Research Article

A New Species of *Allocreadium* Looss, 1900 (Allocreadoidea: Allocreadiidae) in *Wallago attu* Bloch and Schneider, 1801 (Siluriformes: Siluridae) from Yamuna River at Yamuna Nagar, Haryana

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ABSTRACT

Background: *Allocreadium* Looss is a digenetic parasitic trematode of the family Allocreadiidae Stossich and is predominantly found in freshwater catfishes. There are more than 104 species of *Allocreadium* sp. known from a wide range of hosts including 44 Indian species so far. The selected site of the investigation was unexplored in terms of helminth diversity, therefore present investigation was designed to find out and taxonomically evaluate the trematodes population in the fish hosts. **Materials and Methods:** The morphological, microscopic, and morphometric assessment of the recovered endoparasitic worms was performed in the Zoology Laboratory, Department of Bio-Sciences and Technology, M.M. (Deemed to be University), from July 2018 to June 2020. The newly recovered allocreadoidean trematodes were diagnosed as a species of *Allocreadium* Looss (Allocreadoidea: Allocreadiidae) on the basis of the generic diagnostic charades as per the *Key to the Trematoda* during the present investigation from the intestine of freshwater catfish *Wallago attu* Bloch and Schneider (Siluriformes: Siluridae) of river Yamuna at Yamuna Nagar (Haryana), India. **Conclusion:** The morphotaxometric analysis of the present worms with the prior detailed types of similar genera through the advanced statistical tool the Polythetic divisive classificatory system reflected the striking contrasts of taxonomic significance. Therefore, based on the numerous remarkable contrasts in the blend of recognizing features of taxonomic significance, the present worms proposed to be the newer *Allocreadium haryanii* n.sp.

Keywords: *Allocreadium haryanii* n.sp., *Wallago attu*, Trematodes, Morphotaxonomy, Morphotaxometry.

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INTRODUCTION

Parasitology is an on-growing discipline of life sciences. Helminthes parasites are known for their immense diversity in the hosts of diverse habitats and are studied by the different schools of Parasitology worldwide.[1-10] The Helminthes parasites cause major threats to fish and exploit them for food, shelter, and reproduction.[11-14] The digenean are a significant gathering of Helminthes, usually invading the digestive tract of fresh and sea water Piscean hosts.[15-19] *Allocreadium* Looss[20] is the digenetic parasitic trematode of the family Allocreadiidae Stossich[21] and usually found in freshwater catfishes and predominantly found to be distributed in USSR and Asia including India.[22-26]

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The pioneer *Allocreadium* species was coined in the 19th century and proposed as *A. isoporum* Looss[27] that was further recovered and redescribed as *A. annandelei* from *Rhynchobatus djeddesis*.[28] Thereafter, Thapar and Dayal documented and published *Allocreadium* sp. from *Leuciscus indicus*.[29] However, *A. handidi* from *Ophiocephalus punctatus* and *A. nicolli* from *Gobius giuris* were recovered and taxonomically identified by Pande.[30,31] Further Kaw[32] published the *A. nemachilus* from *Nemachilus kashmirensis*. In the subsequent decade, Gupta investigated the genus *Allocreadium* very much and reported *A. thapari* from *Rita rita* and *A. mehri* from *Rhychobdella aculeate*.[33,34] Further *A. raipurensis* from *Clarias batrachus* and *A. spindale* from *Mastacembelus armatus* were recovered and reported by Saxena.[35] Meanwhile, during working with *O. punctatus*, Srivastava published the *A. ophiocephali*.[36] In the same decade, many Parasitologists significantly contributed to the biodiversity of *Allocreadiun* and published *A. dollfusi, A. singhi* and *A. hirani* from fish *Barbus tor*,[37] *A. hetropneustusius* from *Hetropneustes fossilis*[38] as well as Kakaji recorded and published *A. catlai* from *Catla catla, A. fasciatusi* from *Trichogaster fasiatus* and *A. gupti* from *R. rita*.[39-41] In the next decade *A. kasmirensis* from *Schizothorax niger* was studied and reported by Fotedar and Dhar from Kashmir, India.[42] In the subsequent decade a new species of trematodes, *A. fotedari* was reported and published from *S. niger*.[43] Meanwhile, in South India, new species of digenean trematodes including *A. fascitusi* from *Aplocheilus melastigma* and *A. hamdai* from *Channa punctatus* were described.[44,45] Kalyankar and Deshmukh[46] isolated *A. indicum* from *Labeo rohita,* however, Gupta and Puri[47] published *A. calbasii* from *L. calbasu* and *A. manteri* from *Anabas testudineus* in the same year. In the subsequent year, Khan[48] documented and published *A. jainin* from catfish *Wallago attu* and *A. mrigalai* from *Cirrhinus mrigala* at Lucknow (UP), India. Thereafter *A. tigarai* was published by Bhadauria and Dandotia.[49]

In the early 21st century, *A. tonsi* from *Clupisoma garua* and *A. bimaculatusi* from *Ompok bimaculatum* has published in north Indian regions.[50] In the subsequent year *A. bundelkhandesis* and *A. punctatai* were documented from *Channa* sp. of Jhansi (U.P), India.[51] A newer worm of the same trematode *A. tori* from freshwater fish *Tor putitora* was reported well by Anjum *et al*.[52] The preexisting species *A. handiai* was redescribed and published by some schools of Parasitology by gene sequence analysis.[53] Few years back *A. gomtioensis* was extracted by Chandra *et al.* from the fish *Mystus tengara*.[26] But the selected area of river Yamuna in Haryana was unexplored in terms of parasitological investigation in the fish hosts so far. Therefore, the present study intended to find out the parasitic Helminthes in the catfish, *Wallago attu* inhabiting selected sites of the river Yamuna.

