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A morphotaxonomic study of piscian cestodes of freshwater fish from the Bulandshahr region (U.P.), India

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Abstract

The present experimental model of the project has been studied to specify the cladistic taxonomic description and morphological characterization of the rare piscian cestode (tapeworm) species. Freshwater fishes angled and caught by traps on the spot at selected sampling stations with the help of local fishermen and later collected for tapeworm investigations. Mature local freshwater catfish *Clarias batrachus* (Linnaeus, 1758) [3] collected and preserved from Bulandshahr sampling stations and examined for tapeworm parasites as per experimental design. The captured fishes were taken to the Parasitology Laboratory so that they could be properly studied based on their morphological and anatomical characteristics. Parasites derived from infected host fishes retained in 5% formalin and kept to labeled vials with the required information for further investigation. Five cestode parasites were found from the infected intestine and body cavity of the host fish. The morphotaxonomic study of tapeworms shows well that parasitic diversity is facile within the Ganga riverside at Bulandshahr (U.P.) region. Morphotaxonomic characterization reported as ovary H-shaped, situated in the posterior region of the body, testes numerous and well developed like spherical or broadly oval, vitelline follicle - cortical, surrounding testes, extending from base of elongated neck to anterior tip of the ovary, eggs oval and excretory pore terminal. This study provides an approach to understand the diversity of tapeworm parasites of some freshwater fish found in the river Ganges based on morphotaxonomy.

Keywords: Morphotaxonomic, piscian, cestode, tapeworm, Ganga river, Bulandshahr region (U.P.).

Introduction

In the global society for non-veg. eaters, fish is primarily a micronutrient as well as macronutrient source that provides essential nutrients to the human body in the form of proteins, lipids, vitamins, and minerals. Besides, fish is an important source of income and livelihood for people who are involved in fisheries, aquaculture, and fish trade traditionally from many generations. The Government of India periodically undertakes several training schemes and research projects to improve and supply the quality of nutritious fish for the economic benefit of the people dependent on fisheries [1]. A healthy and mature fish of the right weight is considered a nutrient base but when edible fish are found infected with tapeworm parasites that reduce the necessary nutrition from the host fish and secrete harmful substances. So, because of this, the market value of fish is also affected and if consumed, there is a risk of infection [2]. Morphotaxonomic studies of tapeworm parasites are an urgent need today because of all these facts and to obtain more accurate information about the parasite. Compared to other research scenarios, very little scientific exploration has been done on the taxonomy of tapeworm parasites so more experimental work is needed. Done so far many taxonomic and parasitic kinds of research have shown that diversity of generic helminth parasites is found as cestode parasites in freshwater fishes of western Uttar Pradesh. The latest study is proposed as the survey of sampling stations and experimental fieldwork 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 morphotaxonomy of finding cestode parasites of host fishes infected with various sampling stations of the Ganges River in the Bulandshahr region. 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.

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Materials & Methods

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

Sampling site (study area)

