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Morphotaxometry and Ultratopography of *Lytocestus haryanii* n.sp. (Caryophyllidea: Lytocestidae) from the intestine of freshwater catfish *Clarias batrachus* Linnaeus 1758 (Siluriformes: Clariidae) of river Yamuna, Yamuna Nagar, Haryana, India

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Lytocestus haryanii n. sp.

Clarias batrachus

Morphotaxometry

Ultratopography

Histology

Microtomy

ABSTRACT

The present investigation deals with the first report of newer species of caryophyllid cestodes, *Lytocestus haryanii* n.sp. (Caryophyllidea: Lytocestidae) in the freshwater catfish, *Clarias batrachus* Linnaeus 1758 (Siluriformes: Clariidae) of river Yamuna from Yamuna Nagar, Haryana, India from July 2018 to June 2020. These helminthes are the most common cestodes (endoparasites) among the fishes of fresh water, brackish water, and marine habitat worldwide. The recovered newer worms were processed through the standardized protocol for the microscopic observations and morphometry, ultratopographic study through scanning electron microscopy, and anatomical analysis by histology using microtomy techniques followed by the double staining. The findings of the present worms were substantiated and compared with the earlier reported species of the same genera from different hosts shared the common group using advanced numerical taxonomy for the taxometric validation. The present proposed newer worms shared all the common characteristics which helped in the generic diagnosis and are closely related to the species collected from the same host species inhabiting different freshwater bodies. The worms comprised several striking contrasts in the combination of distinguishing characters of taxonomic significance in special reference to shape, size, orientation, distribution, and the dimension of the body (single proglottid), scolex, neck, testes, ovary, cirrus pouch, vitellaria, eggs, and excretory pore. Based on the striking morphological, taxometric, ultratopgraphic, and histological differences summarized here can, therefore, be used to propose the worm as a new species.

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1 Introduction

The *Lytocestus* as genera was recovered and established by Cohn in 1908 with type species *L. adhaerens* from bony fish *C. fuscus* in Hong–Kong. The generic diagnosis of the worm was performed based on an elongated, flat body with a single proglottid without segmentation and bluntly tapering somehow flat anterior extremity with undifferentiated smooth, unarmed scolex followed by a short neck (Yamaguti 1959). The body proper had a divisible cortex and medulla by two layers of longitudinal muscles. The cortical part comprised dispersed vitellaria, however, the reproductive structures like testes and ovary dispersed in the medullary area intended for the placement of the worms in the order Caryophyllidea and Lytocestidae family (Yamaguti 1959). Additionally, the uterine coil and ejaculatory duct housed within a compact parenchymatous bulb and uterine glands confirmed the worms as to the genera *Lytocestus* Cohn 1908. At the outset, Woodland established the type species of this genus as *L. adhaerens* (Woodland 1923) from *Clarias fuscus* and four more species including *L. chalmersius* (Woodland 1924), and *L. cunningtoni* (Furhmann and Bear 1925), *L. indicus* (Moghe 1925, 1931; Mehra 1930; Ash et al. 2011), from *C. batrachus* in India as well as *L. filiformes* (Woodland 1926) from *Mormyrus caschive* in Sudan. Thereafter *L. javanicus* (Bovien 1926), *L. alestesi* (Lynsdale 1956), and *L. birmanicus* (Lynsdale 1956) recovered but later on the *L. alestesi* (Lynsdale 1956) was confirmed as Syn. *L. filiformis* (Woodland 1926). Murhar (1963) reported the *L. moghei*, and Ramadevi (1973) described *L. longicollis* from *C. batrachus*; however, Singh (1975) published *L. fossilis* from *Heteropneusteus fossilis* in India. Shindhe and Deshmukh (1980) re-described two different species from the Marathwada region, however, in 1988 in the Marathwada region of India Shinde and Phad (1988) started to study the cestode fauna in *C. batrachus* and recovered *L. marathwadaensis.* Afterward, Jadhav and Gavhane (1991) find out two species *L. alii* and *L. clariasae* (Sawarkar 2012; Sahay et al. 2019a,b). Later on Kadam et al. (1998) investigated *L. naldurgensis*, Kalse and Shinde (1999) reported *L. chalisgaonesis*, Shinde and Borde (1999) documented *L. kopardaensis* from the same catfish. However, Kolpuke (1999) recovered and published *L. teranaensis* from *Wallago attu* from the same region in the same year.