MATERIALS AND METHODS

The present study was conducted in the Zoology Laboratory, Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala (HR), India from July 2018 to June 2020. The host catfish, *Wallago attu* Bloch, and Schneider, 1801 (Siluriformes: Siluridae) were collected from the three selected sites in a stretch of 5 km up and downstream of river Yamuna from the central point at Yamuna Nagar (Haryana), India (Figure 1). The freshly collected fish were brought to the laboratory for further parasitological investigation. The body cavity, visceral organs, gonads, and alimentary canal of the hosts were carefully examined for encysted larvae, parasitic stages, and adult parasites. The worms were taken out through brush and kept in fresh Petri dishes containing Luke warm water and further thoroughly washed using clean tap water. The collected trematode specimens were fixed in Bouin's fluid and further processed for permanent slide preparation.[54] The permanently mounted trematodes were observed under the light microscope for understanding the morphological characters of taxonomic significance and morphometric measurements in millimeters (mm). The microphotographs were taken using the a unit of image Analysis "MOTIC" through advance microscopic soft tools. The biostatistical calculations like mean and standard error were calculated as per the procedure described by Snedecor and Cochran.[55] The Polythetic Divisive Classificatory System was applied to conduct

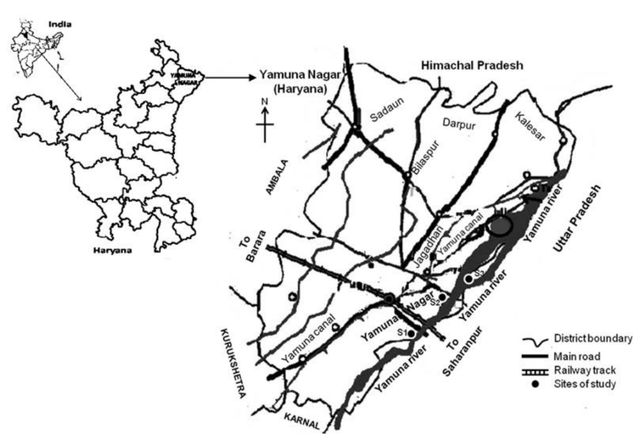

Figure 1: The district map of Yamuna Nagar showing sites (S_1, Near Yamuna bridge; S_2, Dadwa-Fatehpur; S_3, Dadupur) of fish sample collection from river Yamuna (*Map not to scale bar*).

taxometric analysis to establish the newer taxa using various statistical tools.[56,57]

RESULTS

Description (Figures 2, 3)

The endoparasitic trematode worms recovered from the intestine of freshwater catfish host, *Wallago attu* (Siluriformes: Siluridae) was identified on the basis of '*Key to the Trematoda*', as a genus *Allocreadium* Looss (Allocreadoidea: Allocreadiidae). The trematode worms were sub-cylindrical in shape and sub-medium in size 2.769-4.672 (3.377±0.144) x 0.403-1.110 (0.968±0.077) with a tiny protuberance at the anterior extremity 0.133-0.215 (0.185±0.041) x 0.180-0.278 (0.232±0.027) and the body tegument or cuticle observed smooth. The body was anteroposteriorly rounded; oral sucker 0.192-0.492 (0.297±0.098) x 0.239-0.574 (0.393±0.069) in size; however, ventral suckers well developed with a dimension of 0.195-0.672 (0.442-0.096) x 0.328-0.787 (0.528-0.093) and situated at a distance of 0.361-0.880 (0.671±0.197) from the anterior extremity. The digestive tract comprising pharynx 0.166-0.293 (0.223±0.073) x 0.121-0.244 (0.167±0.085) occurred adjacent to oral sucker followed by comparatively elongated esophagus 0.360-0.599 (0.485±0.065) x 0.016-0.048 (0.032-0.003) that further divided into lateral intestinal caeca 1.204-3.319 (2.299±0.126) x 0.160-0.310 (0.232-0.033) in the region of a ventral sucker as well as posterior to gonopore and running to the subterminal region and terminated 0.598-0.713 (0.699±0.106) prior to posterior extremity. The gonopore was 0.098-0.115 (0.100±0.026) in size and situated before but near the forking point of the esophagus. The cirrus sac was larger 0.198-0.319 (0.249±0.096) x 0.148-0.476 (0.287±0.084) with well-developed cirrus and seminal vesicle. The lobed paired testes were larger, spherical, median in position, sub-equal in size arranged one anterior testis 0.806-0.966 (0.886±0.099) x 0.776-0.994 (0.985±0.103) back to the other posterior testis 0.865-0.925 (0.896±0.017) x 0.929-0.989 (0.951±0.150) and sub-equatorial in the location in the body at a distance from anterior extremity 1.325-1.576 (1.431±0.140) and posterior terminal 1.147-1.662 (1.531±0.152). Ovary was also larger 0.249-0.382 (0.267±0.086) x 0.231-0.480 (0.354±0.098) but comparatively smaller than testes as well as located at a distance of 0.765-0.968 (0.876±0.076) from the anterior terminal in between the ventral sucker and anterior testis. The seminal receptacle and Laurer's canal were present, the seminal receptacle voluminous and pear-shaped 0.111-0.380 (0.294±0.068) x 0.079-0.212 (0.137±0.016) arranged in between the ovary and anterior testis. The