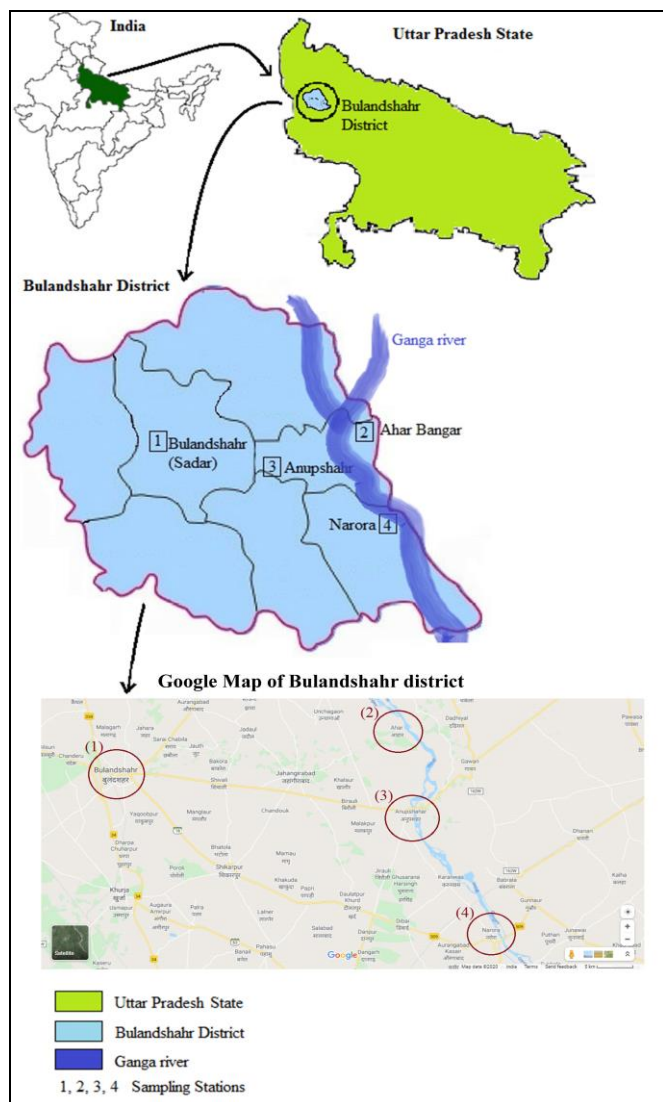


Fig 1: Map showing Ganges river the study area at Bulandshahr region.

The local freshwater catfish namely; *Clarias batrachus* (Linnaeus, 1758)^[3] collected from various sampling stations of Ganga riverside at the Bulandshahr District region of Uttar Pradesh state for the morphotaxonomic study of piscian cestodes. The study area divided into four different geographical zones as Bulandshahr Sadar, Ahar Bangar, Anupshahr and Narora. According to set standards, fishes

angled to caught on the spot at sampling stations with the help of local fishermen and brought to the parasitology laboratory for parasite’s investigation.

Laboratory investigations and data collection

Fishes were dissected in dissecting tray to find out parasitic infection in internal organs; the alimentary canal with various organs of the infected host 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 cases, the parasite's scolex found attached deeply embedded in the fish intestine and body cavity, which was pulled out by scrapping the mucosa of the intestine with the help of sharp-edged needle and scalpel. Meanwhile, the segments of the mucosa and waste tissues attached to the worm were removed by shaking the body in saline water. Some stretched and torsion worms were replaced in lukewarm saline water till changing in normal status. To stable some longer worms and get into the right position, worm's body was lifted several times with the help of long needles to the edges of the Petri dish, and later on, fixed 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. All the physical measurements as Length, width and weight were measured in millimeters and grams respectively. Observation and identification of parasites completed using standard keys of Devi PR, Wardle RA, and McLeod JA ^[10, 16].

Analysis of findings

The calculative analysis of bio-statistical parameters as X1, X2, Y1 and Y2 values, Margolis equations has been adopted to calculate the various bio-statistical parameters of the parasite (Table 1.) [4].

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

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

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

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

Table 1: Showing statistical analysis of the population of host fishes and parasites at sampling stations

Sampling station, District – Bulandshahr (U.P.)								
Sr. No.	Sampling stations	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.	Bulandshahr (Sadar)	30	5	10	0.166	2	0.333	0.055
2.	Ahar Bangar	30	4	9	0.133	2.25	0.3	0.04
3.	Anupshahr	30	6	8	0.2	1.333	0.266	0.053
4.	Narora	30	4	12	0.133	3	0.4	0.053
Total		120	19	39	0.632	8.583	1.299	0.201