Subsequently, *L. govindae* (Patil and Jadhav 2002), *L. batrachusae* (Pawar and Shinde 2002) and *L. clariasae* (Shinde and Pawar 2002), *L. teranaensis*, and *L. shindae* (Khadap et al. 2004), *L. nagapurensis* (Lakhe et al. 2004) recovered, documented and reported from catfish *C. batrachus* as well. Meanwhile, Shomendra et al. (2003) reported *L. bishnupurensis* from *Mystus seenghala* which was critically reevaluated by Singh et al. (2018). Tandon et al. (2005) reported 4 newer species including *L. attenuateus, L. assamensis*, and *L. clariae* from *C. batrachus* and *L. heteropneustii* (Ash et al. 2012) from *H. fossilis* that was later re-described by Sahay et al. (2017) as valid species under the genus *Lytocestus*. Two new species *L. bokaroensis* and *L. mujumdari* (Poonam 2007) and *L. paithanensis* (Shelke 2007) were reported from *C. batrachus*. However, Tripathi et al. (2007) recovered *L. jagtai* from *H. fossilis* in the same year which was further described in detail by Sahay and Ekka (2019). In the next consecutive year Jawalikar et al. (2008), Jadhav et al. (2008), Kaul et al. (2010), Bhure et al. (2010), and Surayawanshi et al. (2010) isolated *L. subhapradhi, L. punensis, L. murharii, L. osmanabadensis,* and *L. follicularae*, and *L. shindei* from *C. batrachus* respectively. Further, Jawale and Borde (2011), Pawar and Hiware (2011) successfully reported new species *i.e. L. khami, L. purnensis,* and *L. vyasaei* from the same catfish, while, Kadam and Dhole (2011) described *L. gariepinusae* from *Clarias gariepinus* of Marathwada region in the same year. Sawarkar and Kale (2012), and Solunke et al. (2012) added *L. thapari* and *L. manjaraensis* from *C. batrachus* respectively; however, Nimbalkar et al. (2012) contributed *L. rekhaensis* from *H. fossilis* that was further critically studied by Sahay and Khalkho (2017). Deshmukh et al. (2015), Pawar and Dandwate (2013), Kale (2017), and Kankale (2017) recovered and reported *L. indica, L. godavariensis, L. paithanensis, L. ambe* from the *C. batrachus* respectively. Further critical studies of many of these species were carried out by Sahay et al. (2018a,b). Meanwhile, a new species *L. mastacembellusi* (Pardeshi 2016) was reported from *Mastacembelus armatus* that was further re-described by Sahay et al. (2019b). Barshe et al. (2018), Dandawate (2018), and Patil (2018) reported *L. elongates, L. mulaansis, L. bharatae* from *C. batrachus* respectively, and further thoroughly studied by Sahay et al. (2018a,b). Recently, Bhavsar et al. (2020), Narayan and Srivastav (2020) reported *L. sahayi* from *C. batrachus* and *L. chhaviensis* from *H. fossils* respectively. The present study aims to morphometrically describe a new species of *Lytocestus* that is characterized and validated through numerical taxometric analysis, ultratopography, and histology.

2 Materials and Methods

The worms were extracted from the freshwater fish, *C. batrachus* Linnaeus 1758 (Siluriformes: Clariidae) from river Yamuna, Yamuna Nagar, Haryana, India. The worms were thoroughly washed in lukewarm water to remove debris and observed under a dissection microscope to identify the group of parasitic helminthes in the Zoology Laboratory, Department of Biotechnology, M.M. (Deemed to be University), Mullana–Ambala (Haryana), India. Specimens were fixed in hot 4% formaldehyde for 20–30 minutes. The fixatives were washed properly under running tap water, stained in an aqueous solution of Mayer's Haemalum, dehydrated using a series of alcohols, and cleared in xylol (dehyadrated ethanol: xylene:: 1:1 *v/v*) followed by xylene and mounted in Canada balsam. The line diagrams were drawn with the aid of camera lucida (SIPCON SP–14), and all the measurements were

recorded in millimeters (mm). The microphotographs were captured using Image Analyzer unit "MOTIC" through Biovis Image Plus software and Nikon Trinocular Computerized microphotography unit. Polythetic Divisive Classificatory System (Malhotra et al. 1981a,b,c) was applied to conduct taxometric analysis to establish various newer taxa (Upadhyay et al. 2009; Kumar et al. 2011; Upadhyay 2012; Upadhyay et al. 2013; Upadhyay 2019) based on Coefficient of Dissimilarity (Bray and Curtis 1957), Coefficient of Divergence (Klauber 1940), Mean Character Difference (Cain and Harrison 1958) and Coefficient of Similarity (Dixit et al. 1979). Some specimens were processed for ultratopography through Scanning Electron Microscopy, SAIF (DST), Department of Anatomy, AIIMS, New Delhi as per the standardized laboratory protocols (Wisse et al. 2010; Malhotra et al. 2012; Upadhyay 2012; Al–Shehadat et al. 2018). Some specific parts of freshly collected worms were processed for the histological studies as per the standardized laboratory protocol (Pearse 1968; Upadhyay 2017, 2018; Upadhyay and Nanware 2020).

