

## Infection and Prevalence of *Senga lucknowensis* (Johri, 1956) in freshwater fish *Channa punctata* (Bloch, 1793) of DVC, a reservoir of Koderma, Jharkhand, India

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### ABSTRACT

This study has been conducted to know the prevalence of fish helminthic endoparasites of freshwater fish hosts. The samples were collected at a regular interval of one week from May 2020 to April 2021 from Tilaiya Dam, a reservoir of Koderma, Jharkhand. Approximately 1800 fish were collected of which 360 species of *Channa punctatus*, 240 species of *N. notopterus*, 240 species of *L. rohita*, 240 species of *C. catla*, 240 species of *O. niloticus*, 240 species of *C. mrigala*, 240 species of *P. sanitwongsei*. The present study has been conducted on seven commercial fishes among which only *C. punctata* was infected. Among these 1800 fish samples, 1600 were non-infected and 200 were infected. The prevalence of *Senga lucknowensis* was recorded in 200 fish species of *C. punctatus* and no helminthic endoparasites *S. lucknowensis* has in other 1600 species of fish. The highest seasonal prevalence has been calculated in pre-monsoon and the highest abundance has been found in pre-monsoon and the highest intensity was also found in pre-monsoon. For the identification of species, we conducted molecular studies using PCR amplification and used 18S rRNA primer. The genomic sequence of the cestodes sample was 100% sequence similarity to the species *S. lucknowensis*. The sequence has been submitted to the NCBI GenBank and the accession number has been given as a representative The accession number for *S. lucknowensis* is ON46856, which has been given by NCBI.

**Keywords:** *S. lucknowensis*, *C. punctata*, 18S RNA, Prevalence, Abundance, Intensity

### INTRODUCTION

For the last few decades, a protein-rich diet for human consumption of fish (both Chondrichthyes and Osteichthyes) has been broadly used across the Indian subcontinent, and so makes a significant contribution to its economy. According to Shukla and Upadhyay [1], about 10 million tonnes of fish are required yearly for fish protein to meet current-day demand in India, compared to the annual production of only 3.5 million tonnes. Approximately 56 million human parasitic infections are due to consuming infected fish products, as reported by the World Health Organization (WHO, 2012). It has been reported that in the current

existence parasite species epitomize a significant portion of the total biodiversity of Earth [2], although quite a few questions stay behind about the degree of diversity of parasites and their distribution all over the world.

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It is found that parasites have been creating great importance to human health. So, there is a need to study helminths whose prevalence is high in our country. Family bothriocephalidea [3], the cestode Platyhelminthes has a wide range of parasites, including numerous families and species that can cause major medical problems in humans, as well as fish, wildlife, and domestic animals. Uncertainty, parasites despite several efforts to determine phylogenetic relationships at the inter-family level. Pathogens are identified to infect and cause disease in fish in both freshwater and marine environments. Helminth parasites found in fish can have a direct or indirect impact on public health [4]. 30,000 helminth species have been approximated to be parasites of fishes, several of which are recognized to be severe hazards to hosts. Cestodes are a class of obligate parasites of intestinal origin that are known to cause major health issues throughout the world, particularly in Asia [5]. [6] Using the available molecular sequence data, a global collaborative effort has been made over the past ten years to link the classification of cestodes to their phylogeny [7]. Among nineteen species of cestode order have been recognized [7]. A few tapeworms of early diverging are different from others by containing bothria on the scolex in one or two pairs, supporting a sucking groove, and assisting the gastrointestinal tract of the host. Parasites that need consecutive hosts to finish off their life cycle are captivating organisms of considerable attention to evolutionary biologists [8, 9].

Family Bothriocephalidea contains a total number of 108 nominal species of 8 genera. From the Indian subcontinent, it was described as perciform and synbranchiform fishes. Only seventeen species of three genera *Bothriocephalus* [10], *Phycobothrium*, and *Senga* [11] have been described by Kuchta [12]. There were fourteen genera and 100 species belonging to the order Caryophyllidean. Eighty-three species were synonymized and seventeen has considered valid [13]. The family Bothriocephalidea has generated by Kuchta [14] as a result of merging the paraphyletic Pseudophyllidea van Benden [15] between two monophyletic clades-Diphyllobothriidea [14], and Bothriocephalidea based on several unique morphological, biological and molecular characteristics [16]. Current bothriocephalidean taxonomy has been recognized by 134 species and 48 genera [17]. In order to accommodate tapeworms with paired bothria on their scales, which were earlier classified in the