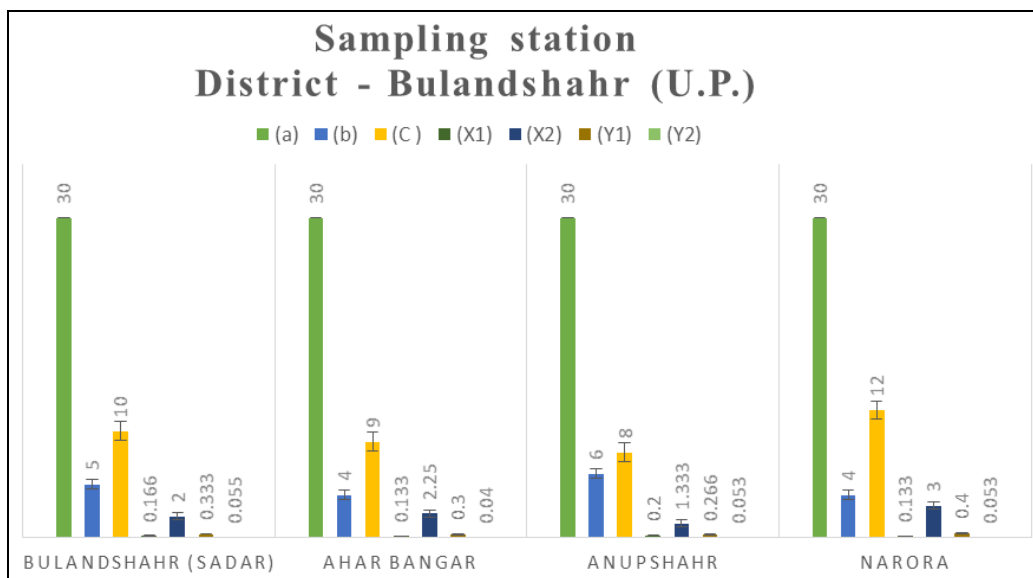


Fig 2: Graphical presentation of sampling stations

Figure 2 showing the Graphical presentation of listed sampling stations of Bulandshahr District. A total of four sampling sites were selected for fish capturing and riverside surveys. All the sampling sites were chosen as being on the banks of

the river Ganges so that the diversity of fish could also be checked in different regions. all gathered data from sampling sites were analyzed and computed based on biometrical, statistical and morphological characters.

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

Sr. No. (Samplingstations)	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	5	<i>Clarias batrachus</i>	Intestine, Gills	197-305	17.4-30.2	10	2	4	4	0
2.	30	4	<i>Clarias batrachus</i>	Intestine	182-320	14.8-17.4	9	1	4	3	1
3.	30	6	<i>Clarias batrachus</i> , <i>Channa punctatus</i>	Intestine, Body cavity	234-373	15.5-23.6	8	1	5	2	0
4.	30	4	<i>Clarias batrachus</i>	Intestine, Body cavity	165-286	18.3-22.5	12	1	6	3	2

Morphometric measurements and comparative analysis

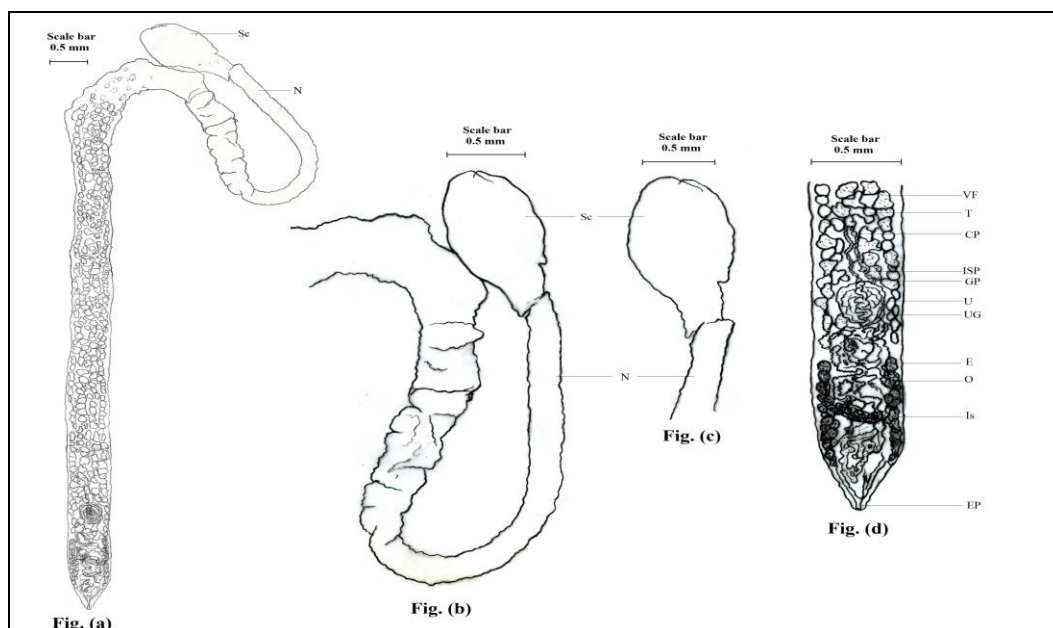


Fig 3: (a) Entire worm, [100x] (b) Anterior and middle part of the worm, [100x] (c) Scolex enlarged, [150x] (d) Posterior end, [100x]

