-7.97 (7.952 \pm 0.02)
Testicular follicles	
(a) Length	0.05-0.06 (0.056 \pm 0.01)
(b) Breadth	0.06-0.08 (0.07 \pm 0.01)
Ovarian lobes	0.03-0.04 (0.036 \pm 0.01)

(a) Length	0.04-0.05 (0.046±0.01)
(b) Breadth	
Pre-testis distance	7.91-7.97 (7.952±0.02)
Pre vitellaria distance	9.15-9.18 (9.171±0.01)
Distance between anterior extent of testes and vitellaria	1.20-1.22 (1.212±0.01)
Position of genital pore from posterior extremity	0.95-1.04 (1.002±0.03)
Eggs	
(a) Length	-
(b) Breadth	
Vitelline follicles	
(a) Length	0.03-0.05 (0.036±0.01)
(b) Breadth	0.02-0.04 (0.026±0.01)

5. *Lytocestus attenuatus* Tandon, Chakravarty and Das 2005 (Figure 5) (Table 5)

Material: A total of 350 specimens of *L. attenuatus* were collected.

Description: Body thin, slender, elongated and flattened, posterior end broader than anterior; scolex smooth, undifferentiated, unarmed with bluntly rounded extremity, followed by a long narrow neck; testes ovoid, longer than vitelline follicles, occupying the medullary region and extending from a little posterior to anterior vitelline follicles caudad up to cirrus sac; cirrus sac medullary, enclosing a thin winding ejaculatory duct and opening separately from, and anterior to, utero-vaginal pore; ovary bilobed, follicular, bent inwards, inverted 'A'-shaped, lobes extending to 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 ovarian isthmus; uterus glandular, extending from behind Mehlis' gland; vagina distinct, straight or slightly convoluted, joining terminal end of uterus to open at utero-vaginal pore; vitelline follicles ovoid, arranged in two rows lateral to testes extending from a little anterior to testes up to cirrus sac, no post-ovarian vitelline follicles present; excretory pore terminal.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E), Damcherra (23.72124°N and 91.74458°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: In India, *L. attenuatus* was reported for the first time from Guwahati (Assam)^[15]. This species comes closer to *L. longicollis*, *L. parvulus*, *L. fossilis*, *L. filiformes* and *L. javanicus* in sharing the characters such as shape of the body, which is thin, slender, elongated and undifferentiated scolex,. However, it differs from each of them in certain characters like: from *L. longicollis* in not having 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; from *L. filiformes* in having oval and large-sized vitelline follicles; from *L. javanicus* in the absence of a long neck and from *L. clariae* in having separate genital apertures. The present study is the first report of the occurrence of *L. attenuatus* from different parts of Tripura, India.

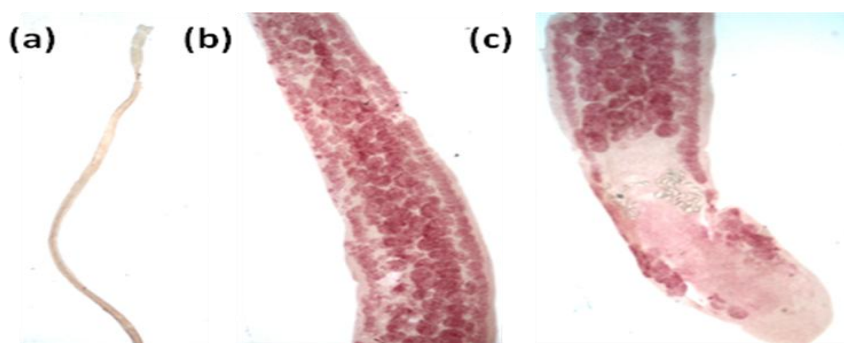


Figure 5: Light microscope images of *Lytocestus attenuatus* (A) Anterior end, (B) Middle region and (c) Posterior end

Table 5: Morphometric measurement (in mm) and characters of the species *Lytocestus attenuatus* (Mean \pm Standard Deviation) reported from Tripura, India

