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Taxonomy of piscian cestodes of the genus *Pseudocaryophyllaeus* (Caryophyllidea- Lytocestidae) from freshwater fishes of Varanasi District (Uttar Pradesh), India

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Abstract
The present morphotaxonomic study has comprised of the regional survey for piscian cestode parasites from freshwater fishes of Ganga river and its tributaries, Varanasi District (U.P.) India. This sampling expedition was conducted from October 2017 to March 2018 in the autumn-winter season in which two Caryophyllidean parasites of the genus *Pseudocaryophyllaeus* from host fish *Clarias batrachus* (Linn.1758) is described here. Under this expedition, all recovered parasitic samples and after preserved as holotype specimens of the parasites have been analyzed, specifying cladistic taxonomic descriptions and morphological characterization of rare piscian cestode (tapeworm) species. During sampling, fishes were angled and caught by traps on the spot at selected water bodies of sampling sites with the help of local fishermen and along with it, few mature freshwater fishes from the local fish market were collected. For morphological and biostatistical analysis the collected fishes were carried out to the laboratory. A total of four cestode parasites were found from the infected intestine of the host fishes. Parasites obtained from the infected host fishes were preserved in 5% formalin using labeled vials for further study as a holotype specimen. Taxonomic studies of tapeworms show that parasitic diversity can be seen at a certain seasonal period in the Ganga river and its tributaries of the Varanasi region. Morphotaxonomic differential characterization of finding species has reported as body elongated, narrow. Scolex slightly distinctly demarcated from the neck. Ovary H-shaped, situated near the posterior end of the body. Uterus present in lateral coils of the median field. Testes ovoid or spherical. Vitelline follicles cortical. Eggs ovoid, smooth and operculated. This study provides a scientific approach to understand the diversity of piscian cestode parasites of some freshwater fish species found in the river Ganges based on cladistic taxonomy.

Keywords: Taxonomy, piscian, cestode, caryophyllidea, lytocestidae, Varanasi district

Introduction
For a large section of the population in the global human society, various edible fish is considered a basic nutritional source of natural food as well as micronutrient and macronutrient supplement that provides essential elements to the body in the form of proteins, lipids, vitamins and minerals. On the economic side, Fish and fisheries are a major source of income and livelihood for those who are traditionally or in some other medium involved in aquaculture and fish trade [1].

Governments of many countries and states are always active to promote and improve healthy edible fishes. for this, periodically many organized and unorganized research institutes work for the benefit of the people associated with fisheries and aquaculture. On the same basis, the Department of Fisheries, Government of India time to time conducts many fish breeding reform and incentive programs to improve and supply the quality of nutritious fish for large scale production and economic benefit of the fishermen [2].

In the fish market, always demands healthy fishes to be of the right weight and length for sale. for healthy nutritious fishes must have correct weight and maturity but it is observed 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 the fishes is affected and there is a risk of parasitic infection to humans [3].

To prevent parasitic infection of any animal, it is essential to first know about the host, infected site (habitat), morphology, anatomy, taxonomic order and demographic diversity of the parasite. In this context, to keep the fish as nutritionally healthy and preventing from parasitic infection, morphotaxonomic studies of tapeworm parasites and helminth diversity update information are important. In comparison to other research activities, very little taxonomic work on piscian tapeworm has been done as per research expeditions and field surveys so more exploration and experimental work are required. Much reliable taxonomic research and field expeditions have shown that helminth parasites diversity exists in host freshwater fishes of the Uttar Pradesh region.

The field expedition has been organized by the authors associated with this project as sampling sites survey and morphotaxonomic investigations to collect important information about tapeworm parasites. According to the experimental protocol, the authors have limited themselves to study morphotaxonomy of retrieved cestode parasites from freshwater fishes of various sampling sites of the Ganges River and its tributaries in the Varanasi district.

Standard morphometric measurements and statistical analysis of host fishes with concerned parasites are imperative for morphotaxonomical study [4]. Observing all the recorded data during the sampling expedition, a statistical analysis has been carried out incorporating all the taxonomical characterization (Table 1 and 2). Besides this, preserved holotype samples of parasites were investigated and listed to ensure taxonomic hierarchy.