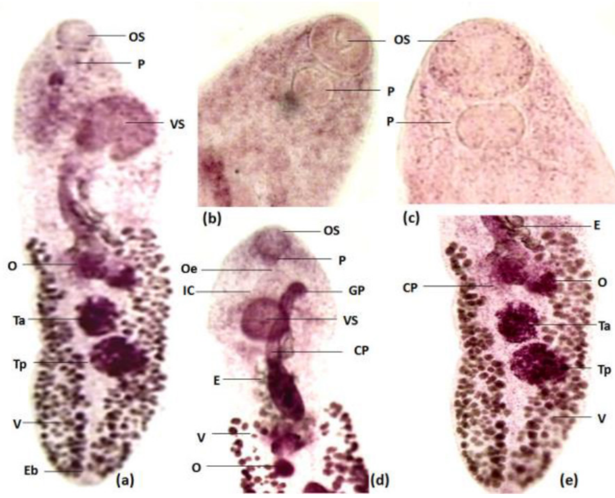

Figure 2: Microphotographs of present *Allocreadium haryanii* n.sp. in *Wallago attu*. A. Whole mount (scale bar: 50X); B, C. Anterior region showing oral sucker and pharynx (scale bar: 200X and 240X respectively); D. Anterior half of mature worms showing genital pore, cirrus pouch and ventral sucker (scale bar: 50X); E. Posterior half showing testes and vitellaria (scale bar: 50X). *Where: OS, oral sucker; P, pharynx; VS, ventral sucker; O, ovary; Ta, anterior testis; Tp, posterior testis; V, vitellaria; Eb, excretory bladder; Oe, oesophagus; IC, intestinal caeca; GP, genital pore; CP, cirrus pouch; E, eggs.*

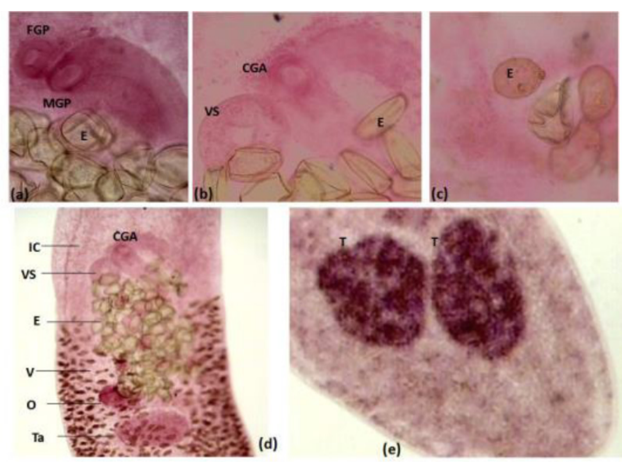

Figure 3: Microphotographs of present *Allocreadium haryanii* n.sp.. A. Genital pores and Eggs (scale bar: 225X); B, Common genital atrium and ventral sucker (scale bar: 225X); C. Eggs with pseudo tuft (scale bar: 225X); D. Middle half of mature worms showing genital atrium, ventral sucker, ovary, anterior testis (scale bar: 60X); E. Posterior region showing testes (scale bar: 225X). *Where: FGP, female genital pore; MGP, male genital pore; CGA, common genital atrium T, testes.*

vitelline glands or vitellaria 0.090-0.131 (0.113±0.012) x 0.096-0.115 (0.109±0.007) were found to immensely develop in the posterior half and back to the testes but these were continuous to oesophageal region and completely fill the body as well. The uterus was very short located in between the ventral sucker and anterior testis and filled with eggs. The eggs were relatively moderate in size 0.035-0.095 (0.073±0.013) x 0.017-0.064 (0.054±0.020) with faint shiny coloration and arranged in a bunch. Posteriorly the body comprised a relatively larger excretory bladder or vesicle 0.310-0.481 (0.363±0.052) x 0.066-0.125 (0.109±0.007) ran from the posterior extremity to the end terminus of caecum.

Systematic Summary

Phylum	-	Platyhelminthes Gegenbaur[58]
Class	-	Trematoda Rudolphi[59]
Order	-	Digenea Beneden[60]
Superfamily	-	Allocreadoidea Looss[61]
Family	-	Allocreadiidae Stossich[21] Odhner[62]
Subfamily	-	Allocreadiinae Looss[61]
Genus	-	*Allocreadium* sp.[20,27]
Species	-	*Allocreadium haryanii* n.sp.

Taxonomic Summary

Type host	:	*Wallago attu* Bloch and Schneider[63]
Type habitat	:	River Yamuna
Type microhabitat	:	Intestine
Type locality	:	Yamuna Nagar (Haryana), India
Deposition of specimens	:	Holotype (BZPLT 101), Paratypes (BZPLT 102) in the Zoology Lab., MM(DU)
Etymology	:	The species name of newer worms proposed after the name of State, Haryana