3 Results and Discussion

3.1 Description of *Lytocestus haryanii* n. sp.

The description of *L. haryanii* has been illustrated in Figures 1–6. The body of recovered worms is made of a single proglottid with 11.52–13.0 x 0.575–1.185 mm in size and undifferentiated, smooth, unarmed, bluntly or pseudo–pointed tapering scolex measured

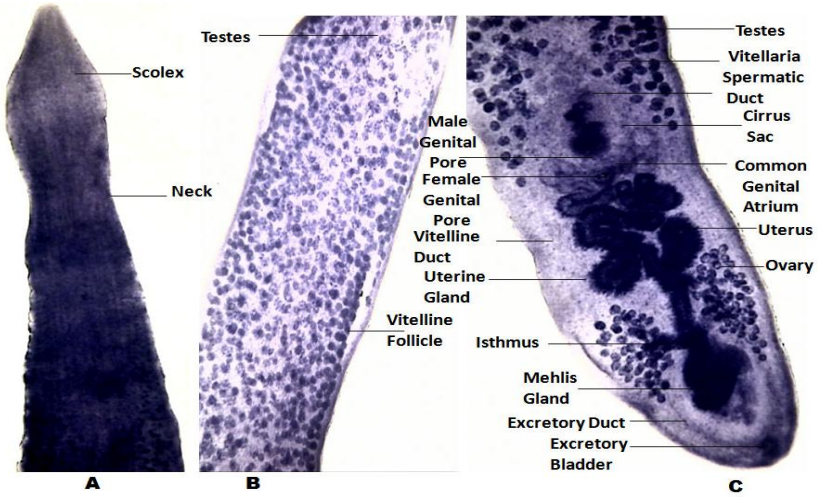

Figure 1 Microphotographs of *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar, (scale bar: 50X); A - Anterior end with pseudo–blunt scolex and an undifferentiated short neck; B - Middle region of mature worm showing the progottid filled with testes and vitellaria; C - Posterior end of mature worms showing male and female reproductive system, terminal excretory bladder.

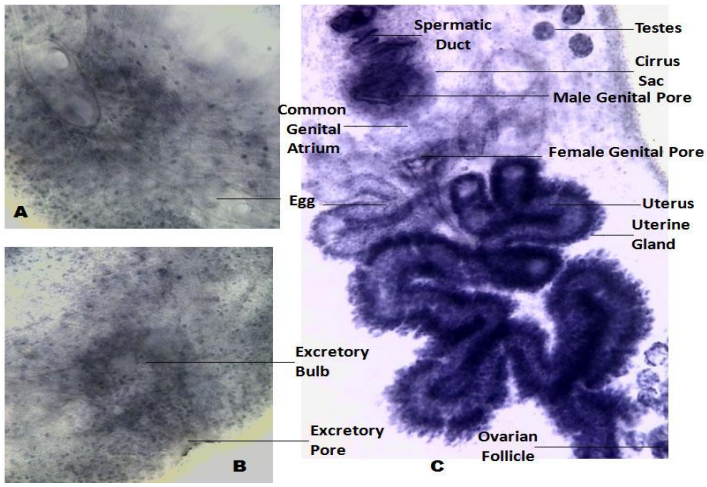

Figure 2 Microphotographs of *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar, (scale bar: 225X); A - Eggs in Uterus; B - Terminal excretory bladder, excretory bulb and excretory pore; C - Posterior end of mature worms showing uterus with uterine glands and cirrus sac with spermatic duct.

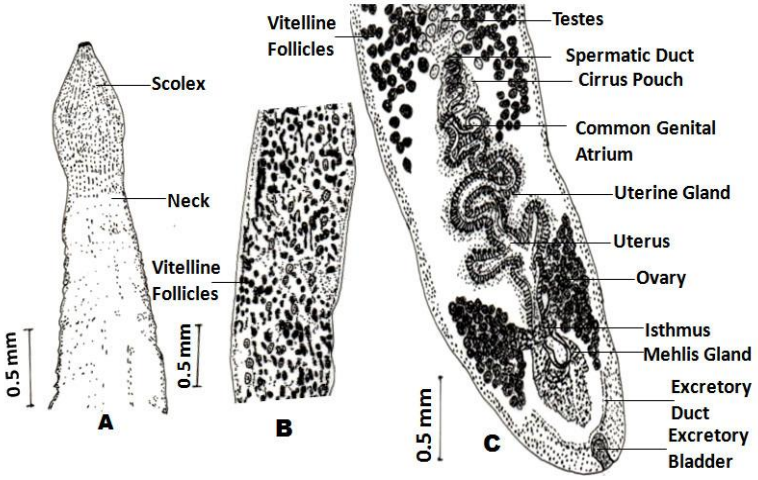

Figure 3 Camera lucida illustrations of *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar, (scale bar: 0.5mm); A - Anterior end with pseudo–blunt scolex and undifferentiated short neck; B - Middle region of mature worm carrying vitelline follicles; C - Posterior end of mature worms showing male and female reproductive system.