suppressed Pseudophyllidea, a new family called the Bothriocephalidea [14] has been proposed. However, little is known about the definite species arrangement, host specificity, and distribution of its members. [18] Kuchta has revised the new order to provide modified generic diagnoses, whereas [12] planned many synonymities of bothriocephalidean species, which include taxa, that have been reported from African freshwater fish. Despite some advancements in the field, the fauna of trematodes and cestodes parasitizing fish in the oriental zoogeographical region in the metacercarial and metacestode phases is still poorly resolved [19], the *Proteocephalus* genus of the cestodes is parasites known to poikilotherm vertebrates, mainly freshwater fishes. The comparative histopathological studies on hosts parasitized by platyhelminth group cestode parasites of the genus *Lytocestus* and *Senga* exhibited high pathological effects which show the way to mortality of the hosts [20, 21].

There is increasing awareness in recent years that parasitism should be investigated in light of particular environmental conditions [22, 23]. The helminth parasites which were primarily found in freshwater fish such as acanthocephalans trematodes, nematodes, and cestodes can harm both vertebrates and invertebrates. They finish off their life cycles to enter their intermediate hosts like fish-eating birds [24] and snails. Snakehead belongs to the genus *Channa* and is vital species of the freshwater environment. Genus *Channa* is carnivorous and fed mostly on animal foods [25]. The earliest data on fish cestodes from India was supplied by British researchers [26,27], followed by Indian helminthologists. In 1934 Dollfus recognized the genus *Senga*, with the type species *S. Bernardi* from *Betta splendens* in Vincennes, France. [28] as *Anchistrocephalus ophiocephalina* from *Ophiocephalus argus* in Taimen, China, and recognized with a form earlier described by Southwell [27]. A total of 38 species under the genus have been reported from India. The most common fish hosts from where the genus *Senga* has been reported from India belong to the genus *Channa*. Amongst all the helminths, it is found that Cestode has adapted to a parasitic mode of life. Almost all vertebrate and invertebrate, larva, and cystic forms of mites, crustaceans, ants, etc are found in the different body organs. Cestodes are endoparasites and characterized by their body as segmented and dorsoventrally flat. The tapeworm looks ribbon-shaped and is the longest among the oldest intestinal parasites that have been found to

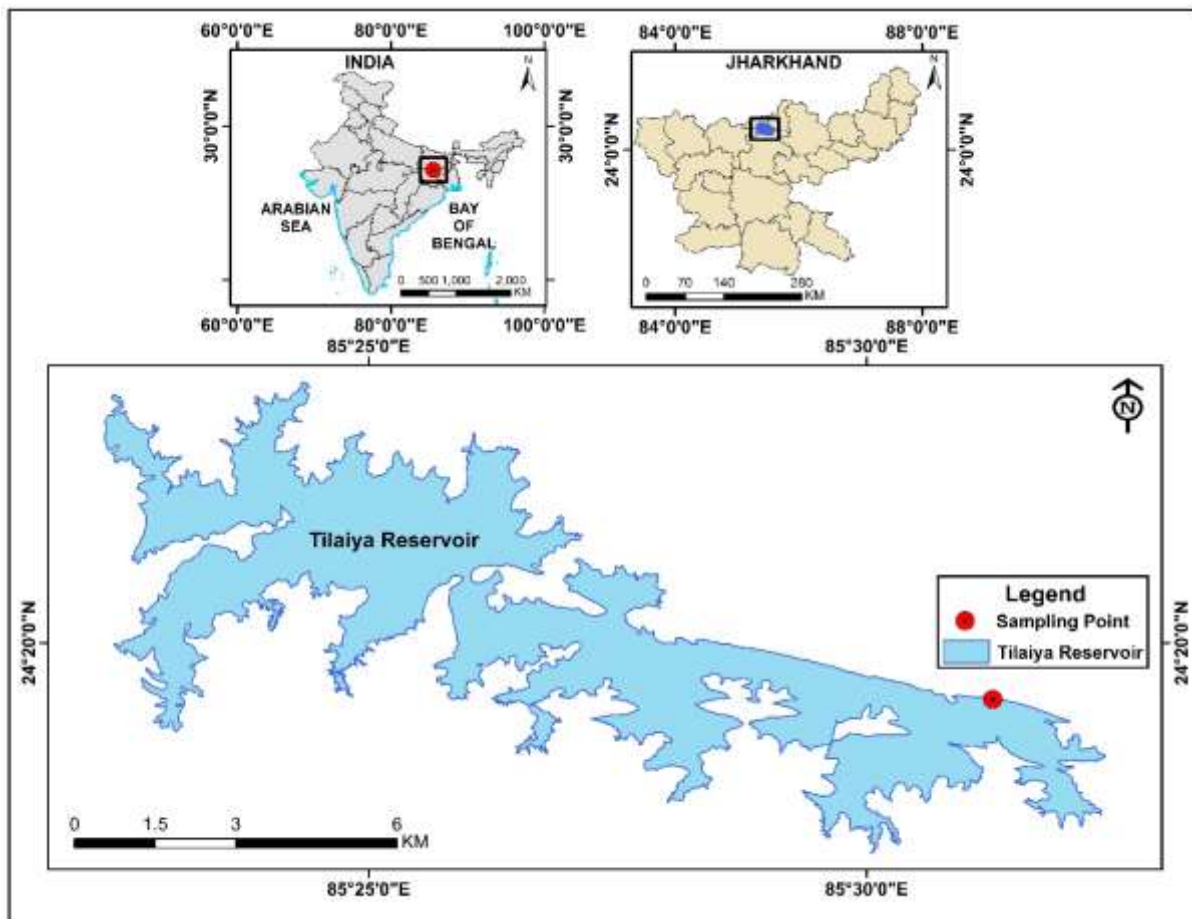
plague humans and other animals since time immemorial. By using morphological and molecular approaches [29, 30] made critical evolution of previously defined species from freshwater fish hosts of India. Because of this, it was possible to classify the species composition, phylogenetic relationships, and host specificity of cestodes found in freshwater fish. They are broad-based in distribution and occur in many forms. Eight of the fourteen Eucestoda orders - Nippotaeniidea, Caryophyllidea, Trypanorhyncha, Pseudophyllidea, Proteocephalidea, Haplobothriidea, Spathebothriidea, and Amphilinidea - can be found in freshwater fish. The India Zoological Survey published an index catalog of cestode parasites of freshwater fish [31].