DISCUSSION

The morphological and morphometric findings of the recovered newer worms were substantiated using the Polythetic divisive classificatory system (C.D., M.C.D., C.Dis., C.S.) and compared with the observations of systematic significance from the reported species including *A. ramai,*[64] *A. handiae,*[53,64,65] *A. gomtiensis,*[64] *A. kasciatusi,*[66] *A. thapari,*[33,66] *A. nicolli,*[64] *A. handi,*[67] *A. kosia,*[64] *A. mehari,*[33,67] *A. heteropnuestisis,*[67,68] *A. tamoroko,*[69] *A. patagonium*[70] sharing the same group of hosts (Tables 1-4). The present worm differs from *A. ramai* in the larger body size (C.D., 2.693; M.C.D., 0.356; C.Dis., 0.827), oral sucker (C.D., 2.516; M.C.D., 0.384; C.Dis., 0.489), ventral sucker (C.D., 2.565; M.C.D., 0.397; C.Dis., 0.596), pharynx (C.D., 2.741; M.C.D., 0.521; C.Dis., 0.957), cirrus pouch (C.D., 2.829; M.C.D., 0.451; C.Dis., 0.479), anterior testis (C.D., 2.661; M.C.D., 0.383; C.Dis., 0.554), posterior testis (C.D., 2.531; M.C.D., 0.306; C.Dis., 0.974) and its position in the body, ovary (C.D., 2.537; M.C.D., 0.356; C.Dis., 0.478), vitelline follicle and eggs (C.D., 2.575; M.C.D., 0.532; C.Dis., 0.918) (Table 1).[64] Similarly the present worms under investigation varied from *A. handiae* in the larger body size (C.D., 2.574; M.C.D., 0.524; C.Dis., 0.789), oral sucker (C.D., 2.303; C.Dis., 0.715), ventral sucker (C.D., 2.679; M.C.D., 0.506; C.Dis., 0.685), pharynx (C.D., 2.843; M.C.D., 0.707; C.Dis., 0.761), cirrus pouch (smaller vs. larger), anterior testis (C.D., 2.509; M.C.D., 0.347; C.Dis., 0.491), posterior testis (C.D., 2.567; M.C.D., 0.770; C.Dis., 0.635) and its position in the body, ovary (C.D., 2.585; M.C.D., 0.766; C.Dis., 0.649), vitelline follicle and eggs (C.D., 2.504; M.C.D., 0.450; C.Dis., 0.681) (Table 1).[53,64,65] Consecutively the trematode worms under study dissimilar from *A. tasnverasale*[71] and *A. gomtiensis* in the larger vs. smaller body size (C.D., 2.706; M.C.D., 0.647; C.Dis., 0.514), oral sucker (C.D., 2.435; M.C.D., 0.362; C.Dis., 0.499), ventral sucker (C.D., 2.453; M.C.D., 0.557; C.Dis., 0.631), pharynx (C.D., 2.759; M.C.D., 0.569; C.Dis., 0.921), cirrus pouch (C.D., 2.772), anterior testis (C.D., 2.706; M.C.D., 0.574; C.Dis., 0.608), posterior testis (C.D., 2.566; M.C.D., 0.574; C.Dis., 0.543) and its position in the body, ovary (C.D is., 0.308), the vitelline follicle (C.Dis., 0.332) and eggs (C.D., 2.806; M.C.D., 0.325; C.Dis., 0.523) (Table 1).[64] The present worms showed remarkable contrast from the *A. neotenicum* from the dytiscid water beetles *Acilius semisulcatus Aubé, Dytiscus sp.* and *Agabus sp.* from forest pools and bogs near Douglas Lake, Michigan, USA; *A. alloneotenicum* and *A. lobatum*[72] in *Semotilus atromaculatus*.[73-75]

In contrast the parasitic trematodes in host fish under study differed from all nine species of *Allocreadium* that have previously been known from Japan: *A. gotoi;*[22] *A. hasu;*[76] *A. japonicum;*[76] *A. tosai;*[77] *A. brevivitellatum;*[78] *A. tribolodontis;*[79] *A. shinanoense;*[80] *A. aburahaya*[80] and *A. tamoroko*.[69,81] The present worms differed from *A. tamoroko* in the smaller body size (C.D., 2.822; M.C.D., 0.393; C.Dis., 0.619), oral sucker (C.D., 2.519; M.C.D., 0.378; C.Dis., 0.519), ventral sucker (C.D., 2.468; M.C.D., 0.501; C.Dis., 0.565), pharynx (C.D., 2.419; M.C.D., 0.541; C.Dis., 0.697), testes (C.D., 2.455; M.C.D., 0.615; C.Dis., 0.595), and its position in the body, ovary (C.D., 2.458; C.Dis., 0.305) and eggs (C.D., 2.367; M.C.D., 0.509; C.Dis., 0.827) (Table 4).[69] The worms under investigation differed from *A. aburahaya* (Shimazu, 2003) in body size, oral sucker, ventral sucker (smaller *vs.* larger), seminal vesicle (tubular *vs.* saccular), seminal receptacle (flask-shaped *vs.* pear-shaped),

Table 1: Polythetic divisive classificatory system-based taxometric analysis from observations of *A. ramai*,[64] *A. handiae*,[53,64,65] *A. gomtiensis*[64] *vis-a-vis* present *Allocreadium haryanii* n.sp.