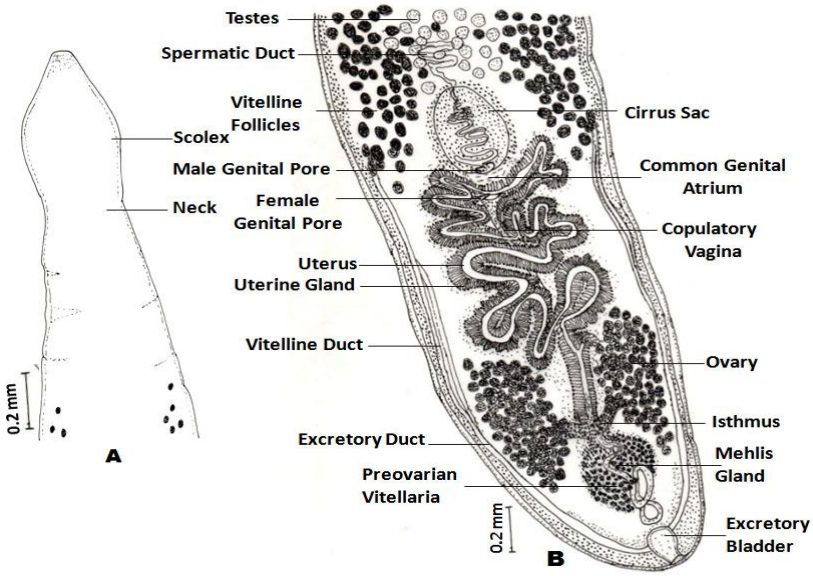

Figure 4 Camera lucida illustrations of *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar, (scale bar: 0.2mm); A - Anterior end with pseudo–blunt scolex and undifferentiated short neck; B - Posterior end of mature worms showing male and female reproductive system, vitelline duct, excretory duct and terminal excretory bladder.

1.176–1.532 x 0.441–0.747 mm. The scolex was followed by a short neck of 0.470–0.952 x 0.529–0.667mm that did not comprise reproductive structures. More than 2/3 of the posterior half of the body comprised numerous testes with the size of 0.032–0.089 x 0.065–0.138 mm that were ovoid, larger than vitelline follicles, and occupied the medullary region, extending from just behind anterior-most vitelline follicles to cirrus sac posteriorly. The cirrus sac is compact, bulbous 0.235–0.380 x 0.148–0.257 mm; the ejaculatory duct opens close to the female genital pore into a shallow common genital atrium 0.190–0.285 x 0.117–0.177 mm.

The ovary is bilobed, follicular, predominantly butterfly–shaped and have a size of 1.115–1.137 x 0.915–1.117, ovarian follicles extending posteriorly beyond Mehlis' gland, ovarian lobes cortical and joined to each other by medullary ovarian isthmus 0.135–0.238 x 0.088–0.118 mm before Mehlis' gland 0.194–0.263 x 0.166–0.205 mm. The uterus was sacular and glandular and had a size of 2.150–3.380 x 0.058–0.127 mm, extending in front of the ovarian isthmus up to the cirrus sac. The conducting vagina with a diameter of 0.030–0.045 mm joined the uterus at a little distal end, however, the copulatory vagina with a diameter of 0.040–0.067 opened together at the shallow atrium.

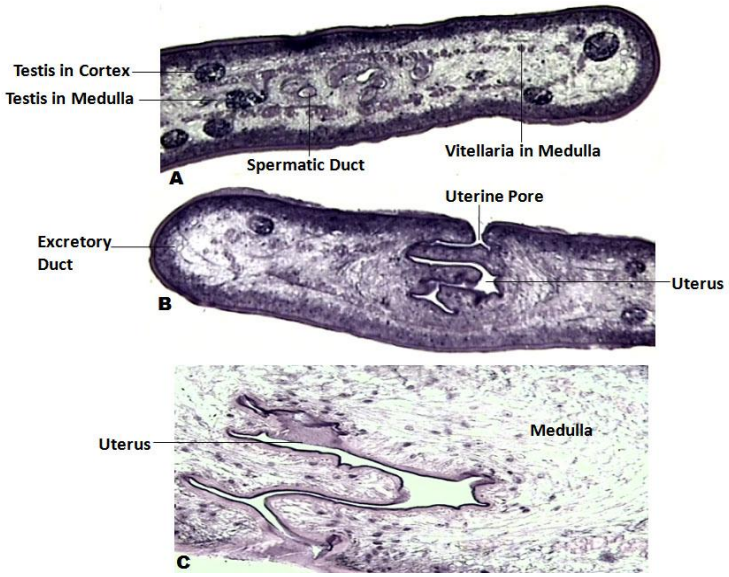

Figure 5 Microphotographs of histology section of mature proglottids of *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar; A - Testes in medullary and cortical region (scale bar: 50X); B - Lateral excretory duct and uterine pore (scale bar: 50X); C - Immense medullary region innervated with huge uterus lined by uterine glands (scale bar: 225X).