Characters	<i>L. attenuatus</i>
Body length	12.98-13.66 (13.531 \pm 0.20)
Max. Breadth	0.67-0.77 (0.732 \pm 0.04)
Length of scolex	0.14-0.16 (0.148 \pm 0.01)
Length of neck	4.98-5.82 (5.724 \pm 0.26)
Testicular follicles	
(a) Length	0.07-0.08 (0.074 \pm 0.01)
(b) Breadth	0.02-0.04 (0.032 \pm 0.01)
Ovarian lobes	
(a) Length	0.04-0.07 (0.056 \pm 0.01)
(b) Breadth	0.03-0.05 (0.041 \pm 0.01)
Pre-testis distance	6.0-6.45 (6.31 \pm 0.12)
Pre vitellaria distance	5.67-5.82 (5.775 \pm 0.05)
Distance between anterior extent of testes and vitellaria	0.50-0.56 (0.4965 \pm 0.16)
Position of genital pore from posterior extremity	0.95-1.04 (1.002 \pm 0.03)
Eggs	
(a) Length	0.02-0.03 (0.024 \pm 0.01)
(b) Breadth	0.01-0.02 (0.014 \pm 0.01)
Vitelline follicles	
(a) Length	0.05-0.07 (0.06 \pm 0.01)
(b) Breadth	0.02-0.03 (0.024 \pm 0.01)

6. *Lytocestus clariae* Tandon, Chakravarty and Das 2005 (Figure 6) (Table 6)

Material: A total of 272 specimens of *L. clariae* were collected.

Description: Body elongated, tapering anteriorly; scolex undifferentiated, smooth and unarmed with bluntly tapering extremity, followed by a short neck, devoid of any reproductive organs; testes numerous, occupying medullary region of body, ovoid in shape, larger than vitelline follicles, extending from a little behind anterior follicles of vitellaria posteriorly up to cirrus sac; cirrus sac a compact parenchymatous bulb, enclosing ductus ejaculatorius; cirrus opening joining distally terminal part of female duct to open to the exterior by a common pore; ovary bi-lobed, H-shaped, follicular, extending behind Mehlis' gland, the lobes cortical in disposition and joined to each other by ovarian isthmus, which is medullary; Mehlis' gland present behind ovarian isthmus; uterus glandular, extending from in front of isthmus up to cirrus sac; vaginal tube joining uterus at its distal end to open unitedly at the shallow atrium; vitelline follicles ovoid in shape, commencing from a short distance anterior to testes up to level of cirrus sac, arranged in two rows lateral to testes; no post-ovarian vitelline follicles present; oval shaped eggs are spinous and operculate; excretory pore terminal.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E), Damcherra (23.72124°N and 91.74458°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: This species was first described from *Clarias batrachus* from Guwahati (Assam)^[15]. When compared with the known species of *Lytocestus*, which stands close to *L. javanicus*, *L. longicollis*, *L. filiformes*, *L. parvulus* and *L. fossilis* in possessing an undifferentiated scolex that tapers anteriorly, in the ovarian lobes behind 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. The present form comes closer to *L. indicus* and *L. birmanicus* in having a short neck and also in the extent of testes and vitellaria. However, it differs from all of them in having confluent genital apertures and spinous eggs. All the species mentioned above have distinctly separated genital apertures and smooth-surfaced eggs.

The present study is the first report of the occurrence of *L. clariae* from different parts of Tripura, India.