Worm Character	Organ Dimension	*A. ramai*				*A. handiae*				*A. gomtiensis*			
		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	2.693	0.356	0.827	0.173	2.574	0.327	0.413	0.587	2.706	0.647	0.514	0.486
	W	2.381	0.409	0.659	0.341	2.417	0.524	0.789	0.211	2.660	0.749	0.701	0.291
Oral Sucker	L	2.348	0.384	0.432	0.568	2.214*	0.142*	0.715	0.285	2.435	0.362	0.499	0.501
	W	2.516	0.364	0.489	0.511	2.303	0.152*	0.745	0.255	2.123*	0.317	0.416	0.584
Ventral Sucker	L	2.306	0.397	0.596	0.405	2.499	0.543	0.709	0.291	2.415	0.426	0.631	0.369
	W	2.565	0.323	0.475	0.525	2.679	0.506	0.685	0.315	2.453	0.557	0.465	0.535
Pharynx	L	2.741	0.521	0.957	0.043	2.843	0.707	0.761	0.239	2.409	0.569	0.992	0.008
	W	2.717	0.474	0.909	0.091	2.869	0.688	0.904	0.096	2.759	0.983	0.921	0.079
Cirrus Sac	L	2.829	0.363	0.479	0.521	–	–	–	–	2.181*	0.164*	0.183*	0.817*
	W	2.565	0.451	0.472	0.528	–	–	–	–	2.772	0.118*	0.288	0.712*
Anterior Testis	L	2.505	0.353	0.554	0.446	2.509	0.347	0.491	0.509	2.706	0.350	0.227*	0.773
	W	2.661	0.383	0.413	0.587	2.505	0.343	0.415	0.585	2.641	0.574	0.608	0.392
Posterior Testis	L	2.531	0.249*	0.251*	0.849*	2.468	0.315	0.418	0.582	2.566	0.239*	0.287*	0.713*
	W	2.253	0.306	0.974	0.026	2.567	0.770	0.635	0.335	2.427	0.574	0.543	0.457
Ovary	L	2.537	0.356	0.478	0.522	2.585	0.485	0.649	0.351	2.152*	0.259*	0.308	0.692
	W	2.180*	0.213*	0.241*	0.759*	2.277*	0.766	0.336	0.664	2.138*	0.278*	0.205*	0.795*
Egg	L	2.575	0.532	0.918	0.082	2.197*	0.346	0.419	0.581	2.806	0.316	0.523	0.477
	W	2.238*	0.233*	0.181*	0.919*	2.504	0.450	0.681	0.319	2.469	0.325	0.474	0.526
Follicle	L	–	–	–	–	–	–	–	–	2.138*	0.173*	0.332	0.678
	W	–	–	–	–	–	–	–	–	2.102*	0.475*	0.295*	0.705*

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.

the extension of vitelline follicles (less *vs.* more) and showed similarity in shape and size of operculate eggs and excretory vesicle. The newer worms in the study varied from *A. brevivitellatum* in body shape and size (smaller and slender *vs.* larger and obese), testes (entire and smooth *vs.* irregular and lobed), seminal receptacle (clavate and small *vs.* pear-shaped and larger), excretory vesicle (larger overlapping some part of posterior testis *vs.* smaller and ending in post testicular region from the posterior testis) and reflected similarity in fairly equal size of ovary and extension of vitelline follicles in the body.[78] The current worms showed a striking contrast from *A. gotoi* in body size (longer and slender *vs.* smaller and obese), cirrus pouch (clavate *vs.* saccular), testes (elliptical *vs.* spherical), seminal receptacle (flask-shaped *vs.* pear-shaped), the extension of vitelline follicles (post pharyngeal *vs.* ovarian), excretory vesicle (larger overlapping some part of posterior testis *vs.* smaller and ending in post testicular region from the posterior testis).[22,25,77] The present worms differed from *A. hasu* in body size (larger and broader *vs.* smaller and slender), oral sucker and acetabulum (small *vs.* large), pharynx (narrower *vs.* broader), cirrus pouch (larger and elliptical *vs.* smaller and saccular), testes (larger and smooth *vs.* smaller and lobular), ovary (larger and lobed *vs.* smaller and smooth), seminal receptacle (larger and flask-shaped *vs.* smaller and pear-shaped), eggs (larger *vs.* smaller), excretory vesicle (larger overlapping some part of posterior testis *vs.* smaller and ending in post testicular region from the posterior testis).[25,76,77] The morphometric measurements and microscopic observations of present worms showed differences from *A. japonicum* in body size (fairly larger and obese *vs.* smaller and slender), oral sucker and acetabulum (broader *vs.* narrower), bifurcation of oesophagus (ad-acetabular *vs.* post-acetabular), seminal receptacle (S-shaped *vs.* pear-shaped), eggs (larger *vs.* smaller), vitellaria (more extended *vs.* less extended); however, showed similarity to size and shape of testes.[23,25,76,77,82] The morphometric numerical assessment and morphological observations of present worms showed variability from *A. shinanoense* in body size (smaller and slender *vs.* fairly larger and obese), oral sucker and acetabulum (narrower *vs.* broader), cirrus pouch and seminal vesicle (claviform *vs.* saccular), seminal receptacle (ovate *vs.* pear-shaped), eggs (larger *vs.* smaller), excretory vesicle (larger overlapping some part of posterior testis *vs.* smaller and ending in post testicular region from the posterior testis).[80] The present newer worms showed differences from *A. tosai*[77] and *A. tribolodontis*[79] in body size (smaller and ovate *vs.* fairly larger, elongated and obese), eggs (larger *vs.* smaller), excretory vesicle (larger overlapping some part of posterior testis *vs.* smaller and ending in post testicular region from the posterior testis), ovary (elliptical *vs.* spherical), testes (smooth and smaller *vs.* irregular and larger).[77,79]

