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A. Morphotaxonomic study of piscian cestodes of the Genus Lytocestus (Caryophyllidea-Lytocestidae) from freshwater fishes of Lucknow District (Uttar Pradesh), India

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Abstract
The present morphotaxonomical study has comprised of the regional survey for piscian cestode parasites from freshwater fishes of Gomti river, Lucknow District (U.P.) India. This expedition was conducted from September 2019 to March 2020 in the spring winter season from which one Caryophyllidean species of the genus *Lytocestus* from host fish *Heteropneustes fossilis* (Bloch) is described here. In this study, all retrieved specimens and holotype models of parasites have been studied to specify the cladistic taxonomic description and morphological characterization of the rare piscian cestode (tapeworm) species. During the field survey, mature freshwater fishes were collected from the local fish market and sometimes angled and caught by traps on the spot at selected water bodies of sampling sites with the help of local fishermen. The collected fishes as holotype specimens were taken to the laboratory so that they could be properly studied based on their morphological characteristics. Parasites obtained from infected host fishes were maintained in 5% formalin filled labeled vials with the necessary information for further investigation. A total of six cestode parasites were found from the infected intestine and body cavity of the host fishes. Morphotaxonomic studies of tapeworms show that Parasitic diversity can be seen at a certain period in the Gomti River of Lucknow region. Morphotaxonomic differential characterization of finding species has reported as body elongated, scolex differentiated, ovary bilobed and H-shaped, large saccular uterus, testes numerous and well developed, vitelline follicle are cortical and annular surrounding testes, eggs are ovoid and non-filamentous. This study provides an approach to understand the diversity of tapeworm parasites of some freshwater fish species found in the Gomti river based on morphotaxonomy.

Keywords: morphotaxonomic, piscian, cestode, caryophyllidea, lytocestidae, gomti river, Lucknow

Introduction
For a large population in the global society, edible fish is considered a basic source of natural food as a micronutrient and macronutrient supplement that provides essential nutrients to the human body in the form of proteins, lipids, vitamins and minerals. As an economic aspect, fish and fisheries is a major source of income and livelihood for people who are involved in aquaculture and fish trade traditionally or any other way [1].

From time to time, many organized and unorganized institutes work for the benefit of the people associated with fisheries. On the same basis, the Department of Fisheries, Government of India periodically conducts several training programs and research trials to improve and supply the quality of nutritious fish for the economic benefit of the fishermen [2].

A nutritious healthy fish requires its correct weight and maturity but it is seen that fishes are found infected with tapeworm parasites that reduce the nutrition from their body and secrete harmful substances. Due to which the market price of fishes is affected as well as the risk of parasitic infection if consumed [3].

To keep the fish healthy and nutritious and preventing from parasitic infection, knowledge of the piscian parasites and helminth diversity in the animal kingdom is very important. Keeping all these facts in focus, morphotaxonomic studies of tapeworm parasites are urgently needed in present to obtain up to date more accurate information about the parasites.

Although Compared to other research scenarios, very little expedition projects have been done on the taxonomic study of tapeworm parasites so more exploration and experimental work is needed. Many taxonomic and parasitic explorative research have shown that helminth parasites diversity in host freshwater fishes of Uttar Pradesh is observed. The latest study is proposed as the regional survey of sampling sites and morphotaxonomic investigation by the authors associated with this project to collect anecdotal information about tapeworm parasites. In the inclusion of this experimental protocol, the authors have limited themselves to the morphotaxonomic study of finding cestode parasites from infected host fishes with various sampling sites of the Gomti river in the Lucknow region.

Morphometric measurements with statistical analysis of host fishes and concerned parasites are imperative for morphotaxonomic study [4]. Keeping all the records gathered during the research, an analysis has been carried out incorporating all the taxonomic aspects and statistical parameters (Table 1 and 2). Holotype and paratype of tapeworms were reported and listed to ensure taxonomic hierarchy.

Materials & Methods
The methodology has been adopted in this study with the following steps as per experimental design –

Sampling site (study area)

Fig 1: Map showing Gomti riverside study area at Lucknow District region.

The local freshwater fishes collected from various sampling sites of Gomti riverside at the Lucknow District region of Uttar Pradesh state for the morphotaxonomic study of piscian cestodes. The study area divided into five different geographical locations as Janeshwar Mishra Park Lake, Gomti Fish Hatchery, Gomti River Weir, Telibagh Fish Market and Kaiserbagh Fish Market. According to set standards, fishes angled to caught on the spot at some sampling sites with the help of local fishermen and brought to the laboratory for parasite's investigation.