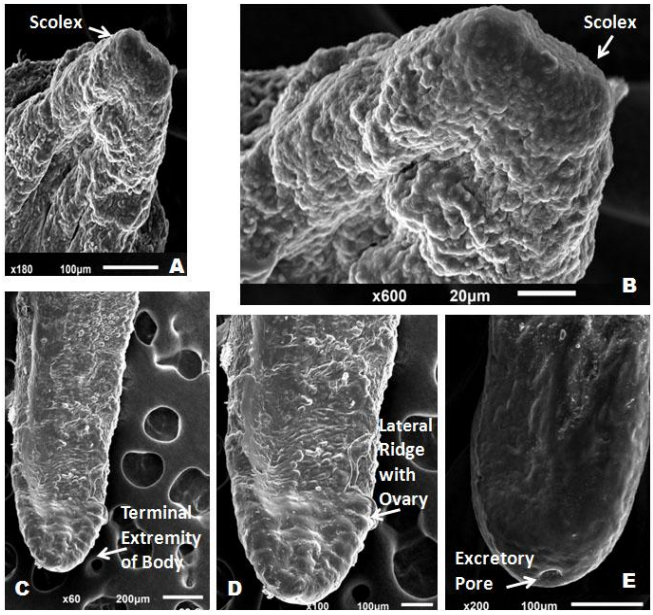

Figure 6 Scanning Electron Microphotographs showing ultratopography *L. haryanii* n.sp. in *C. batrachus* from river Yamuna at Yamuna Nagar; A - Anterior end with pseudo–blunt scolex (scale bar: 100µm; 180X); B - Anterior end with broad scolex (scale bar: 20µm; 600X); C - Posterior end of mature worms showing terminal extremity (scale bar: 200µm; 60X); D - Posterior end of mature worms showing lateral ridge with ovary (scale bar: 100µm; 100X); E - Posterior extremity of mature worms showing terminal excretory pore (scale bar: 100µm; 200X).

The vitelline follicles were ovoid to subovoid 0.027–0.078 and 0.032–0.079 mm in size and emerged a little distance anterior to the testes and traveled up to the level of the cirrus sac. The vitelline ducts diameter was 0.035–0.055 mm and the excretory ducts diameter was reported 0.025–0.040 mm running laterally connected to vitelline glands and excretory bladder 0.125–0.240 x 0.050–0.150 mm respectively. The excretory bladder carries a short excretory bulb-like structure with a 0.080–0.149 mm diameter and followed by a terminal excretory pore with a size of 0.045–0.095 mm through which it opens outside. Eggs were numerous, oval, rough 0.253–0.350 x 0.095–0.125 mm in size, and somewhat operculate in the high magnification microscopic observations.

Systematic Summary

Phylum	–	Platyhelminthes Gegenbaur 1859
Class	–	Cestoda Yamaguti 1961
Order	–	Caryophyllidea Woodland 1926
Family	–	Lytocestidae Wardle and McLeod 1952
Subfamily	–	Lytocestinae Hunter 1927
Genus	–	*Lytocestus* Cohn 1908
Species	–	*haryanii* n. sp.

The present newer worm under investigation comprised a single proglottid body with spatulate, elongated, blunt-ended flat terminal, undifferentiated scolex, short undifferentiated neck, and numerous testes in the medullary region with sparse distribution in the cortical region confirmed through the histological investigation. The follicular vitellaria running more than two third of the body and found to be suspended in the cortical region and reflecting its quite closer similarity to typical *Lytocestus* species including *L. adhaerens* (Cohn 1908), *L. filiformis* (Woodland 1923), *L. chalisgaonensis* (Kalse and Shinde 1999), *L. govindae* (Patil and Jadhav 2002), *L. shindae* (Khadap et al. 2004), *L. clariae*, and *L. assamensis* (Tandon et al. 2005), *L. paithanensis* (Shelke 2007), *L. punensis* (Jadhav et al. 2008), *L. murharii* (Kaul et al. 2010), *L. follicularae* (Bhure et al. 2010), *L. gariepinusae* (Kadam and Dhole 2011), *L. khami* (Jawale and Borde 2011), *L. manjaraensis* (Solunke et al. 2012), *L. godavariensis* (Pawar and Dandwate 2013), *L. mastacembellusi* (Pardeshi 2016), *L. mulaansis* (Dandawate 2018), *L. bharatae* (Patil 2018), *L. chaaviensis* (Narayan and Srivastav 2020), *L. heteropnuestii* (Ash et al. 2012), *L. vyasaei* (Pawar and Hiware 2011), *L. longicolis* (Ramadevi 1973), *L. purnensis* (Jawale and Borde 2011), *L. attenuates* (Tandon et al. 2005), *L. lativitellarium* (Furtado and Kim–Low 1973), *L. purvulus* (Furtado 1963), *L. indicus* (Moghe 1925, 1931; Ash et al. 2011) and *L. sahayi* (Bhavsar et al. 2020). The present worm differs from *L. sahayi* (Bhavsar et al. 2020) in the shape of scolex (undifferentiated vs. differentiated), from *L. shindei* (bluntly differentiated vs. tapering anteriorly), from *L. manjaraensis* (Solunke et al. 2012) (bluntly differentiated vs. cylindrical); and similar to *L. adhaerens* (Woodland 1923), *L. filiformis* (Woodland 1926), *L. assamensis* (Tandon et al. 2005), *L. paithanensis* (Shelke 2007) in shape and size of an undifferentiated, short neck. The present tapeworm is quite similar in appearance of scolex to *L. chalisgaonensis* (Kalse and Shinde 1999), *L. shindae* (Khadap et al. 2004), *L. clariae* (Tandon et al. 2005), *L. murharii* (Kaul et al. 2010) and *L. nagapurensis* (Lakhe et al. 2004) in the shape of the scolex (blunt-ended, spatulate, elongated, elyptical and somehow flat at anterior-most extremity) confirmed through the ultratopography using scanning electron micrography. The present form, differs from *L. assamensis* (Tandon et al. 2005) in the shape of ovary (butterfly shaped vs. inverted 'A' shaped), and differs from *L. paithanensis* (Shelke 2007) in the shape of the cirrus pouch (oval vs. cylindrical), differs in the orientation of cirrus pouch from

Table 1 Polythetic divisive classificatory system based on taxometric analysis from observations of *L. mulaansis* (Dandawate 2018), *L. khami* (Jawale and Borde 2011), *L. chaaviensis* (Narayan and Srivastav 2020) *vis–a`–vis L. haryanii* n.sp.