Materials and Methods

The methodology has been adopted in this study with the following steps as per experimental design – Sampling site (study area)

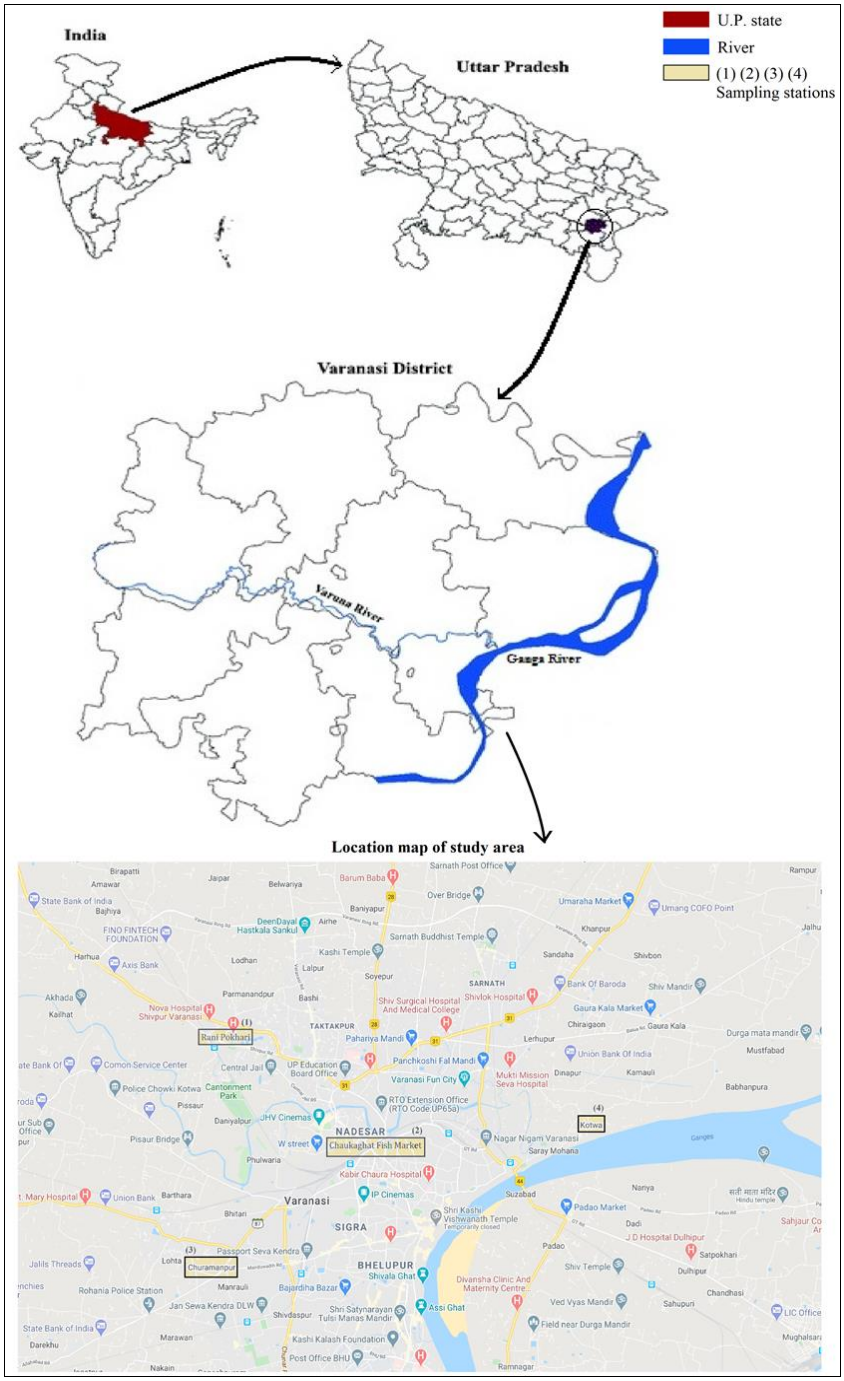

Fig 1: Map showing Ganga riverside study area at Varanasi District region

The local freshwater fishes collected from various sampling sites of Ganga riverside and its tributaries at the Varanasi District's lowland region of Uttar Pradesh state for the morphotaxonomic study of piscian cestodes. The study area selected at five different locations as Rani Pokhari lake, Chaukaghat Fish Market, Churamanpur Village, Kotwa Village. According to the research protocol, fishes angled to caught on the spot at sampling sites with the collaboration of local fishermen and brought to the laboratory for parasite's investigation.