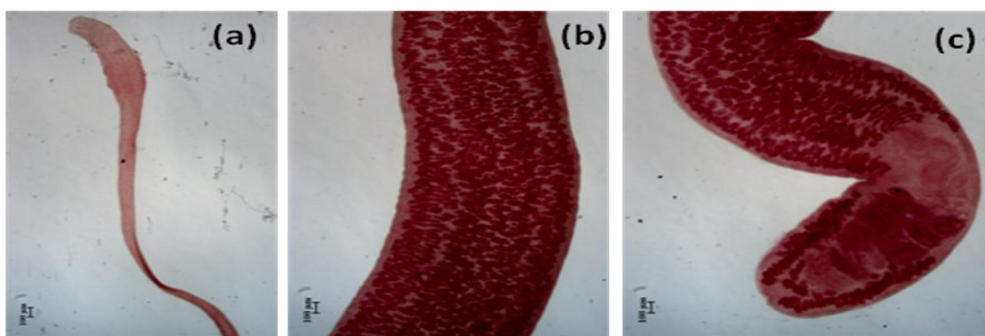


Figure 6: Light microscope images of *Lytocestus clariae* (a) Anterior end, (b) Middle region and (c) Posterior end

Table 6: Morphometric measurement (in mm) and characters of the species *Lytocestus clariae* (Mean \pm Standard Deviation) reported from Tripura, India

Characters	<i>L. clariae</i>
Body length	13.59-14.02 (13.929 \pm 0.14)
Max. Breadth	1.13-1.28 (1.221 \pm 0.05)
Length of scolex	0.87-0.96 (0.925 \pm 0.04)
Length of neck	6.90-6.92 (6.912 \pm 0.01)
Testicular follicles	
(a) Length	0.03-0.06 (0.44 \pm 0.01)
(b) Breadth	0.11-0.16 (0.141 \pm 0.02)
Ovarian lobes	
(a) Length	0.03-0.05 (0.042 \pm 0.01)
(b) Breadth	0.06-0.09 (0.076 \pm 0.01)
Pre-testis distance	8.67-8.69 (8.683 \pm 0.01)
Pre vitellaria distance	7.85-7.87 (7.863 \pm 0.01)
Distance between anterior extent of testes and vitellaria	0.80-0.82 (0.813 \pm 0.01)
Position of genital pore from posterior extremity	0.86-0.89 (0.88 \pm 0.01)
Eggs	
(a) Length	-
(b) Breadth	-
Vitelline follicles	
(a) Length	0.02-0.04 (0.03 \pm 0.01)
(b) Breadth	0.06-0.11 (0.077 \pm 0.01)

7. *Lytocestus filiformes* (Woodland 1923) Fuhrmann and Baer 1925 (Figure 7) (Table 7)

Material: A total of 7 specimens of *L. filiformes* were collected.

Description: Body flat, ribbon-like, elongated, posterior end broader than anterior end; scolex smooth, undifferentiated, variable in shape, either flat or pointed in some; neck long and slender; testes numerous, occupying the medullary region of the body, spherical or oval in shape extending from behind neck up to cirrus sac posteriorly; cirrus lined by a thin muscular wall, opening separately from utero-vaginal pore; ovary bi-lobed, follicular, cortical, two lobes joined to each other by an ovarian isthmus; Mehlis' gland well developed, behind ovarian isthmus; uterine coils glandular, extending from behind isthmus beyond anterior horns of ovary up to cirrus sac; vagina distinct, joins uterus distally to open at utero-vaginal pore; vitellaria cortical, smaller than testes, spherical or oval in shape, forming a crescent around testes; no post-ovarian vitelline follicles present; eggs smooth, operculate and oval in shape; excretory pore terminal.

Location: Intestine

Locality: Pecharthal (24.189879°N and 92.099274°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: *L. filiformes* was first described as *Caryophyllaeus filiformes* from a mormyrid fish host, *Mormyrus cashive*, from the river Nile at Khatoum^[33]. Later, it was shifted to the genus *Lytocestus*, on

the basis of cortical disposition of vitellaria and medullary disposition of testes^[36]. This species was reported from the same fish host from Guwahati (Assam) and provided the measurements of various organs to supplement the original description^[15].

The present study is the first report of the occurrence of *L. filiformes* from Tripura, India, thus form new locality records.