Worm Character	Organ dimension	*L. mulaansis*				*L. khami*				*L. chaaviensis*			
		C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.
Body	L	2.499	0.944	0.561	0.439	2.292*	0.086*	0.091*	0.909*	2.126*	0.198*	0.225*	0.775*
	W	2.517	0.604	0.370	0.630	2.638	0.373	0.363	0.637	2.128*	0.796	0.323	0.677
Scolex	L	2.838	0.201	0.559	0.441	2.760	0.465	0.307	0.693	2.758	0.204*	0.235*	0.765*
	W	2.735	0.589	0.848	0.152	2.770	0.554	0.390	0.610	2.476	0.595	0.861	0.139
Testes	L	2.345	0.527	0.385	0.615	2.317	0.370	0.458	0.542	2.484	0.349	0.445	0.555
	W	2.262*	0.276*	0.263*	0.737*	2.167*	0.189*	0.198*	0.802*	2.401	0.412	0.519	0.481
Ovary	L	2.307	0.540	0.748	0.252	2.505	0.421	0.552	0.448	2.556	0.387	0.513	0.487
	W	2.372	0.761	0.339	0.661	2.540	0.623	0.563	0.437	2.349	0.518	0.504	0.496
Cirrus Pouch	L	2.495	0.344	0.397	0.603	2.160*	0.129*	0.139*	0.861*	2.155*	0.082*	0.932	0.068
	W	2.775	0.333	0.407	0.593	2.115*	0.318	0.318	0.682	2.452	0.349	0.358	0.642
Egg	L	2.319	0.177*	0.193*	0.807*	–	–	–	–	2.679	0.322	0.251*	0.749*
	W	2.123*	0.212*	0.248*	0.752*	–	–	–	–	2.461	0.380	0.329	0.671

C.D. - Coefficient of divergence; M.C.D. - Mean character difference; C.Dis. -Coefficient of dissimilarity; C.S. - Coefficient of similarity;
* Non–significant; – Observations not available

Table 2 Polythetic divisive classificatory system based on taxometric analysis from observations of *L. clariae* (Tandon et al. 2005), *L. heteropnuestii* (Ash et al. 2012), *L. assamensis* (Tandon et al. 2005) *vis–a`–vis L. haryanii* n.sp.

Worm Character	Organ dimension	*L. clariae*				*L. heteropnuestii*				*L. assamensis*			
		C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.
Body	L	2.072*	0.172*	0.061*	0.939*	2.167*	0.557	0.394	0.606	2.518	0.554	0.392	0.608
	W	2.200*	0.320	0.412	0.588	2.336	0.782	0.499	0.501	2.539	0.613	0.900	0.100
Testes	L	2.250*	0.739	0.586	0.414	–	–	–	–	2.728	0.781	0.642	0.358
	W	2.895	0.752	0.617	0.383	–	–	–	–	2.408	0.734	0.598	0.402
Ovary	L	2.584	0.619	0.900	0.100	2.774	0.619	0.904	0.096	2.830	0.629	0.927	0.073
	W	2.667	0.819	0.731	0.269	2.737	0.646	0.579	0.421	2.806	0.844	0.515	0.485
Vitellaria	L	2.303	0.925	0.867	0.133	2.105*	0.691	0.577	0.423	2.395	0.460	0.627	0.373
	W	2.398	0.411	0.583	0.417	2.222*	0.208*	0.955	0.045	2.322	0.584	0.833	0.167
Excretory Pore	L	2.498	0.141*	0.153*	0.847	–	–	–	–	–	–	–	–
	W	2.894	0.320	0.608	0.392	–	–	–	–	–	–	–	–
Egg	L	2.266*	0.700	0.393	0.607	2.125*	0.250*	0.286*	0.714*	2.375	0.325	0.393	0.607
	W	2.335	0.630	0.400	0.600	2.333	0.467	0.629	0.371	2.381	0.333	0.400	0.600

C.D. - Coefficient of divergence; M.C.D. - Mean character difference; C.Dis. - Coefficient of dissimilarity; C.S. - Coefficient of similarity; * Non–significant; – Observations not available

Table 3 Polythetic divisive classificatory system based on taxometric analysis from observations of *L. vyasaei* (Pawar and Hiware 2011), *L. longicolis* (Ramadevi 1973), *L. purnensis* (Jawale and Borde 2011) *vis–a`–vis L. haryanii* n.sp.