Laboratory investigations and data collection

Physical expertise and morphological testing of the samples was completed in a controlled environment of the laboratory. Collected sample fishes were dissected in dissecting tray to find out parasitic infection in internal organs. The infected host fish's alimentary canal, intestine and internal organs were cut and transferred into 0.9% (w/v) aqueous isotonic saline water-filled petri dish. All internal organs kept in lukewarm saline water were shaken several times and the associated waste materials were removed. The alimentary canal, intestine, gills, stomach and body cavity of fishes were examined thoroughly under a dissecting microscope to ensured that no one parasites are left. Occasionally, it was found that the parasite's scolex penetrated deeply into the intestine and internal organs of the host which is carefully pulled out of the intestinal mucosa with the help of a sharp-edged needle and scalpel. During this procedure, the differentiated pieces of the mucosa and waste tissues attached to the worm were separated by shaking in normal saline water. Some of these coiled stretched and torsion worms were placed in lukewarm saline water till getting in the normal situation. The elongated worm's body was stirred many times with the help of long needles on the sides of the Petri dish. This made the worms quiet and stable which were later subsequently preserved in 5% formalin. After the dehydration process for whole-mount staining of worm, Harris' hematoxylin solution and eosin (H&E) used then cleared in xylene and finally mounted in Canada balsam. Camera lucida drawings were prepared using a 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*., & Cohn [17, 8, 7].

Analysis of findings

For bio-statistical analysis of the infected host population with prevalence parameters as X1, X2, Y1 and Y2 values, Margolis equations have been adopted to solve various endemic problems of the parasitic infection (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 – Varanasi (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.	Rani Pokhari lake	30	3	5	0.1	1.666	0.166	0.0166
2.	Chaukaghat Fish Market	30	2	4	0.066	2	0.133	0.0088
3.	Churamanpur Village	30	5	6	0.166	1.2	0.2	0.0333
4.	Kotwa Village	30	4	4	0.133	1	0.133	0.0177
Total		120	14	19	0.465	5.866	0.632	0.0764