The first and latest molecular phylogenetic investigation, which determined the interrelationship of the family Bothriocephalidea, used the nuclear rRNA gene sequence of 17 bothriocephalidean species [32]. Kuchta [33] has

been given a rationalized list of applicable species and Kuchta has conceded a taxonomical review of the order, following the family classification by Vertebrates [34]. The analyses of molecular and phylogenetics have to a great extent influenced our thoughts in delineating species complexes and has been evaluated traditional taxonomic groups and estimated relationships among taxa [35]. The usefulness of 18S rRNA in describing, recognizing, and the phylogenetic conclusion is well explained in diverse forms of organisms like cestodes, digenean, and nematodes [36, 37]. It is acknowledged that definite species of parasites are a little bit more effective in causing an infection as compared to other species of the same genus [38].

The present study has been conducted with the following which is to carry out an all-inclusive molecular phylogenetic evaluation of the family Bothriocephalidea, used a gene database of 18S rRNA, and study the congruence of the traditional classification based on morphological characters

**Fig 1. GIS map showing the sampling site, DVC Dam, Tilaiya Reservoir, Koderma, Jharkhand. The Arc GIS 10.2.1 framework was used to make the map for collecting fish sample**



with the newly generated sequences. This study was conducted in the DVC Dam, a reservoir of Koderma, in which a total of 44 fish species have been found, representing 05 orders and 16 families. There were 44 different species of fish, of which 22 belonged to the order Cypriniformes, 11 to the order Perciformes, 2 to the order Osteoglossiformes, 6 to the order Siluriformes, and 2 to the order Synbranchiformes. Some fish species are commonly found such as *L. rohita*, *C. punctata*, *N. notopterus*, *C. mrigala*, *O. niloticus*, *C. catla*, *P. sanitwongesei*, etc. These fish species are used for commercial purposes and local people are dependent on this reservoir for purchasing fish as a food source. A present study has been conducted to find the prevalence of *S. lucknowensis* in some commercial fishes, which generally people use as food sources.

## MATERIAL AND METHODS

### Study area

This study has been conducted in the reservoir of Tilaiya Dam (24°19'26" N 85°31'16") (Fig 1). It is a hydroelectric power station which is constructed by DVC (Damodar Valley Corporation) across the Barakar River, district of Koderma in the Indian state of Jharkhand. The dam is 64.4 km downstream, across the Barakar River, and rises 30.2 m above the riverbed. DVC Dam Tilaiya occupied 984 sq. km and generates electricity [39]. The catchment region is made up of hilly terrains with communities, wastelands, cultivated land, pastures, and forested areas [40].