Worm Character	Organ dimension	*L. vyasaei*				*L. longicolis*				*L. purnensis*			
		C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.
Body	L	2.224*	0.189*	0.094*	0.904*	2.274*	0.168*	0.185*	0.815*	2.092*	0.137*	0.148*	0.852*
	W	2.384	0.374	0.478	0.522	2.389	0.456	0.595	0.405	2.576	0.506	0.678	0.322
Scolex	L	2.333	0.254*	0.299*	0.701*	–	–	–	–	2.635	0.250*	0.288*	0.712*
	W	2.526	0.374	0.539	0.461	–	–	–	–	2.572	0.712	0.605	0.395
Testes	L	2.113*	0.114*	0.121*	0.879*	2.280*	0.336	0.427	0.573	2.584	0.616	0.380	0.620
	W	2.357	0.520	0.714	0.286	2.505	0.347	0.441	0.559	2.404	0.511	0.581	0.419
Ovary	L	2.723	0.345	0.458	0.542	2.278*	0.375	0.494	0.506	2.651	0.582	0.518	0.482
	W	2.647	0.736	0.648	0.352	2.642	0.531	0.104*	0.896*	2.594	0.854	0.769	0.231
Vitellaria	L	–	–	–	–	2.391	0.711	0.594	0.406	–	–	–	–
	W	–	–	–	–	2.515	0.501	0.700	0.300	–	–	–	–
Cirrus Pouch	L	2.547	0.549	0.879	0.121	2.341	0.165*	0.184*	0.816*	2.689	0.839	0.409	0.591
	W	2.879	0.266*	0.203*	0.797*	2.438	0.312	0.379	0.621	2.705	0.517	0.485	0.515
Excretory pore	L	–	–	–	–	2.755	0.665	0.898	0.102	–	–	–	–
	W	–	–	–	–	2.200*	0.289*	0.453	0.547	–	–	–	–
Egg	L	2.838	0.560	0.969	0.031	–	–	–	–	2.029*	0.181*	0.201*	0.799*
	W	2.756	0.557	0.787	0.213	–	–	–	–	2.260*	0.098*	0.017*	0.983*

C.D. - Coefficient of divergence; M.C.D. - Mean character difference; C.Dis. - Coefficient of dissimilarity; C.S. - Coefficient of similarity; * Non–significant; – Observations not available

Table 4 Polythetic divisive classificatory system based on taxometric analysis from observations of *L. attenuates* (Tandon et al. 2005), *L. lativitellarium* (Furtado and Kim–Low 1973), *L. purvulus* (Furtado 1963) *vis–a`–vis L. haryanii* n.sp.

Worm Character	Organ dimension	*L. attenuates*				*L. lativitellarium*				*L. purvulus*			
		C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.
Body	L	2.046*	0.389	0.558	0.442	2.198*	0.154*	0.168*	0.832*	2.446	0.526	0.328	0.672
	W	2.011*	0.477	0.627	0.373	2.399	0.885	0.526	0.474	2.693	0.623	0.924	0.076
Testes	L	2.422	0.653	0.985	0.015	2.375	0.714	0.556	0.444	2.462	0.558	0.800	0.200
	W	2.118*	0.414	0.707	0.293	2.320	0.767	0.743	0.257	2.690	0.592	0.550	0.450
Ovary	L	2.532	0.605	0.871	0.129	–	–	–	–	–	–	–	–
	W	2.609	0.706	0.624	0.276	–	–	–	–	–	–	–	–
Vitellaria	L	2.405	0.814	0.699	0.301	2.778	0.571	0.878	0.122	–	–	–	–
	W	2.545	0.733	0.584	0.416	2.469	0.534	0.750	0.250	–	–	–	–
Egg	L	2.353	0.305	0.343	0.657	2.433	0.312	0.592	0.408	0.845	0.321	0.362	0.638
	W	2.408	0.333	0.400	0.600	2.387	0.322	0.476	0.524	0.761	0.655	0.524	0.476

C.D. - Coefficient of divergence; M.C.D. - Mean character difference; C.Dis. - Coefficient of dissimilarity; C.S. - Coefficient of similarity;
* Non–significant; – Observations not available

L. punensis (Jadhav et al. 2008) (vertically placed vs. transversely placed), *L. thapari* (Sawarkar and Kale 2012) (vertically placed vs. obliquely placed), *L. godavariensis* (Pawar and Dandwate 2013) (vertically *vs.* transverse orientation) and differs from *L. murharii* (Kaul et al. 2010) in the vagina (coiled *vs.* slightly curved). The present form is quite similar to *L. gariepinusae* (Kadam and Dhole 2011) in the shape of the scolex, and presence of the neck but differed in the number of testes. However, the *L. khami* (Jawale and Borde 2011) and *L. thapari* (Sawarkar and Kale 2012) shared a similar shape of receptacle seminalis (present), vitellaria (follicular) and differed in the eggs (undifferentially operculated *vs.* nonoperculated). The present worm corroborated the vitellaria shape (follicular) with *L. indicus* (Moghe 1925, 1931; Ash et al. 2011), *L. birmanicus* (Lynsdale 1956), *L. moghei* (Murhar 1963), *L. longicollis* (Ramadevi 1973), *L. fossilis* (Singh 1975), *L. marathawadaensis* (Shinde and Phad 1988), *L. alii* (Sawarkar 2012), *L. clariasae* (Kadam et al. 1998; Shinde and Pawar 2002), *L. kopardaensis* (Shinde and Borde 1999), *L. teranaensis* (Kolpuke 1999), *L. batrachusae* (Pawar and Shinde 2002), *L. subhapradhi* (Jawalikar et al. 2008), *L. osamnabadensis* (Bhure et al. 2010), *L. vyasaei* (Pawar and Hiware 2011), *L. purnensis* (Jawale and Borde 2011), *L. ambe* (Kankale 2017) and *L. elongates* (Barshe et al. 2018). The present worm showed close resemblance with *Lytocestus indicus* (Moghe 1925) Woodland 1926 redescribed by Ash et al. (2011) in generic diagnostic characters, shape of neck, excretory duct, numerous medullary testes and vitelline follicle predominantly in cortex region, spherical to oval cirrus sac; however carried several striking contrasts including body size (smaller and narrower vs. larger and obese), shape and size of scloex (smaller blunt pseudoscloex with somewhat flat anterior extremity vs. larger, digitiform with rounded anterior terminal), cirrus pouch (smaller vs. larger), ovary follicular (ovarian follicles loose, broad 'H' or butterfly shaped vs. compact, dumbbell or butterfly shaped), uterus fantastically looped (dense and larger uterine glands vs. numerous but comparative smaller uterine glands) and well marked excretory bladder comprised tiny tube and opened outside through shallow excretory pore.