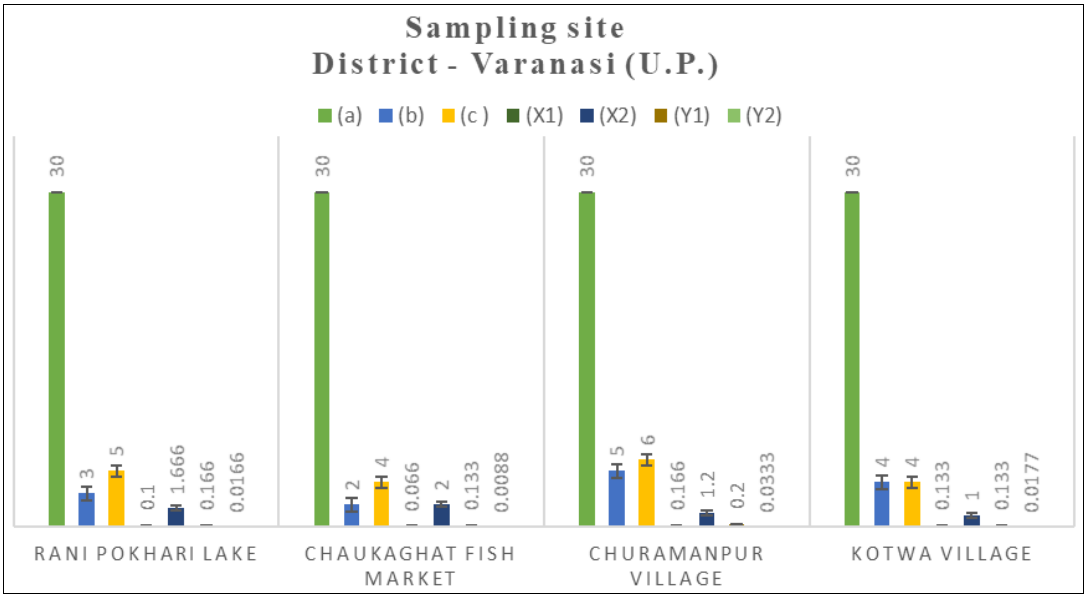

Fig 2: Graphical presentation of sampling sites

Figure 2 showing the Graphical presentation of listed sampling sites of Varanasi District. A total of four sampling sites were selected for fish capturing and riverside surveys. Some sampling sites were selected as nearby banks of the river Ganges, its tributaries and lakes so that the fish population diversity, as well as parasitic diversity and infection, could be checked. All required data from sampling sites were recorded and analyzed basis 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	3	*Clarias batrachus, Labeo rohita, Heteropneustes fosIllis*	Intestine, Gills	164-287	15.1-22.5	5	1	3	1	0
2.	30	2	*Clarias batrachus, Channa punctatus, Heteropneustes fosIllis*	Intestine	178-271	13.5-20.8	4	0	2	1	1
3.	30	5	*Clarias batrachus, Heteropneustes fosIllis, Channa punctatus, Labeo rohita*	Intestine, Body cavity	190-302	14.2-23.6	6	2	3	1	0
4.	30	4	*Clarias batrachus, Channa punctatus, Heteropneustes fosIllis*	Intestine, Gills, Body cavity	143-264	12.8-21.5	4	1	2	1	0