### Sample Collection and Preservation

Fishes have been collected from the selected study area for one year. The selected fish samples were *C. punctata*, *N. notopterus*, *L. rohita*, *C. mrigala*, *O. niloticus*, *C. catla*, and *P. sanitwongesei*. Approximately 1800 fish were collected from the different fishing sites of Tilaiya Dam in the reservoir of Koderma which 360 species of *Channa*, 240 species of *Notopterus*, 240 species of *Labeo*, 240 species of *Catla*, 240 species of *Oreochromis*, 240 species of *Cirrhinus*, 240 species of *pangasius*. The present study has been conducted on seven commercial fishes among which only *C. punctata* were infected. Among these 1800 fish samples, 1600 were non-infected and 200 were infected. The prevalence was recorded in 200 fish of *Channa* species and no helminthic endoparasites are in the other 1600 fishes. For the study, fish samples

have been kept in plastic containers which were filled with water and transported alive to the laboratory of (VBU) university for identification and examination to recover endoparasites. For this, remove excess mucus from the fish body, and the samples were dissected through the abdomen by making an incision along the midventral line from the anus using a sterile blade (HiMedia, India). The alimentary tract, gills, and liver were isolated and stretched out and the alimentary tract was grouped into the esophagus, stomach, and intestine. This part of the alimentary canal was placed in five separate Petri dishes which contained 0.6% saline and examined at 10× magnification under a microscope (Magnüs MLXi PLUS, India). Then the helminthic endoparasites were collected from a salt solution with the help of a brush and washed in PBS (HiMedia, India). It was preserved in 95% alcohol (HiMedia, India) for molecular study and kept at -20°C.

### Morphological examination of isolated cestode

For the morphological study, the washed worms were flattened by placing them between the slides and fixed overnight and kept for a few days (depending on the size and thickness of the parasites) in AFA solution (Acetic acid, formalin, and alcohol). After fixation, whole-mount preparation was done by staining in Semichon's acetocarmine (distained in acid water for the parasites that were overstrained), dehydrated in graded series of alcohol (70%, 80%, 95%, and 100%), cleared in toluene and mounted in domar gum. The prevalence, abundance, and intensity of the helminth endoparasite of selected species were calculated by using [41] Margolis formula.

### Extraction of DNA from cestode

The total genomic DNA of the sample was extracted with the help of the DNeasy Blood and Tissue kit (Qiagen, Germany). DNA has been isolated, for this, the samples have been washed (Fig 4) in Phosphate buffered saline (PBS) (HiMedia, India), and 1.5 ml microtubes were used. About 300 µl of TAE (10mm, Tris-HCl; 100mm NaCl; 10ml EDTA) buffer was added. After this, homogenized using a homogenizer, and incubated overnight in the lysis buffer at 37 °C containing 0.2% sodium dodecyl sulfate, 10µl proteinase K (Sigma-Aldrich, Germany). After that, we used equal amounts of Tris saturated phenol (250 µl), chloroform, and isoamyl alcohol

**Table 1.** Primer detail, used for amplification of cestode parasites.

Primer name	Primer sequence 5'-3'	Size of Product(bp)	Target gene	Reference
UEP-F	5'-ACCTGGTTGATCCTGCCAG-3'	424 bp	18S rRNA	[43]
UEP-R	5'-CTTCCGCAGGTTACCTACGG-3'			

**Table 2.** Similarities of species between the Genus *Senga* Dolfus, 1934. All measurements are in millimeters.

Species	<i>S. lucknowensis</i> [45]	<i>S. lucknowensis</i> (Present study)
Host	<i>M. armatus</i>	<i>C. Punctata</i>
Locality	India	India
Whole specimen		
Shape	Broadens posteriorly	Broadens posteriorly
length	210-212	202-208
Scolex		
Shape	Pear-shaped	
Length	1.24-1.95	1.32- 1.78
Apical disc		
Shape	Two ½ crowns	Figure-5
Marginal hooks		
Number (large+ rudimentary)	36-48 (Total)	41- 45
Length of large	0.060-0.079	0.065 – 0.074
Length of rudimentary	0.020-0.050	0.029- 0.043
Diameter	0.004-0.015	0.09-0.017
Bothrium		
Bothrium: Scolex	3:4	3:4
Proglottids		
Segmentation	Distinct and indistinct	Distinct
Shape	Maximum breadth>Length	Maximum breadth>Length
Testes		
Diameter	0.040-0.046	0.039- 0.043
Ovary		
Shape	Bilobed	Bilobed
Position	Post-equatorial	Post-equatorial

(250µl) (Sigma-Aldrich, Germany) and was added and centrifuged (Tarsons, India) at 10,000 rpm for 10 min, this process has done in three times. The DNA has been precipitated with 100% ethanol (HiMedia, India) and washed with 70% ethanol. Now it was centrifuged, dried out, and liquified in 25 µl TE buffer (pH 8.0) [42]. The PCR amplification has been performed using the primer of the 18S rRNA region.