Laboratory investigations and data collection

All the experimental work completed in the laboratory's-controlled environment. Collected fishes were dissected in dissecting tray to find out parasitic infection in internal organs; the alimentary canal with various internal organs of the infected host fish was cut and removed in 0.9% (w/v) aqueous isotonic saline water in the petri dish. All organs placed in lukewarm saline water were shaken several times and many of the associated waste materials were removed. The intestine, gills, stomach and body cavity of parasites were examined thoroughly under a dissecting microscope to ensure that no one parasites are left without screening. In some case studies, it was observed that the parasite's scolex is found deeply embedded in the host's intestine and internal body cavity, which was necessary to pull out of the intestinal mucosa carefully with the help of a sharp-edged needle and scalpel. Under a simple procedure, the differentiated segments of the mucosa and waste tissues attached to the worm were separated from the body by shaking in normal saline water. Some of these coiled stretched and torsion worms were replaced in lukewarm saline water till getting in normal status. The worm's body was moved around several times with the help of long needles on the sides of the Petri dish to stabilize and fixing of some long worms and later subsequently preserved in 5% formalin. To stain the whole worm specimen, Harris' hematoxylin solution and eosin (H&E) used then cleared in xylene and finally mounted in Canada balsam. Camera lucida drawings were made using stage micrometer with an ocular micrometer. In biostatistical and morphometrical analysis all the physical measurements as Length, width and weight were measured in millimeters and grams. Observation and identification of parasites completed using standard keys of Mackiewicz et.al., Wardle et.al., and Cohn [17, 8, 7].

Analysis of findings

For bio-statistical analysis of infected host population parameters as X1, X2, Y1 and Y2 values, Margolis equations have been adopted and various endemic problems of the parasitic infection solved (Table 1) [5].

$$\text{Incidence of infection (X1)} = \frac{\text{No.of infected hosts (b)}}{\text{No.of examined hosts (a)}}$$

$$\text{Intensity of infection (X2)} = \frac{\text{No.of collected parasites(c)}}{\text{No.of infected hosts (b)}}$$

$$\text{Density of infection (Y1)} = \frac{\text{No.of collected parasites (c)}}{\text{No.of examined hosts (a)}}$$

$$\text{Index of infection (Y2)} = \frac{\text{No.of infected hosts (b))} \times \text{(No.of collected parasites (c)}}{\text{No.of examined hosts } (a^2)}$$

Table 1: Showing statistical analysis of parasitic infection in the host fish population with concerned parasites from listed sampling sites.

Sampling site, District – Lucknow (U.P.)								
Sr. No.	**Sampling sites**	**No. of examined hosts (a)**	**No. of infected hosts (b)**	**No. of collected parasites (c)**	**Incidence of infection (X1)**	**Intensity of infection (X2)**	**Density of infection (Y1)**	**Index of infection (Y2)**
1.	Janeshwar Mishra Park Lake	30	4	8	0.133	2	0.266	0.035
2.	Gomti Fish Hatchery	30	6	6	0.2	1	0.2	0.04
3.	Gomti River Weir	30	5	7	0.166	1.4	0.233	0.038
4.	Telibagh Fish Market	30	3	4	0.1	1.333	0.133	0.013
5.	Kaiserbagh Fish Market	30	4	6	0.133	1.5	0.2	0.026
Total		150	22	31	0.732	7.233	1.032	0.152

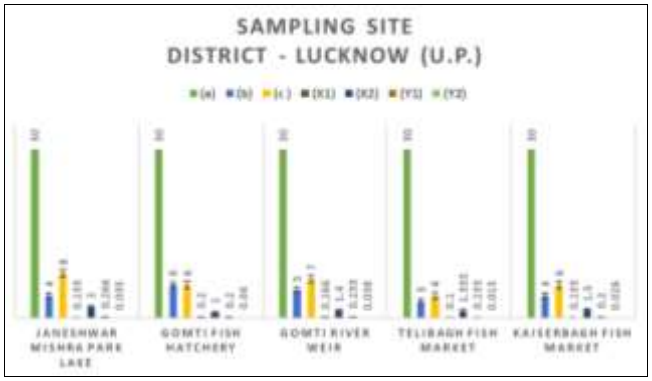

Fig 2: Graphical presentation of sampling sites.

Figure 2 showing the Graphical presentation of listed sampling sites of Lucknow District. A total of five sampling sites were selected for fish capturing and riverside surveys. Some sampling sites were selected as nearby on the banks of the river Gomti and lakes so that the fish population diversity and parasitic infection could be checked in different sites. All gathered data from sampling sites were recorded and analyzed based on biostatistical, morphological and endemic tapeworm infection parameters.