Morphometric measurements and comparative analysis

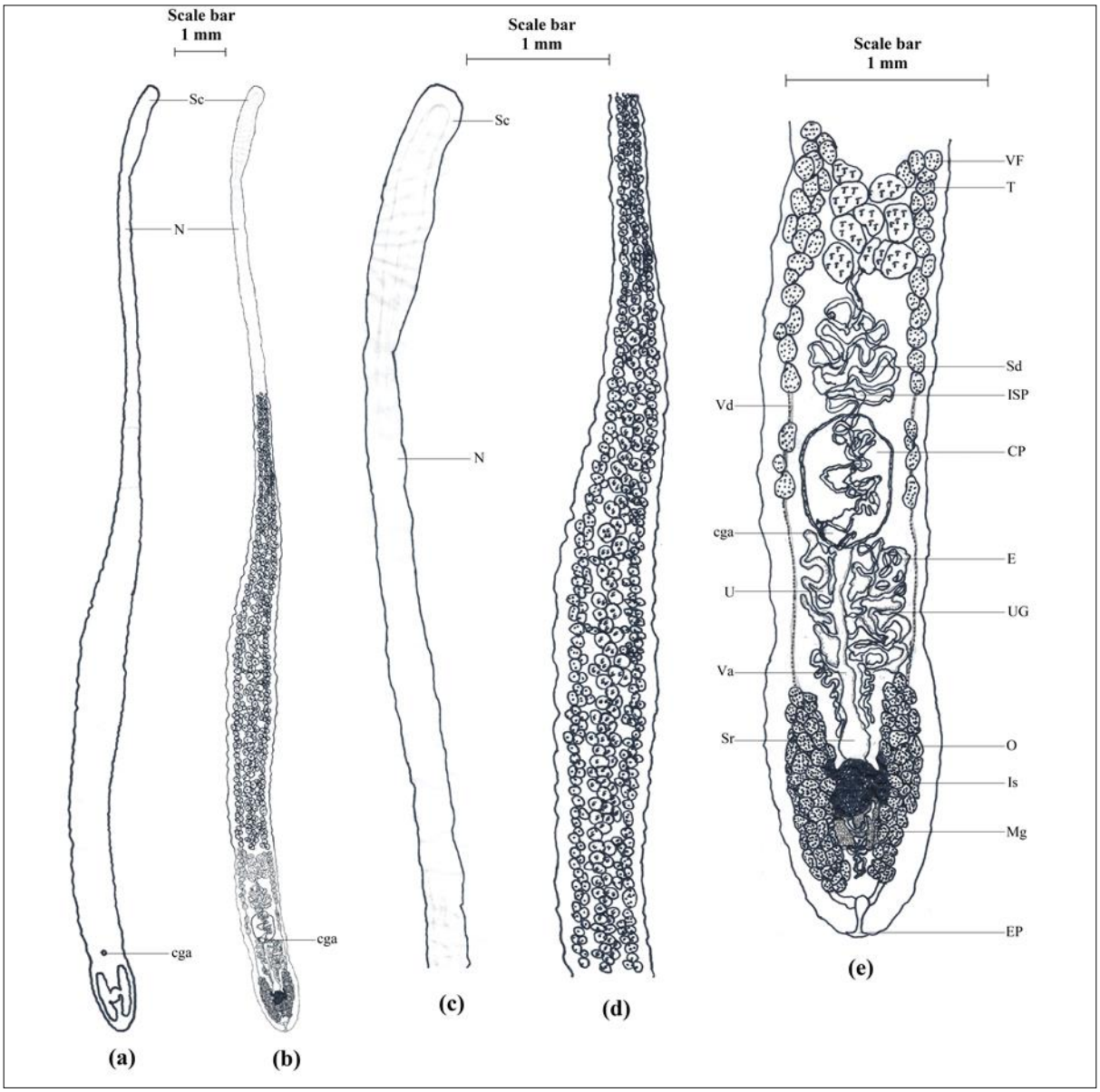

Fig 3: (a) Whole-mount worm (outline structure), [100X] (b) Entire worm, [100X] (c) Scolex enlarged with neck, [150X] (d) Middle part of body [150X] (e) Posterior half of body, [150X]

Over here a detailed account of the remarkable tapeworm parasite of host freshwater catfish (*Clarias batrachus*, Linn.1758) [6] is described. Shape, size, morpho-micrometry, infection site, etc. has been observed under a compound microscope and camera lucida figures are drawn (Fig.3, Tables 2 and 3). All morphometrical measurements and dimensions have been 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 Family Lytocestidae (Wardle and McLeod, 1952) [7] tapeworms under the generic description.

Sr. No.	Characters	Newfound species *(Under Investigation)*	*Lytocestus osmanabadensis* (Bhure *et al*., 2010) [8, 9]	*Lytocestus marathwadensis* (Shinde *et al*, 1988) [10]	*Lytocestus follicularae* (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 Fuhrmann *et al*., 1925) [15]	*Lytocestus attenuatus* (Tandon *et al*., 2005) [16]	*Lytocestus longicollis* (Redescribed) (Singh *et al*., 2020) [1]
1.	Length of the worm	18.71	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	0.92	0.82	0.90	0.70	0.95	0.80	0.69	0.85	0.96	1.05	0.60
3.	Neck	4.28×0.28	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	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	0.571×0.285	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	0.285×0.142	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	0.214×0.785	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 18.06-19.36×0.79-1.05 (18.71×0.92) in length×width. Body elongated and flat, narrow, tapering anteriorly to posterior. Scolex not distinctly demarcated from the neck, unspecialized and blunt, not broader than the body at the top measured 1.796-1.918×0.312-0.402 (1.857×0.357). Neck present, long, narrow and constricted with measurement 3.86-4.70×0.21-0.35 (4.28×0.28). Proglottids absent. Male and female gonopores at beginning of last eight of body length on the ventral surface. Common genital atrium present. Numerous testes ovoid or spherical, medullary, extending from slightly behind anterior-most vitellaria to coils of vas deferens, measured 0.571-0.857×0.428-0.571 (0.714×0.499). Vas deferens visible as much convoluted narrow tube. External and internal seminal vesicles absent. Cirrus pouch ovoid, anterior to the ovary, opening on the ventral body surface as male gonopore measured 2.625-2.945×1.832-2.024 (2.785×1.928). Vitelline follicles cortical and annular, external to inner longitudinal muscles in two lateral rows, pre-ovarian, measured 0.523-0.619×0.296-0.274 (0.571×0.285), post ovarian vitelline follicles absent. Uterus normal and saccular, laterally coiled medullary, not extending anterior to cirrus pouch, uterine glands present. Seminal receptacle large, well developed, closely anterior to the ovarian isthmus. Common genital atrium present. Mehlis' gland complex and large, posterior to isthmus in between two ovarian lobes. Ovary H-shaped, situated near the posterior end of body, lateral ovarian arms situated in medullary or partly cortical regions measured 0.9-1.1 long, follicular, cortical, connected by a transverse medullary isthmus. Genital pore present at the posterior region of the worm. Isthmus medullary and slightly curved in the medullary region. Excretory pore measured 0.022-0.024×0.032-0.038 (0.285×0.142). Eggs are some oval and non-filamentous, broader than length, the operculate structure measured 0.237-0.333×0.127-0.157 (0.285×0.142).