#### Amplification and Sequencing of target gene

The extracted DNA samples were used for PCR amplification. The genetic markers used in this procedure are 18S rRNA regions. The 18S rRNA gene for detecting *S. lucknowensis* was amplified with the forward primer, 18S-RNA UEP-F 5'-ACCTGGTTGATCCTGCCAG-3', and reverse primer 18S-rRNA UEP-R 5'-CTTCCGCAGGTTACCTACGG-3' with the PCR protocol (Table 1). The PCR master mixture was water (14 µl), Taq buffer (1 µl), dNTPs (2.5 µl), MgCl<sub>2</sub> (2.5 µl), Taq polymerase (1 µl), and genomic DNA (2

μl). The PCR protocol of early denaturation at 94 °C for 5m, followed by 35 cycles for the 30s at 94 °C, 30s at 54 °C, and 30s at 72 °C with a final extension at 72 °C for 5m. The concentration of DNA was quantified using a UV-visible spectrophotometer (Eppendorf, India). The PCR products has been separated by gel electrophoresis on 1.8 % agarose, and EtBr (ethidium bromide) was used to dye them for visualization, trans-illuminated inside ultraviolet light, and then photographed. The genomic sequence of the cestodes sample was 100% sequence similarity to the species *S. lucknowensis*. The sequence has been submitted to the NCBI GenBank and the accession number has been given as a representative The accession number for *S. lucknowensis* is ON46856, which has been given by NCBI (Table 3).

### Sequence and phylogenetic analysis

The generated genomic sequence has been compared with another genomic sequence that is available in GenBank used. For the generated sequence of *S. lucknowensis*, a Similarity search was carried out with the help of the BLAST Search tool. The generated sequence has been aligned with the help of MEGA11 software [44]. All of the data available in the public domain on the 18S rRNA sequence of *S. lucknowensis* were recovered from GenBank; available taxa have been taken to represent key genera of *Senga*. Phylogenetic analysis has been carried out for a dataset consisting of 18S rRNA sequences from species of *S. lucknowensis*.

### Parasite Prevalence, abundance, and intensity estimation

Selected fish has been isolated from the reservoir of Koderma Jharkhand. During the study period, 200 fishes out of 1800 were identified to be infected with cestode parasites *S. lucknowensis*, indicating approximately 55.5 % of fishes were affected. The fish samples were measured at 10 to 18cm and 50-120kg in weight (Fig 2). Prevalence, intensity, and abundance of infection were calculated and used a simple percentage (%).

Prevalence of parasite infestation: The prevalence of parasite infestation was calculated using the formula given by [41] Margolis.

$$\text{Prevalence(\%)} = \frac{\text{No. of fish host infected}}{\text{total no. of fish host examined}} \times 100$$

Prevalence based on sex: The prevalence of parasite infestation on basis of the sex of fish was calculated followed by [41] Margolis.

$$\text{Prevalence(\%)} = \frac{\text{No. of particular sex of fish infected}}{\text{total no. of particular sex of fish examined}} \times 100$$

Prevalence based on the season: The prevalence of parasite infestation based on the season has been estimated followed by [41] Margolis.

$$\text{Prevalence (\%)} = \frac{\text{No. of infected fish in a particular season}}{\text{Total no. of examined fish in a particular season}} \times 100$$

Abundance of parasites: The abundance of parasites was calculated using a formula by [41] Margolis.

$$\text{Abundance} = \frac{\text{No. of parasites recovered}}{\text{Total no. of host examined}}$$

Intensity of parasite: The intensity of parasites has been calculated using the formula of [41] Margolis.

$$\text{Intensity} = \frac{\text{No. of parasites recovered}}{\text{No. of hosts infected}}$$

## RESULTS

### Morphological character

The species *S. lucknowensis* belongs to the order Pseudophyllidea [15], family Bothriocephalidae [3], and genus *Senga*. It is isolated from the intestine of *C. punctatus* (Fig. 3) from the reservoir of Koderma, Jharkhand. The species of the genus *Senga* has been first isolated from fish labyrinthiform and cypriniform in South East Asia. It has been reported by S. Bernardi Dolfus, 1934 from fighting fish *Betta splendens*, in an aquarium, in France. *S. lucknowensis* is elongated with thin musculature, containing the scolex, immature, mature, and gravid proglottids. Some of the anterior proglottids are immature and gravid proglottids are much wider than long. The scolex is medium in size, pear-shaped structure, broader at the base, and tapering towards the anterior end. The scolex contains bothria, extending from the anterior end to the posterior end of the scolex. The scolex anterior end terminals into a rostellum, which is armed with hooks, round to oval in shape. The hooks are 41- 45 in number. The body is long and segmented, the mature segment width and immature segment were present. Proglottids are acraspedote wide. Testes are numerous, small, and



Figure-2. *C. punctata* collected from DVC Dam, a reservoir of Koderma Jharkhand.