Table 2: Biometric characteristic (weight and total length) of freshwater host fishes with Prevalence of Cestode/ Nematode/ Trematode/ Acanthocephala parasites.

Sr. No. (Sampling sites)	No. of examined hosts	No. of infected hosts	Host fishes	Infected site (Habitat)	Weight (gm)	Total Length (cm)	No. of collected Parasites	Parasites			
								C	N	T	A
1.	30	4	*Clarias batrachus, Heteropneustes fosIllis*	Intestine, Gills	188-312	14.2-21.6	8	2	3	2	1
2.	30	6	*Clarias batrachus, Heteropneustes fosIllis*	Intestine	175-298	14.5-20.4	6	1	4	1	0
3.	30	5	*Clarias batrachus, Channa punctatus*	Intestine, Body cavity	218-324	16.5-22.8	7	2	4	1	0
4.	30	3	*Clarias batrachus, Heteropneustes fosIllis, Channa punctatus*	Intestine, Body cavity	184-276	13.8-19.5	4	0	3	1	0
5.	30	4	*Clarias batrachus, Channa punctatus, Heteropneustes fosIllis*	Intestine, Gills, Body cavity	212-304	16.2-25.3	6	1	3	2	0

Morphometric measurements and comparative analysis

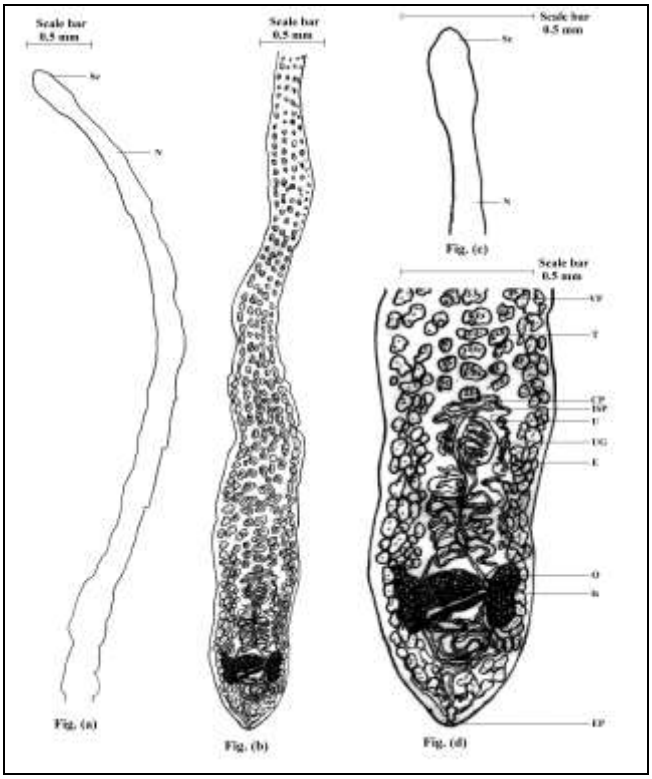

Fig 3: (a) Anterior half of the body, [100x] (b) Posterior half of the body, [100x] (c) Scolex enlarged, [150x] (d) Posterior end, [150x]

A detailed account of the remarkable tapeworm parasite of host freshwater catfish (*Heteropneustes fossilis*, Bloch) [6] is described. Shape, size, morpho-micrometry, infection site, etc. are observed under a compound microscope and camera lucida figures are drawn (Fig.3, Tables 2 and 3). All morphometrical measurements and dimensions are measured in millimeters using a stage micrometer and an ocular micrometer.

Table 3: Showing morphometric measurement L×W (in mm) and cladistic taxonomical characters of Genus *Lytocestus* Cohn, 1908 [7] tapeworms under the generic description.