The present worms differed from *A. cyprini*[83] in body size (larger *vs.* smaller); *A. gachuai,*[84] *A. kawi,*[83] and *A. tori*[52] in body size (smaller and slender *vs.* larger and obese), oral sucker, pharynx, ventral sucker, ovary, testes, eggs (smaller *vs.* larger), seminal vesicle (acetabular *vs.* postacetabular), genital pore (lateral *vs.* submedian in acetabular and pre-oesophageal bifurcation region). On the other hand, the worms in host fish under study varied from *A. wallagoensis* in the smaller body size, and larger oral sucker, ventral sucker, pharynx, cirrus pouch, testes and its position in the body, ovary, vitelline follicle, and eggs.[83] The present worms showed a striking contrast from *A. kasciatusi* in the body size (C.D., 2.825; M.C.D., 0.487; C.Dis., 0.645), oral sucker (C.D., 2.826; M.C.D., 0.525; C.Dis., 0.721), ventral sucker (C.D., 2.620; M.C.D., 0.780; C.Dis., 0.685), pharynx (C.D., 2.383; M.C.D., 0.695; C.Dis., 0.869), anterior testis (M.C.D., 0.381; C.Dis., 0.525), posterior testis (C.D., 2.448; M.C.D., 0.677; C.Dis., 0.592) and its position in the body, ovary (C.D., 2.531; M.C.D., 0.494; C.Dis., 0.667) and eggs (M.C.D., 0.583; C.Dis., 0.824) (Table 2).[66] The worms worked out reflected the differences from *A. thapari* in the body size (C.D., 2.584; M.C.D., 0.463; C.Dis., 0.603), oral sucker (C.D., 2.504; M.C.D., 0.447; C.Dis., 0.596), ventral sucker (C.D., 2.539; M.C.D., 0.481; C.Dis., 0.695), pharynx (C.D., 2.383; M.C.D., 0.580; C.Dis., 0.791), anterior testis (C.D., 2.614; M.C.D., 0.646; C.Dis., 0.528), posterior testis (C.D., 2.509; M.C.D., 0.625; C.Dis., 0.554) and its position in the body, ovary (C.D., 2.527; M.C.D., 0.435; C.Dis., 0.556) and eggs (C.D., 2.452; M.C.D., 0.746; C.Dis., 0.605) (Table 2).[33,66]
The trematodes reported from host fish during the present investigation noticed differences from *A. nicolli* in the body size (C.D., 2.798; M.C.D., 0.806; C.Dis., 0.334), oral sucker (C.D., 2.538; M.C.D., 0.389; C.Dis., 0.467), pharynx (C.D., 2.462; M.C.D., 0.535; C.Dis., 0.522), testes (C.D., 2.536; M.C.D., 0.476; C.Dis., 0.835) and ovary (C.D., 2.541; C.Dis., 0.709) (Table 2).[64] The gut fluke microfauna documented from host fish during the study showed variability from *A. kosia* in the body size (C.D., 2.858; M.C.D., 0.367; C.Dis., 0.346), oral sucker (C.Dis., 0.618), ventral sucker (M.C.D., 0.663), pharynx (C.D., 2.462; M.C.D., 0.566; C.Dis., 0.479) and testes (C.D., 2.535; M.C.D., 0.475; C.Dis., 0.839) (Table 3).[64] The trematodes morphometrically enumerated from *Wallago attu* showed differences from *A. mehrai* in the body size (C.D., 2.788; M.C.D., 0.455; C.Dis., 0.589), oral sucker (C.D., 2.340; M.C.D., 0.462;

Table 2: Polythetic divisive classificatory system-based taxometric analysis from observations of *A. kasciatusi*,[66] *A. thapari*,[33,66] *A. nicolli*[64] *vis-a-vis* present *Allocreadium haryanii* n.sp.

Worm Character	Organ Dimension	*A. kasciatusi*				*A. thapari*				*A. nicolli*			
		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	2.825	0.487	0.645	0.355	2.584	0.463	0.603	0.397	2.798	0.806	0.334	0.666
	W	2.596	0.771	0.725	0.275	2.570	0.476	0.716	0.284	2.417	–	–	–
Oral Sucker	L	2.826	0.525	0.721	0.279	2.504	0.423	0.573	0.427	2.504	0.389	0.454	0.546
	W	2.213*	0.505	0.681	0.319	2.213*	0.447	0.596	0.404	2.538	0.359	0.467	0.533
Ventral Sucker	L	2.411	0.612	0.931	0.069	2.419	0.481	0.695	0.305	–	–	–	–
	W	2.620	0.780	0.685	0.315	2.539	0.649	0.575	0.425	–	–	–	–
Pharynx	L	2.365	0.726	0.676	0.324	2.362	0.526	0.396	0.604	2.462	0.535	0.443	0.557
	W	2.383	0.695	0.869	0.131	2.383	0.580	0.791	0.209	2.331	0.534	0.522	0.478
Anterior Testis	L	2.093*	0.241*	0.525	0.475	2.250*	0.646	0.528	0.472	2.307	0.476	0.835	0.165
	W	2.109*	0.381	0.474	0.526	2.614	0.392	0.491	0.509	2.536	0.192*	0.222*	0.778*
Posterior Testis	L	2.448	0.339	0.477	0.523	2.509	0.492	0.241	0.759	–	–	–	–
	W	2.373	0.677	0.592	0.408	2.490	0.625	0.554	0.446	–	–	–	–
Ovary	L	2.531	0.494	0.667	0.333	2.527	0.435	0.556	0.444	2.541	0.223*	0.709	0.291
	W	2.209*	0.396	0.509	0.491	2.208*	0.355	0.438	0.562	2.437	0.176*	0.294*	0.706*
Egg	L	2.298*	0.551	0.837	0.163	2.262*	0.603	0.915	0.085	–	–	–	–
	W	2.245*	0.583	0.824	0.176	2.452	0.746	0.605	0.395	–	–	–	–

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 taxometric analysis from observations of *A. handi*,[67] *A. kosia*,[64] *A. mehrai*[33,67] *vis-a-vis* present *Allocreadium haryanii* n.sp.