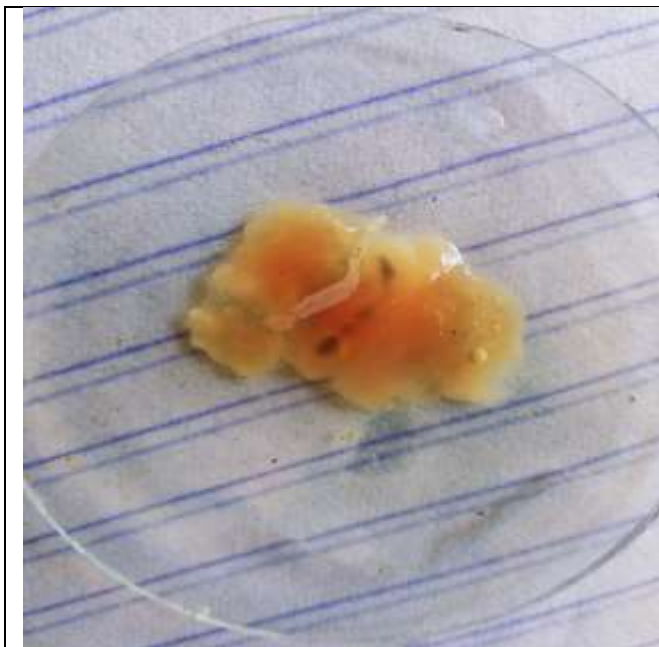


Figure-3. Cestode found in waste of small intestine, collected from the *C. punctata*.



Figure-4. Endohelminthic parasites Cestode isolated from the intestine of *C. punctata*

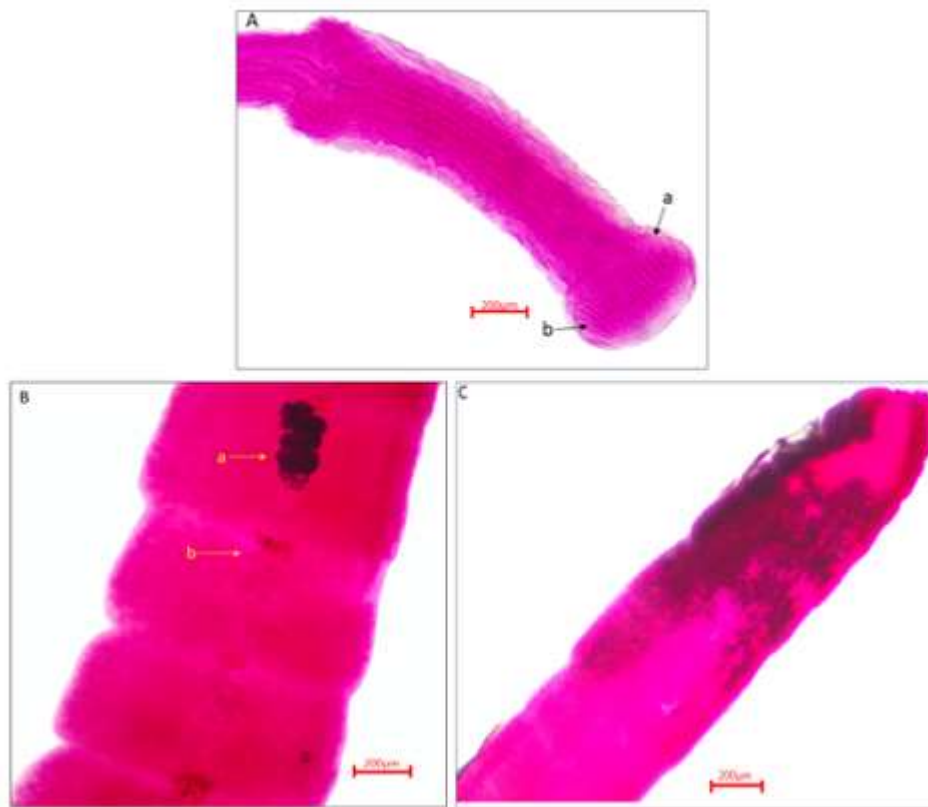
rounded. The ovary was lobulated. Fertilized egg was dispersed in the posterior region, present in gravid proglottid (Fig 5).