Sr. No.	Characters	*Lytocestus fossilis* (Redescribed)	*Lytocestus osmanabadensis* (Bhure et. al., 2010) [8, 9]	*Lytocestus marathwadensis* (Shinde et. al, 1988) [10]	*Lytocestus folliculare* (Bhure et. al., 2010) [8, 9]	*Lytocestus puranensis* (Kasar et. al., 2010) [11]	*Lytocestus parvulus* (Kasar et. al., 2010) [11]	*Lytocestus Clariae* (Redescribed) (Singh et. al., 2020) [12]	*Lytocestus birmanicus* (Lynsdale, 1956) [13]	*Lytocestus filiformis* (Woodland, 1923 [14] and Fuhrman et. al., 1925) [15]	*Lytocestus attenuatus* (Tandon et.al., 2005) [16]	*Lytocestus longicollis* (Redescribed) (Singh et. al., 2020) [1]
1.	Length of the worm	11.09	11.52	12.22	12.26	14.69	12.46	12.06	11.58	12.67	13.20	16
2.	Maximum breadth of the worm	0.64	0.82	0.90	0.70	0.95	0.80	0.69	0.85	0.96	1.05	0.60
3.	Neck	0.59×0.12	0.61×0.22	0.53×0.23	0.46×0.18	0.49×0.24	0.68×0.19	0.69×0.16	0.62×0.20	0.50×0.23	0.65×0.31	3.73×0.27
4.	Ovary: shape	H shaped like the Bow tie	H shaped	Butterfly shaped	H shaped	Inverted U shaped	X shaped	H shaped like a butterfly	H shaped	Inverted U shaped	Butterfly shaped	Cortical, H shaped with closely packed follicles
5.	Genital aperture (pore)	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
6.	Vitelline follicles	0.095×0.071	0.6×0.13	0.5×0.12	0.5×0.12	0.6×0.12	0.05×0.12	0.05×0.13	0.6×0.12	0.6×0.13	0.4×0.10	0.075×0.125
7.	Cirrus Pouch	Ovoid	Round to oval	Round	Symmetrical oval	Round to oval	Round to oval	Well developed, oval to round	Round to oval	Round to oval	Round	well-developed oval to round with ISV
8.	Eggs	0.011×0.012	0.04×0.02	0.04×0.01	0.05×0.03	0.04×0.03	0.06×0.03	0.05×0.02	0.04×0.02	0.04×0.04	0.05×0.01	0.024×0.038
9.	Excretory pore	0.023×0.035	0.03×0.07	0.04×0.7	0.03×0.06	0.05×0.08	0.04×0.9	0.03×0.08	0.03×0.08	0.05×0.09	0.04×0.7	0.022×0.063

Tapeworms were measured 9.10-13.08×0.50-0.78 (11.09×0.64) in length×width. Body elongated and flat. Scolex is slightly differentiated and blunt without any major groove, anterior end stumpy rounded, broader and parallel than neck at the top measured 0.17-0.15×0.34-0.40 (0.16×0.37). Neck present and constricted with measurement 0.57-0.61×0.10-0.14 (0.59×0.12). Proglottids absent. Male and female gonopores are separated and closely open in the mid posterior region. Numerous testes are rounded or oval in the medullary region, extending from the nearest part of the ovary to anterior most vitelline follicles measured 0.05-0.08×0.10-0.13 (0.065×0.115). Vas deferens visible and convoluted in front of the cirrus pouch. Cirrus pouch ovoid. Internal seminal vesicle present and External seminal vesicle absent. Vitelline Follicles are cortical and annular, surrounding testes and anterior tip of the ovary measured 0.090-0.100×0.067-0.075 (0.095×0.071), some VF surrounds touches the ovarian lobes. No post ovarian vitelline follicles.

Uterus large saccular, laterally coiled medullary and extends the posterior region to the ovary, uterine glands present. No seminal receptacle. Cirrus pouch well-developed, oval to round in shape, median measured 0.05-0.08×0.08-0.10 (0.06×0.09). Absence of common genital atrium, Mehlis' gland complex and large, posterior to isthmus in between two ovarian lobes. Ovary medullary to cortical, symmetrically lobed, H shaped like the Bow tie whose lateral arms are closely packed follicular, both arms even, lateral lobes of ovary situated in medullary or partly cortical regions measured 0.48-0.42×0.27-0.25 (0.45×0.26). Genital pore present at the posterior region of the worm. Isthmus medullary and slightly curved in the medullary region. Anterior uterus measured 0.02-0.03×0.15-0.16 (0.02×0.15). Excretory pore measured 0.022-0.024×0.032-0.038 (0.023×0.035). Eggs are some oval and non-filamentous, broader than length, the operculate structure measured 0.009-0.013×0.014-0.010 (0.011×0.012).

Results and Discussion

Standard Key to taxonomic Identification

Order: Caryophyllidea (After Mackiewicz and Blair, 1978) [17]

Testes and vitellaria follicular either completely in medullary parenchyma or partially in medullary and cortical parenchyma; testes, vitellaria follicular or both internal to inner longitudinal muscles that separate medullary testes from cortical vitellaria.

Family: Lytocestidae (Wardle and McLeod, 1952) [18]

Body elongated, sometimes filiform, variable in length. Scolex with suctorial grooves of different shapes marked off from the body or not. Testes medullary, anterior to ovary and uterus. Cirrus pouch preuterine, post testicular. Male genital pore in the anterior or posterior half of the body. Ovary symmetrically lobed, medullary or partly cortical, post uterine; usually near posterior extremity. Vitellaria medullary, cortical or partly cortical and partly medullary. Uterus preovarian maybe partly posted ovarian, Eggs ovoid, operculated, containing unsegmented ova.