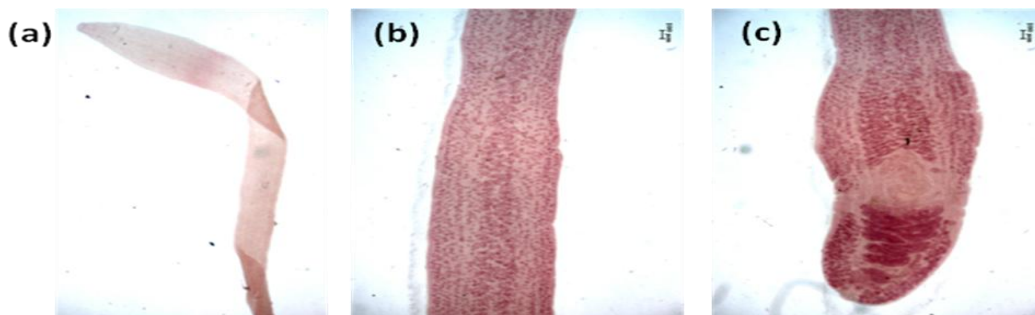


Figure 7: Light microscope images of *Lytocestus filiformes* (a) Anterior end, (b) Middle region and (C) Posterior end

Table 7: Morphometric measurement (in mm) and characters of the species *Lytocestus filiformes* (Mean ± Standard Deviation) reported from Tripura, India

Characters	<i>L. filiformes</i>
Body length	16.4-17.0 (16.83±0.19)
Max. Breadth	0.49-0.62 (0.568±0.05)
Length of scolex	0.87-0.96 (0.925±0.04)
Length of neck	5.0-5.5 (5.21±0.17)
Testicular follicles	0.03-0.06 (0.044±0.01)
(a) Length	0.11-0.16 (0.141±0.02)
(b) Breadth	
Ovarian lobes	0.03-0.04 (0.036±0.01)
(a) Length	0.04-0.05 (0.046±0.01)
(b) Breadth	
Pre-testis distance	1.90-1.98 (1.94±0.03)
Pre vitellaria distance	5.67-5.82 (5.775±0.05)
Distance between anterior extent of testes and vitellaria	0.80-0.82 (0.813±0.01)
Position of genital pore from posterior extremity	0.09-0.10 (0.096±0.01)
Eggs	-
(a) Length	
(b) Breadth	
Vitelline follicles	0.05-0.07 (0.06±0.01)
(a) Length	0.02-0.03 (0.024±0.01)
(b) Breadth	

Genus: *Djombangia*

8. *Djombangia penetrans* Bovien 1926 (Figure 8) (Table 8)

Materials: A total of 29 specimens of *D. penetrans* were collected.

Description: Body short, flask-shaped, broad and fleshy; scolex globular with a terminal sucker; neck marked off from body; testes spherical or ovoid, extending in two lateral rows, from some distance behind neck up to level just in front of ovary; cirrus pouch not well defined, opening into common atrium close to posterior extremity, just in front of ovarian isthmus; ovary bi-lobed, two lobes joined to each other by ovarian isthmus; uterus partly glandular, its coils large in median field of medulla, reaching cephalad up to 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.

Location: Intestine

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Kumarghat (24.1583°N and 92.0297°E) and Agartala (23.8333°N and 91.2667°E)

Remarks: Bovien^[37] established the genus *Djombangia* with *D. penetrans* as its type species, with *C. batrachus*, as type host. The diagnostic features of this genus are body short, broad, flask-shaped distinct sucker at the tip of the scolex, neck marked off from the body, no post-ovarian vitelline follicles, cirrus sac not prominent and spinose eggs. Satpute and Agarwal^[38] described another species *D. indica* from *C. batrachus* in Raipur with a characteristics of no post-ovarian vitelline follicles and in the absence of a prominent cirrus sac and receptaculum seminis. Two more Indian species added to the genus are *D. caballeroi* Sahay and Sahay, 1977 from *H. fossilis* in Bihar and *D. clariae* Kundu, Bhattacharya and Datta, 1985 from *C. batrachus* in West Bengal both of which were reported to be having smooth, non-spinous eggs. In the opinion of Mackiewicz^[39], the genus *Djombangia* is represented by 2 species in India, i.e., *D. penetrans* and *D. indica*. *D. penetrans* has earlier been reported from *C. batrachus* in Guwahati (Assam) in Northeast India by Chakravarty and Tandon^[15]. The present study is the first report of the occurrence of *D. penetrans* from different parts of Tripura, India.