**Etymology:** Bearing in mind all these differences which were matched in morphological characteristics from all the species of the genus *Senga* studied, a little bit difficult to consider new to science, and the authors face difficulties to identify in species level so authors suggest going further study.

So further study has been analyzed based on the molecular study.

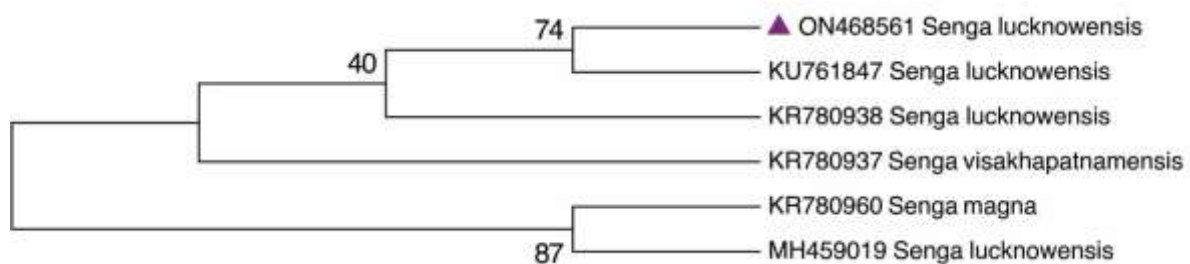
#### Molecular identification of isolated cestode

Cestode *S. lucknowensis* from the *C. punctata* were identified by using the tool NCBI BLAST. It is isolated from the mucus of the intestine from the species of *C. punctatus*. The cestode showed maximum identity from the cestode species *S. lucknowensis*. The 18S gene of *S. lucknowensis*



**Figure-5. Light microscopic (Magnus MLX1 PLUS, India) images of *S. lucknowensis* (stained Semichon's acetocarmine)**

- A) Anterior region of the body (a) showing scolex with pear-shaped bothria (b) hooks present in scolex.  
 B) Mid-region of the body showing body metamerism (a) Mature proglottids from regions of the mature strobila showing testicular field (b) Eggs present in the mature proglottids.  
 C) Posterior end of the body.



**Figure-6. Evolutionary relationships of genus *Senga* have been analyzed using the Mega version 6.0 software package.**

taxonomy has three gaps and a mismatch, according to a multiple sequence alignment. The similarity index matrix exposed with the sequence of our species is 100% matching to *S. lucknowensis* of Vietnamese isolate. The variation is almost negligible. The evolutionary tree shows that this similarity index between species exists. The diversity of the phylogenetic tree of *S. lucknowensis* found in DVC Dam Tilaiya in Koderma, Jharkhand. It is not

showing more diversity as compared to species which is found earlier in India.

The Neighbour-Joining approach was used to infer the evolutionary history [46]. The perfect tree has shown a branch length of a total of 0.00674898. The percentage of duplicate trees in which the pertinent taxa clustered during the bootstrap test (1000 repetitions) is displayed next to the branches [47]. The phylogenetic tree is shown to scale, and the



**Table 3. 18S RNA sequence of *Senga* species used for phylogenetic inference and sequence analysis by different researchers from a different place.**

Sl.No	Name of species	Accession number	Locality	Host
1.	<i>S. lucknowensis</i>	ON468561*	India	<i>C. punctata</i>
2.	<i>S. lucknowensis</i>	KR780938	Vietnam	<i>Mastacembelarmats</i>
3.	<i>S. lucknowensis</i>	KU761847	India	<i>C. punctata</i>
4.	<i>S. vishakapatnamensis</i>	KR780937	India	<i>C. punctata</i>
5.	<i>S. magna</i>	KR780960	Russia	<i>Sinipercachautsi</i>

\*-Present study

**Table 4. Prevalence (%), abundance, and intensity of parasites *S. lucknowensis* in various commercial fish species of DVC Dam, a reservoir of Koderma, Jharkhand**

S. No.	Name of fishes	No. of fishes examined	No. of infected fish	No. of parasite	Prevalence (%)	Abundance	Intensity
01	<i>Channa punctatus</i>	360	200	234	55.5%	0.65	1.17
02	<i>Cirrhinus mrigala</i>	240	-	-	-	-	-
03	<i>Notopterus Notopterus</i>	240	-	-	-	-	-
04	<i>Catla catla</i>	240	-	-	-	-	-
05	<i>Oreochromis niloticus</i>	240	-	-	-	-	-
06	<i>Labeo rohita</i>	240	-	-	-	-	-
07	<i>Pangasius sanitwongsei</i>	240	-	-	-	-	-
<b>Total</b>		<b>1800</b>	<b>200</b>	<b>234</b>	<b>-</b>	<b>-</b>	<b>-</b>