Worm Character	Organ Dimension	*A. handi*				*A. kosia*				*A. mehrai*			
		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	2.693	0.510	0.686	0.314	2.858	0.367	0.346	0.654	2.788	0.455	0.589	0.411
	W	2.445	0.645	0.971	0.029	2.462	–	–	–	2.422	0.573	0.848	0.152
Oral Sucker	L	2.504	0.538	0.743	0.257	2.504	–	0.618	0.382	2.168*	0.462	0.628	0.372
	W	2.493	0.895	0.776	0.224	2.154*	–	0.498	0.502	2.340	0.412	0.548	0.452
Ventral Sucker	L	2.431	0.595	0.866	0.134	–	0.663	–	–	2.228	0.458	0.666	0.334
	W	2.404	0.740	0.647	0.353	–	0.105*	–	–	2.734	0.739	0.693	0.307
Pharynx	L	2.472	0.707	0.473	0.527	2.462	0.566	0.479	0.521	2.524	0.642	0.676	0.324
	W	2.558	0.634	0.665	0.335	2.210*	0.190*	0.399	0.601	2.608	0.730	0.898	0.102
Anterior Testis	L	2.622	0.600	0.503	0.407	2.308	0.475	0.745	0.255	2.595	0.656	0.507	0.493
	W	2.675	0.325	0.387	0.613	2.535	0.226*	0.839	0.161	2.500	0.415	0.532	0.468
Posterior Testis	L	2.543	0.353	0.505	0.495	–	–	–	–	2.453	0.233*	0.286	0.714*
	W	2.529	0.756	0.708	0.292	–	–	–	–	2.645	0.717	0.648	0.352
Ovary	L	2.423	0.451	0.584	0.416	–	–	–	–	2.410	0.473	0.626	0.374
	W	2.208*	0.478	0.677	0.323	–	–	–	–	2.395	0.394	0.505	0.495
Egg	L	2.525	0.433	0.692	0.308	–	–	–	–	2.443	0.495	0.763	0.237
	W	2.324	0.487	0.802	0.198	–	–	–	–	2.110*	0.382	0.778	0.222

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.

C.Dis., 0.628), ventral sucker (C.D., 2.734; M.C.D., 0.739; C.Dis., 0.693), pharynx (C.D., 2.608; M.C.D., 0.730; C.Dis., 0.898), anterior testis (C.D., 2.595; M.C.D., 0.656; C.Dis., 0.507), posterior testis (C.D., 2.645; M.C.D., 0.717; C.Dis., 0.648) and its position in the body, ovary (C.D., 2.410; M.C.D., 0.473; C.Dis., 0.626) and eggs (C.D., 2.443; M.C.D., 0.495; C.Dis., 0.763) (Table 3).[33,67] The present worms differed from *A. heteropnuestisis* in the body size (C.D., 2.714; M.C.D., 0.528; C.Dis., 0.833), oral sucker (C.D., 2.557; M.C.D., 0.474; C.Dis., 0.643), ventral sucker (C.D., 2.482; M.C.D., 0.709; C.Dis., 0.868), pharynx (C.D., 2.750; M.C.D., 0.793; C.Dis., 0.718), anterior testis (C.D., 2.619; M.C.D., 0.666; C.Dis., 0.587), posterior testis (C.D., 2.598; M.C.D., 0.704; C.Dis., 0.625) and its position in the body, ovary (C.D., 2.493; M.C.D., 0.501; C.Dis., 0.681) and eggs (C.D., 2.336; M.C.D., 0.411; C.Dis., 0.676) (Table 4).[67,68] The newer worms under consideration varied from *A. patagonium* in body size (C.D., 2.661; M.C.D., 0.344; C.Dis., 0.433), oral sucker (M.C.D., 0.363; C.Dis., 0.502), ventral sucker (C.D., 2.524; M.C.D., 0.652; C.Dis., 0.654), pharynx (C.D., 2.574; M.C.D., 0.647; C.Dis., 0.865), anterior testis (C.D., 2.490; M.C.D., 0.613; C.Dis., 6.05), posterior testis (C.D., 2.425; M.C.D., 0.577; C.Dis., 0.948) and its position in the body, ovary (C.D., 2.507; M.C.D., 0.381; C.Dis., 0.471) and eggs (C.D., 2.506; M.C.D., 0.553; C.Dis., 0.586) (Table 4).[70]

Thus the present worms under conversation with other specimens viz. *A. handai;*[64] *A. thapari;*[33] *A. nemachilus;*[32] *A. ophiocephali;*[36] *A. mukundi;*[84] *A. heteropneustusius;*[68] *A. mrigalai;*[85] *A. saranai;*[85] *A. saranai;*[85] *A. jaini;*[48] *A. fotedari;*[43] *A. bimaculatusi*[50] and *A. punctatai*[51] having the contrast of anterior protuberance, larger but intra-caecal ventral sucker, pre-pharynx and showed similarity in the position of the pre-equatorial ovary, pear-shaped seminal receptacle.[33,64,68,84] The present worms differed from *A. fotedari* in the extension of vitellaria (post-ventral sucker *vs.* pre-ovarian)[43] from *A. mrigalai* and *A. jaini* (post-pharynx *vs.* pre-ovarian);[48,85] and similar in the receptaculum seminalis post-ovarian and subdiagnol to the ovary.[43] However, the present form may also differ from *A. punctatai* in having receptaculum seminalis (post-ovarian *vs.* pre-ovarian).[51] The worms under investigation may also showed a contrast between *A. jaini* and *A. thapari* in having non-operculated eggs instead of operculated;[33,48] from *A. saranai* in cirrus sac (coiled large tubular *vs.* larger saccular) and receptaculum seminalis (conical *vs.* oval or pear-shaped).[85] The newer worms showed striking contrast from *A. ophiocephali* in oesophagus (absence *vs.* presence), receptaculum seminalis (pre-ovarian *vs.* post-ovarian), vesicula seminalis (cone shape *vs.* pear shape) and similarity in the position of genital pore prior to the intestinal caeca.[36] The worms showed quite more similarity in the extension and distribution of vitellaria mid-level of the ovary in *A. bimaculatusi*, genital pore just above the intestinal caeca, oesophagus (absence *vs.* very little but present), cirrus sac larger and saccular, while vesicula seminalis saccular only.[50]