A detailed account of rare tapeworm parasite of freshwater catfish (*Clarias batrachus* Linnaeus, 1758) [3] is described. Shape, size, morpho-micrometry, infection site, etc. are observed under a compound microscope and camera lucida

figures are drawn (Fig.3, Table 2 and 3). All morphometrical measurements 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^[17] tapeworms under the generic description

Sr. No.	Characters	<i>Lytocestus longicollis</i> (Redescribed)	<i>Lytocestus osmanabadensis</i> (Bhure et al., 2010) [5,7]	<i>Lytocestus marathwadensis</i> (Shinde et al., 1988) [6]	<i>Lytocestus follicularae</i> (Bhure et al., 2010) [5,7]	<i>Lytocestus puranensis</i> (Kasar et al., 2010) [8]	<i>Lytocestus parvulus</i> (Kasar et al., 2010) [8]	<i>Lytocestus Clariae</i> (Redescribed) (Singh et al., 2020) [11]	<i>Lytocestus birmanicus</i> (Lynsdale, 1956) [12]	<i>Lytocestus filiformis</i> (Woodland, 1923 [13] and Fuhrmann et al., 1925) [14]	<i>Lytocestus attenuatus</i> (Tandon et al., 2005) [9]	<i>Lytocestus heteropneustii</i> (Tandon et al., 2005) [9]
1.	Length of the worm	16	11.52	12.22	12.26	14.69	12.46	12.06	11.58	12.67	13.20	11.80
2.	Maximum breadth of the worm	0.60	0.82	0.90	0.70	0.95	0.80	0.69	0.85	0.96	1.05	0.62
3.	Neck	3.73×0.27	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	0.60×0.26
4.	Ovary: shape	Cortical, H shaped with closely packed follicles	H shaped	Butterfly shaped	H shaped	Inverted U shaped	X shaped	H shaped like a butterfly	H shaped	Inverted U shaped	Butterfly shaped	Inverted A-shaped
5.	Genital aperture (pore)	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
6.	Vitelline follicles	0.075×0.125	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	0.6×0.9
7.	Cirrus Pouch	well-developed oval to round with ISV	Round to oval	Round	Symmetrical oval	Round to oval	Round to oval	Well developed, oval to round	Round to oval	Round to oval	Round	Mostly oval
8.	Eggs	0.024×0.038	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	0.05×0.03
9.	Excretory pore	0.022×0.063	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	0.05×0.08

Tapeworms were measured 14-18×0.45-0.75 (16×0.60) in length×width. Scolex is undifferentiated and blunt without any major groove and bothria, "swollen pear-shaped" in holotype specimens. Narrow at the top measured 1.25-1.40×0.70-0.25 (1.32×0.47). Neck present with measurement 3.15-4.32×0.20-0.35 (3.73×0.27). Proglottids absent. Numerous testes are present in the medullary region, numerable 120 to 147 in numbers, spherical or broadly oval to round in shape, measured 0.05-0.10×0.07-0.09 (0.075×0.08). Vas deferens visible, much convoluted, and followed by a long ejaculatory duct. Vitelline Follicle is present around of testes, extending from the base of neck to anterior tip of the ovary, present in the cortical and medullary region measured 0.05-0.10×0.10-0.15 (0.075×0.125). Some VF surrounds touches the ovarian lobes. Uterus extends the posterior region to the ovary. Uterus long, non-glandular, coiled medullary, situated posterior and anterior to the ovarian isthmus. Cirrus pouch well-developed, oval to round with internal seminal vesicle lined by a thin muscular wall. Median measured 0.06-0.08×0.08-0.10 (0.07×0.09). Genital aperture present. Mehlis' gland present, posterior to isthmus in between two ovarian lobes. Ovary cortical, H shaped with closely packed follicles, both arms even, lateral lobes of ovary situated in cortical and medullary regions measured 0.42-0.52×0.76-0.80 (0.46×0.78). Genital pore present at the posterior region of the worm. Isthmus medullary and slightly curved in the medullary region. Anterior uterus measured 0.02-0.04×0.15-0.18 (0.03×0.16). Excretory pore measured 0.021-0.024×0.062-0.065 (0.022×0.063). Eggs are some oval and unembryonated, broader than length, the operculate structure measured 0.021-0.028×0.035-0.042 (0.024×0.038).