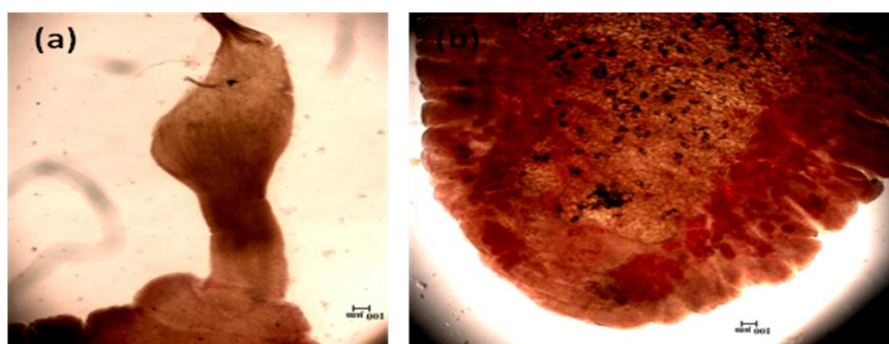


Figure 8: Light microscope images of *Djombangia penetrans* (a) Anterior end and (b) Posterior end

Table 8: Morphometric measurement (in mm) and characters of the species *Djombangia penetrans* (Mean \pm Standard Deviation) reported from Tripura, India

Characters	<i>D. penetrans</i>
Body length	6.1-7.5 (6.8 \pm 0.45)
Max. Breadth	3.0-4.0 (3.51 \pm 0.32)
Length of scolex	0.82-1.96 (1.3 \pm 0.39)
Length of neck	0.29-0.62 (0.445 \pm 0.11)
Testicular follicles	
(a) Length	0.06-0.09 (0.072 \pm 0.01)
(b) Breadth	0.04-0.07 (0.049 \pm 0.01)
Ovarian lobes	
(a) Length	0.21-0.64 (0.471 \pm 0.15)
(b) Breadth	0.99-1.32 (1.224 \pm 0.12)
Pre-testis distance	1.91-2.42 (2.126 \pm 0.24)
Pre vitellaria distance	1.98-2.91 (2.445 \pm 0.49)
Distance between anterior extent of testes and vitellaria	0.45-0.53 (0.504 \pm 0.03)
Position of genital pore from posterior extremity	0.81-1.32 (1.025 \pm 0.17)
Eggs	
(a) Length	0.06-0.07 (0.064 \pm 0.01)
(b) Breadth	0.03-0.04 (0.036 \pm 0.01)
Vitelline follicles	
(a) Length	0.06-0.12 (0.095 \pm 0.03)
(b) Breadth	0.04-0.10 (0.075 \pm 0.03)

Phylum- Nematelminthes
Class- Nematoda
Order- Ascaridida
Family- Anisakidae
Genus- Anisakis

9. Anisakis SP. L3 larvae (Figure 9) (Table 9)

Material: A total of 2127 specimens of *Anisakis* sp. L3 larvae were collected.

Description: Cylindrical in shape, attenuated at both ends; larvae covered with a rigid cuticle that has an annular transverse striation; lips inconspicuous with prominent boring tooth at the anterior extremity; four small labial papillae (two dorso-lateral and two ventro-lateral) were surrounding the tri-radiate mouth opening; esophagus had anterior muscular part and a glandular ventriculus with an oblique esophago-intestinal junction; Long intestinal caeca with clear demarcation; excretory duct runs from the excretory pore, which is situated ventrally below the larval tooth; rectum surrounded by rectal glands opening by anal opening; mucron present at the caudal end.