CONCLUSION

The current investigation assessed and approved through the advanced biostatistical morphotaxometric tools utilizing the polythetic divisive classificatory system with extraordinary reference to taxonomically important feature based on the prior revealed perceptions of *A. ramai*, *A. handiae*, *A. gomtiensis, A. kasciatusi*, *A. thapari*, *A. nicolli*, *A. handi*, *A. kosia*, *A. mehrai*, *A. heteropnuestisis*, *A. tamoroko*, *A. patagonium vis-a-vis* proposed newer worm reflected taxonomically significant and striking differences in relation to the statistically substantiated dimensions and size. Based on the noticed, looked at, and examined leaned toward likenesses to assess the conventional conclusion and striking differences for the validation and approval of the- newer worm, authors assume to propose the current worms of the conversation as a newer species *Allocreadium haryanii* n.sp. under the same genera.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest among themselves or with any other individual, organization, etc. throughout the entire duration of this study.

ABBREVIATIONS

C.D: Coefficient of divergence; **C.Dis:** Coefficient of dissimilarity; **C.S:** Coefficient of similarity; **M.C.D:** Mean character difference; **L:** Length; **W:** Width; **OS:** Oral sucker; **P:** Pharynx; **VS:** Ventral sucker; **O:** Ovary; **Ta:** Anterior testis; **Tp:** Posterior testis; **V:** Vitellaria; **Eb:** Excretory bladder; **Oe:** Oesophagus; **IC:** Intestinal caeca; **GP:** Genital pore; **CP:** Cirrus pouch; **E:** Eggs;

Table 4: Polythetic divisive classificatory system-based taxometric analysis from observations of *A. heteropnuestisis*,[67,68] *A. tomoroko*,[69] *A. patagonium*[70] *vis-a-vis* present *Allocreadium haryanii* n.sp.

Worm Character	Organ Dimension	*A. heteropnuestisis*				*A. tamoroko*				*A. patagonium*			
		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	2.714	0.528	0.833	0.167	2.822	0.246*	0.324	0.676	2.661	0.344	0.433	0.567
	W	2.494	0.665	0.515	0.485	2.425	0.393	0.619	0.381	2.512	0.464	0.701	0.299
Oral Sucker	L	2.148*	0.474	0.643	0.357	2.519	0.378	0.519	0.481	2.158*	0.363	0.502	0.498
	W	2.557	0.336	0.457	0.543	2.175*	0.304	0.423	0.577	2.197	0.322	0.443	0.557
Ventral Sucker	L	2.482	0.596	0.868	0.132	2.468	0.368	0.565	0.435	2.545	0.453	0.659	0.341
	W	2.464	0.709	0.642	0.352	2.441	0.501	0.972	0.028	2.524	0.652	0.654	0.346
Pharynx	L	2.600	0.519	0.718	0.282	2.393	0.541	0.413	0.587	2.542	0.526	0.396	0.604
	W	2.750	0.793	0.632	0.364	2.419	0.511	0.697	0.303	2.574	0.647	0.865	0.135
Anterior Testis	L	2.591	0.666	0.587	0.413	2.455	0.615	0.595	0.405	2.323	0.613	0.605	0.395
	W	2.619	0.354	0.431	0.569	2.209*	0.319	0.378	0.622	2.490	0.232	0.267*	0.733*
Posterior Testis	L	2.598	0.329	0.455	0.545	–	–	–	–	2.425	0.109*	0.116*	0.884*
	W	2.542	0.704	0.625	0.375	–	–	–	–	2.153*	0.577	0.948	0.052
Ovary	L	2.493	0.501	0.681	0.319	2.389	0.241*	0.305	0.695	2.507	0.381	0.471	0.529
	W	2.108*	0.175*	0.198*	0.802*	2.458	0.168*	0.305	0.695	2.306	0.268*	0.309	0.691
Egg	L	2.336	0.411	0.676	0.324	2.153*	0.467	0.730	0.270	2.399	0.469	0.733	0.267
	W	2.192*	0.462	0.775	0.225	2.367	0.509	0.827	0.173	2.506	0.553	0.586	0.414

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.

FGP: Female genital pore; **MGP:** Male genital pore; **CGA:** Common genital atrium; **T:** Testes.

SUMMARY

The two years (July 2018 to June 2020) study was conducted for taxonomic substantiation of endoparasitic trematodes in the catfish, *Wallago attu* inhabiting selected sites of the river Yamuna in Haryana that was unexplored in terms of parasitological investigation in the fish hosts so far. The host fish sample was collected by netting and angling methods from the river Yamuna. The collected samples were brought to the Zoology Laboratory, Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala (HR), India for the extraction of gastrointestinal parasitic helminths and its further identification, morphometric substantiation, and systematic validation using advance numerical taxonomic tools. The extracted worms were diagnosed as trematode and identified as *Allocreadium* Looss (Allocreadoidea: Allocreadiidae) on the basis of sub-cylindrical and sub-medium body with a tiny protuberance along with the taxonomically significant characteristics based on the Key to Trematoda. The further evaluation of newly recovered worms using the polythetic divisive classificatory system with earlier reported species of the same genera reflected statistically significant and systematically striking contrasts. The observed and noticeable favored similarities helped in the generic diagnosis while striking contrasts provided the scientifically sound ground for taxonomic validation of the present newer worm, as *Allocreadium haryanii* n.sp.

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