Results and Discussion

This tapeworm species comes closer to genera *Lytocestus*. This species differs from *Pseudolytocestus* in having scolex undifferentiated like "swollen pear-shaped" with no bothria, external seminal vesicle, and internal seminal vesicle present, ovary cortical, 'H' shaped with closely packed follicles [10, 15].

1. The present form differs from *Lytocestus osmanabadensis* in having a pear-shaped scolex with neck, ovary H shaped, and oval eggs [5, 7].
2. The present form differs from *Lytocestus marathwadensis* in having the full length of worm with developed scolex, H shaped ovary with closely packed follicles, uterus extends up to post ovarian region and bilobed, operculated oval eggs [6].
3. The Present form differs from *Lytocestus follicularae* having the full length of the worm, H shaped ovary in the presence of the Mehlis gland [5, 7].
4. The present form differs from *Lytocestus puranensis* in having undifferentiated scolex without bothria, long neck, internal seminal vesicle present, and H shaped ovary [8].
5. This form differs from *Lytocestus clariae* (Re-described) in having long worm, presence of long neck without segment, presence of internal seminal vesicle, and H-shaped ovary [11].
6. The present form differs from *Lytocestus parvulus* in having an H shaped ovary and broader than long, operculate eggs [8].
7. The present form differs from *Lytocestus birmanicus* in having H shaped ovary, both arms even, follicles are closely packed, broader than longer oval-shaped eggs [12].
8. The present form differs from *Lytocestus filiformis* in having H shaped ovary, round to oval eggs, Internal seminal vesicle present, external seminal vesicle present [13].
9. The present form differs from *Lytocestus attenuates* in having thin and medullary curved Isthmus, big H shaped ovary, Internal seminal vesicle present, broader than longer ovular eggs [9].
10. The present form differs from *Lytocestus heteropneustii* in having a well-developed curved ovary, a uterus with uterine pore, internal seminal vesicle, Mehlis' gland visible, excretory pore present [9].

Based on taxonomic comparison with morphometrical characters present tapeworm holds a specific classified hierarchy in the Lytocestidae family.

Table 4: Taxonomic Summary of *Lytocestus longicollis*

Taxonomic Summary	
Kingdom	Animalia
Phylum	Platyhelminthes
Class	Cestoda
Order	Caryophyllidea
Family	Lytocestidae (Wardle et McLeod, 1952) ^[16]
Genus	<i>Lytocestus</i> (Cohn, 1908) ^[17]
Species	<i>longicollis</i> (Redescribed)
Host	<i>Claris batrachus</i> (Linn. 1758) ^[3]
Habitat	Intestine, Body cavity
Locality	Ganga riverside at Ahar Bangar, Anupshahr and Narora, District - Bulandshahr (U.P.) India
Number of Parasites	03/05

Conclusion

In cladistic morphotaxonomic records, the *Lytocestus longicollis* is considered as uncommon Caryophyllidea cestode of *Clarias batrachus* host fish. The morphological cladistic analysis of this cestode population has observed and reported in listed sampling stations of the Bulandshahr region. A comparative study of this parasite with other known tapeworms reveals the recent status of its demographic diversity with biometrical and statistical analysis. The anatomical structure and morphotaxonomic studies are particularly needed to prevent parasites infection in edible fishes. In the case of tapeworm parasitic infection in the piscian host, intestine, gills, and body cavity has observed the most impact by worm's scolex and hooks penetration. Morphotaxonomical vital characterization and anatomical exploration with morphometry of the cestode parasites including its host fishes have a considerable requirement.

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