The present work was also evaluated and validated through the numerical morphotaxometry using a polythetic divisive classificatory system (C.D., M.C.D., C.Dis., C.S.) with special reference to taxonomically significant characters based on the earlier reported observations of *L. mulaansis* (Dandawate 2018), *L. khami* (Jawale and Borde 2011), *L. chaaviensis* (Narayan and Srivastav 2020), *L. clariae* (Tandon et al. 2005), *L. heteropnuestii* (Ash et al. 2012), *L. assamensis* (Tandon et al. 2005), *L. vyasaei* (Pawar and Hiware 2011), *L. longicolis* (Ramadevi 1973), *L. purnensis* (Jawale and Borde 2011), *L. attenuates* (Tandon et al. 2005), *L. lativitellarium* (Furtado and Kim–Low 1973), *L. purvulus* (Furtado 1963), *L. indicus* (Moghe 1925, 1931; Ash et al. 2011), *L. sahayi* (Bhavsar et al. 2020), *L. mastacembellusi* (Pardeshi 2016) *vis–a–vis* present newer worm that showed significant contrast characters in term of the statistical dimension and size (Tables 1 to 5). Based on the observed, compared, and discussed favored similarities to evaluate the generic diagnosis and striking contrasts for the substantiation and validation of the newer worm, the authors suppose to propose the present worms of the discussion as a new species i.e. *L. haryanii* n.sp.

Taxonomic Summary

Type host : *Clarias batrachus* Linnaeus 1758

Type habitat : River Yamuna

Table 5 Polythetic divisive classificatory system based on taxometric analysis from observations of *L. indicus* (Moghe 1925, 1931; Ash et al. 2011), *L. mastacembellusi* (Pardeshi 2016), *L. sahayi* (Bhavsar et al. 2020) *vis–a`–vis L. haryanii* n.sp.

Worm Character	Organ dimension	*L. indicus*				*L. mastacembellusi*				*L. sahayi*			
		C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.	C.D.	M.C.D.	C.Dis.	C.S.
Body	L	2.578	0.398	0.402	0.598	2.409	–	–	–	2.424	0.404	0.484	0.516
	W	2.476	0.423	0.545	0.455	2.459	0.305	0.395	0.605	2.843	0.382	0.488	0.512
Testes	L	2.620	0.323	0.556	0.444	2.462	0.171*	0.186*	0.814*	2.756	0.309	0.432	0.568
	W	2.400	0.767	0.743	0.257	2.368	0.362	0.453	0.547	2.568	0.619	0.601	0.399
Ovary	L	–	–	–	–	2.341	0.287*	0.396	0.604	2.579	0.312	0.422	0.578
	W	–	–	–	–	2.319	0.567	0.573	0.427	2.553	0.573	0.532	0.468
Vitellaria	L	2.577	0.571	0.782	0.218	–	–	–	–	2.302	0.508	0.849	0.151
	W	2.650	0.534	0.750	0.250	–	–	–	–	2.349	0.431	0.607	0.393
Egg	L	2.774	0.127*	0.238*	0.762*	–	–	–	–	2.813	–	–	–
	W	2.381	0.560	0.432	0.568	–	–	–	–	2.718	–	–	–
Cirrus Pouch	L	–	–	–	–	2.107*	–	–	–	–	–	–	–
	W	–	–	–	–	2.317	0.442	0.465	0.535	–	–	–	–

C.D. - Coefficient of divergence; M.C.D. - Mean character difference; C.Dis. - Coefficient of dissimilarity; C.S. - Coefficient of similarity; * Non–significant; – Observations not available

Type microhabitat : Intestine

Type locality : Yamuna Nagar, Haryana, India

Deposition of specimens : Holotype (BZPLC 105), Paratypes (BZPLC 106) in Zoology Laboratory, MM(DU), Haryana, India.

Etymology : The species name of newer worms proposed after the locality State, Haryana.

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Conflicts of Interest

The authors declare no conflict of interest.

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