Genus: *Lytocestus* (Cohn, 1908) [7]

Body elongated, tapering anteriorly. Scolex undifferentiated. Numerous testes in the broad medullary region of preuterine medulla. Vas deferens are convoluted. Cirrus with a strong muscular wall opening into a deep narrow midventral pit. The ovary is bilobed with lateral lobes outside of
the inner longitudinal muscle sheath. No post ovarian vitellaria follicles. Uterus looped behind the Mehlis gland and then closely coiled between the ovary and male Terminalia.

This tapeworm species comes closer to genera *Lytocestus*. This species differs from *Pseudolytocestus* in having scolex slightly differentiated and blunt without any major groove and internal seminal vesicle present, ovary 'H' shaped with closely packed follicles [19].

1. The present form differs from *Lytocestus osmanabadensis* in having a slightly differentiated, blunt scolex with Parallel neck, ovary H shaped medullary or partly cortical, and oval eggs [8, 9].
2. The present form differs from *Lytocestus marathwadensis* in having the long worm with developed scolex, ovary H shaped like the Bow tie whose lateral arms are closely packed follicular, both arms even, uterus extends up to post ovarian region, operculated oval eggs [10].
3. The Present form differs from *Lytocestus follicularae* having an elongated body and the full length of the worm, H shaped ovary, Mehlis gland present [8, 9].
4. The present form differs from *Lytocestus puranensis* in having differentiated scolex without bothria, long neck, H shaped ovary, internal seminal vesicle present and external seminal vesicle absent [11].
5. This form differs from *Lytocestus clariae* (Re-described) in having long worm, presence of long straight neck without any groove, presence of internal seminal vesicle, and H-shaped medullary or partly cortical ovary [12].
6. The present form differs from *Lytocestus parvulus* in having an H shaped ovary and broader than long vitelline follicles, Isthmus medullary and slightly curved, ovoid eggs [11].
7. The present form differs from *Lytocestus birmanicus* in having H shaped medullary to the partly cortical ovary, both bilobed arms even, follicular are closely packed, spherical to ovoid-shaped eggs [13].
8. The present form differs from *Lytocestus filiformis* in having H shaped bilobed ovary, round to ovoid operculated eggs, Isthmus medullary and partly curved, Internal seminal vesicle present, separate gonopores present [14, 15].
9. The present form differs from *Lytocestus attenuates* in having elongated straight body, slightly curved Isthmus, big H shaped medullary to the partly cortical ovary, ISV present, ESV absent, broader than longer ovoid eggs [16].
10. The present form differs from *Lytocestus longicollis* in having differentiated complex scolex, neck long and narrow to symmetrical, ovary H shaped like the bow tie, large saccular uterus, testes numerous, VF cortical and annular, ISV present, ESV absent, ovoid eggs [1].

Based on taxonomic comparison with morphometrical characters present tapeworm holds a specific classified hierarchy as *Lytocestus* gen. *fossilis* sp.

Table 4: Taxonomic Summary of *Lytocestus fossilis.*

Taxonomic Summary	
Kingdom	Animalia (Animal, animaux, animals)
Subkingdom	Bilateria
Infrakingdom	Protostomia
Superphylum	Platyzoa
Phylum	Platyhelminthes
Subphylum	Neodermata
Class	Cestoda - tapeworms
Subclass	Eucestoda
Order	Caryophyllidea (after Mackiewicz and Blair, 1978) [17]
Family	Lytocestidae (Wardle and McLeod, 1952) [18]
Genus	*Lytocestus* (Cohn, 1908) [7]
Species	*fossilis* [20] (Redescribed)
Host	*Heteropneustes fossilis* (Bloch, 1794) [6]
Habitat	Intestine
Locality	Gomti river, District - Lucknow (U.P.) India
Number of Parasites	03/06

Conclusion

In cladistic morphotaxonomic records, the *Lytocestus fossilis* is considered as uncommon Caryophyllidea-Lytocestidae-Lytocestus cestode of *Heteropneustes fossilis* host fish. In the case of tapeworm parasitic infection, in host fishes, some internal organs as intestine, gills, and body cavity have observed the most impact by worm's scolex and hooks penetration. The taxonomic hierarchy of this cestode parasite has been reported and summarized according to NCBI and ITIS data. A comparative study of this parasite with other known tapeworms reveals the recent status of its demographic diversity with biometrical and statistical analysis. The morphotaxonomic studies of parasites might be beneficial to prevent parasitic infection in edible fishes.

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