Habitat: Body cavity, mesenteries

Locality: Dharmanagar (24.3700°N and 92.1700°E), Pecharthal (24.189879°N and 92.099274°E), Agartala (23.8333°N and 91.2667°E) and Udaipur (23.5300°N and 91.4800°E)

Remarks: Dujardin^[40] created the genus *Anisakis* as a subgenus of the genus *Ascaris* Linnaeus, 1758. Olson et al.^[41] and Dixon^[42] characterized the third stage larvae (L3) of *Anisakis simplex* as a small worms, (9- 36 mm in length) with a straight anterior gut consisting of esophagus, ventriculus, and intestine, cuticle obviously striated transversely, lips inconspicuous but having prominent boring tooth on anterior end, the live larvae white or cream in color and encysted in capsules of host origin, coiled like a watch- spring.

The present study is the first report of the occurrence of *Anisakis* sp. L3 larvae from the freshwater catfish *C. batrachus* of Tripura, India.

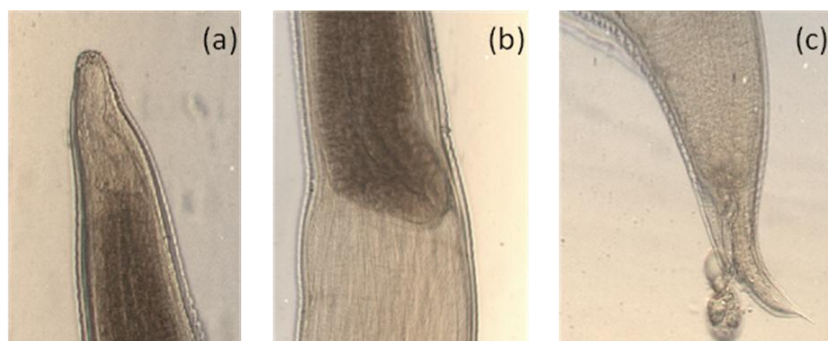


Figure 9: Light microscope images of *Anisakis* SP. L3 larvae (a) Anterior end, (B) Middle region and (C) Posterior end

Table 9: Morphometric measurement (in mm) and characters of the *Anisakis* SP. L3 larvae (mean ± Standard Deviation) reported from Tripura, India

Characters	<i>Anisakis</i> sp. L3 Larvae
Body length	13.9-14.7 (14.25±0.30)
Breadth/ Diameter	0.49-0.61 (0.537±0.05)
Oral sucker	-
(c) Length	-
(d) Breadth	-
Pre-pharynx	-
(c) Length	-
(d) Breadth	-

Pharynx	-
(c) Length	-
(d) Breadth	-
Oesophagous	1.87-2.89 (2.247±0.39)
(c) Length	-
(d) Breadth	-
Acetabulum	-
(c) Length	-
(d) Breadth	-
Anterior testes	-
(c) Length	-
(d) Breadth	-
Posterior testes	-
(c) Length	-
(d) Breadth	-
Cirrus sac	-
Ovary	-
(c) Length	-
(d) Breadth	-
Length of Mucron	0.01-0.03 (0.021±0.01)

Conclusion

The present study revealed the occurrence of nine different helminth parasites from the freshwater catfish, *Clarias batrachus* of Tripura, India, namely, *Astiotrema reniferum*, *Lytocestus indicus*, *L. birmanicus*, *L. longicollis*, *L. attenuatus*, *L. filiformes*, *L. clariae*, *Djombangia penetrans* and *Anisakis* sp. L3 Larvae. In the present study *Anisakis* sp. L3 Larvae were reported for the first time from *C. batrachus* whereas the occurrence of *L. indicus*, *L. birmanicus*, *L. longicollis*, *L. attenuatus*, *L. filiformes*, *L. clariae* and *D. penetrans* are new locality records from Tripura, India.

Acknowledgements

The authors are thankful to the DSA (UGC-SAP) program for the infrastructural support to the Department of Zoology, NEHU, Shillong. Ruma Koiri also acknowledges UGC Non-NET Fellowship for the financial support.

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