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) [7].

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

Genus: *Pseudocaryophyllaeus* (Gupta, 1961) [18].

Scolex oval, well defined, unspecialized, distinctly marked off from body; neck present, long narrow; testes numerous, strewn in medulla anterior to cirrus sac; uterine coils not extending anterior to cirrus sac; post ovarian vitelline follicles absent.

This tapeworm species comes closer to genera *Pseudocaryophyllaeus*. This species differs from *Pseudolytocestus* in having unspecialized scolex, distinctly marked off from the body, blunt shape without any major groove; Internal seminal pouch present; ovary 'H' shaped with closely packed follicles [19].

1. The present form differs from *Lytocestus osmanabadensis* in having unspecialized and blunt scolex, not broader than the body with a long neck; ovary H shaped medullary or partly cortical; ovoid operculate eggs [8, 9].
2. The present form differs from *Lytocestus marathwadensis* in having the long worm with broader and blunt scolex; Ovary H shaped, lateral arms are closely packed follicular, both arms even; Uterus normal and saccular, laterally coiled medullary; operculated oval eggs [10].
3. The Present form differs from *Lytocestus follicularae* having an elongated flat body; H shaped ovary; Mehlis gland complex and large [8, 9].
4. The present form differs from *Lytocestus puranensis* in having long flatworm; Scolex was not distinctly demarcated from the neck, unspecialized and blunt; long neck; H shaped ovary; Common genital atrium present [11].
5. This form differs from *Lytocestus clariae* (Redescribed) in having long flatworm; the presence of long straight parallel neck without any groove; H-shaped medullary or partly cortical ovary [12].
6. The present form differs from *Lytocestus parvulus* in having 'H shaped' ovary; Vitelline follicles cortical and annular, external to inner longitudinal muscles in two lateral rows; 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, Vaginal tube narrow and straight; Mehlis' gland large and complex; spherical to ovoid-shaped eggs [13].
8. The present form differs from *Lytocestus filiformis* in having bilobed H shaped ovary, ovoid operculated eggs; Isthmus medullary and partly curved; Internal seminal pore present; common genital atrium present [14, 15].
9. The present form differs from *Lytocestus attenuates* in having a straight flat elongated body; large H shaped medullary to the partly cortical ovary; medullary and slightly curved Isthmus; ISP present; uterine glands present; broader than longer operculate ovoid eggs [16].
10. The present form differs from *Lytocestus longicollis* (Redescribed) in having unspecialized and blunt scolex; neck long and symmetrical; ovary H shaped; large saccular uterus; testes numerous and medullary; VF cortical and annular; ISP present; post ovarian vitelline follicles absent; ovoid operculate eggs [1].

Based on taxonomic comparison with morphometrical characters present tapeworm holds a specific classified hierarchy as *Pseudocaryophyllaeus* gen., *Bovienia indica* sp. The taxonomic order of this cestode parasite has been reported and summarized according to GBIF and ITIS data (Table 4) [21].

Table 4: Taxonomic Summary of *Bovienia indica* [21].

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) [7]
Genus	*Pseudocaryophyllaeus* (Gupta, 1961) [18]
Species	*Bovienia indica* (Niyogi, Gupta & Agarwal, 1982) [20] (Redescribed)
Host	*Clarias batrachus* (Linn., 1758) [6]
Habitat	Intestine
Locality	Ganga river, District - Varanasi (U.P.) India
Number of Parasites	02/04
Global Biodiversity Information Facility ID (GBIF)	9398058

Conclusion

In cladistic morphotaxonomic records, the *Bovienia indica* is considered as uncommon Caryophyllidea-Lytocestidae-*Pseudocaryophyllaeus* cestode of *Clarias batrachus* host fish. Depending on the tendency of this parasite's morphological modulation, the evolutionary changes and parasitic biodiversity can be understood in the water ecosystem. In the case of tapeworm parasitic infection of host fishes, mostly internal organs as intestine and gills observed the most impact by worm's scolex and hooks penetration. The morphotaxonomic comparative study of this parasite with other known tapeworms reveals the recent status of its demographic diversity with biometrical and statistical analysis. As per research findings, morphotaxonomic studies of parasites might be beneficial to prevent parasitic infection in edible fishes.

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