**Table 5. Prevalence, abundance, and intensity of *Channa punctatus* on the basis of sex**

Sex of fish	No. of fish examined	No. of infected fish	No. of parasites recovered	Prevalence (%)	Abundance	Intensity
Male	190	110	128	57.89	0.67	1.16
Female	170	90	106	52.9	0.62	1.17

branch lengths are in the same units as the evolutionary distances used to estimate it. MEGA6 [48] was used to conduct an evolutionary analysis (Fig 6).

### Statistical analyses

Approximately 1800 fishes have collected 360 species of *C. punctata*, 240 species of *N. notopterus*, 240 species of *L. rohita*, 240 species of *C. catla*, 240 species

of *O. niloticus*, 240 species of *C. mrigala*, and 240 species of *P. sanitwongsei*. Among these species of fish, only *C. punctatus* was infected. Using a simple percentage (%), the prevalence, abundance, and intensity of the infection were calculated. Within the samples, length range frequencies in relation to prevalence were examined.

**Table 6. The seasonal prevalence, abundance, intensity of infestation, and intensity of parasites in *C. punctatus* are based on the season.**

Season	Months	No. of examined fish	No. of infected fish	No. of parasites collected	Prevalence (%)	Abundance	Intensity
Pre-monsoon	February	30	22	26	73.3	0.86	1.18
	March	30	20	25	66.6	0.83	1.25
	April	30	18	22	60.0	0.73	1.22
	may	30	20	28	66.6	0.93	1.40
Monsoon	June	30	14	15	46.6	0.50	1.07
	July	30	13	14	43.3	0.46	1.07
	August	30	11	15	36.6	0.50	1.36
	September	30	12	13	40.0	0.43	1.08
Post-monsoon	October	30	15	17	50.0	0.56	1.13
	November	30	18	20	60.0	0.66	1.11
	December	30	17	18	56.6	0.60	1.05
	January	30	20	21	66.6	0.70	1.05

#### Prevalence of parasites in *C. punctatus* has based on sex.

This research investigated the prevalence of infection that has been evaluated in *C. punctata* based on sex. A total of 200 *C. punctatus* have been found to be infected and 234 parasites were recovered of which 128 parasites were recovered from males and 106 parasites were recovered from females. The prevalence of infestation in male hosts was 57.89% and in female hosts was 52.9 % respectively (Table 5). The abundance of parasites is higher in males as compared to females. The intensity of the parasite was slightly higher in females (1.17) than in males (1.16). Males have higher prevalence, abundance, and females have higher intensity, according to the current study. The present study has exposed that male hosts were found to be more infected compared to female hosts (Fig 7 & 8). The fundamental cause for the differences in parasite load with sex, according to [49] is a physiological condition.

#### Seasonal variation in prevalence, abundance, and mean intensity of helminth parasites

Seasonal variation of helminth parasites, including parasites of public health importance in fishes, has been studied by many authors from different parts of the world, and their existence is influenced by many factors [50, 51, 52, 53]. The most important factor that influence seasonality is the temperature which has a direct impact on free-living

developmental stages or the parasite within the hosts.

The present study revealed variations in the prevalence, abundance, and infection intensity of *S. lucknowensis* in *C. punctatus*. The seasonal data has been presented in (Table 6). The highest percentage of prevalence of the parasite was recorded in pre-monsoon than in monsoon and post-monsoon. The intensity of the parasite was slightly higher in pre-monsoon than in monsoon and post-monsoon (Fig 9 and 10).

## DISCUSSION

All the valid species which belong to the family Bothriocephalidea require critical evolution. It is thought that monogeneans are the closest relative of cestodes but it is still a matter of discussion of phylogenetic relationship and classification of the cestodes [55, 56], on the other hand, broad approval is achieved on some points on the entire as a result of widespread phylogenetic study based on the morphology, including ultrastructure and molecular data [56, 57]. Phylogenetic studies and testing of the evolutionary link between hosts and cestodes indicate a lengthy period of cestode vertebrate coevolution, probably reaching back to the Devonian, or 350 - 420 million years ago. Cestodes lack a fossil record [5]. The evidence of the still-existing cestode group indicates that it originated as fish parasites and spread to parasitize all main

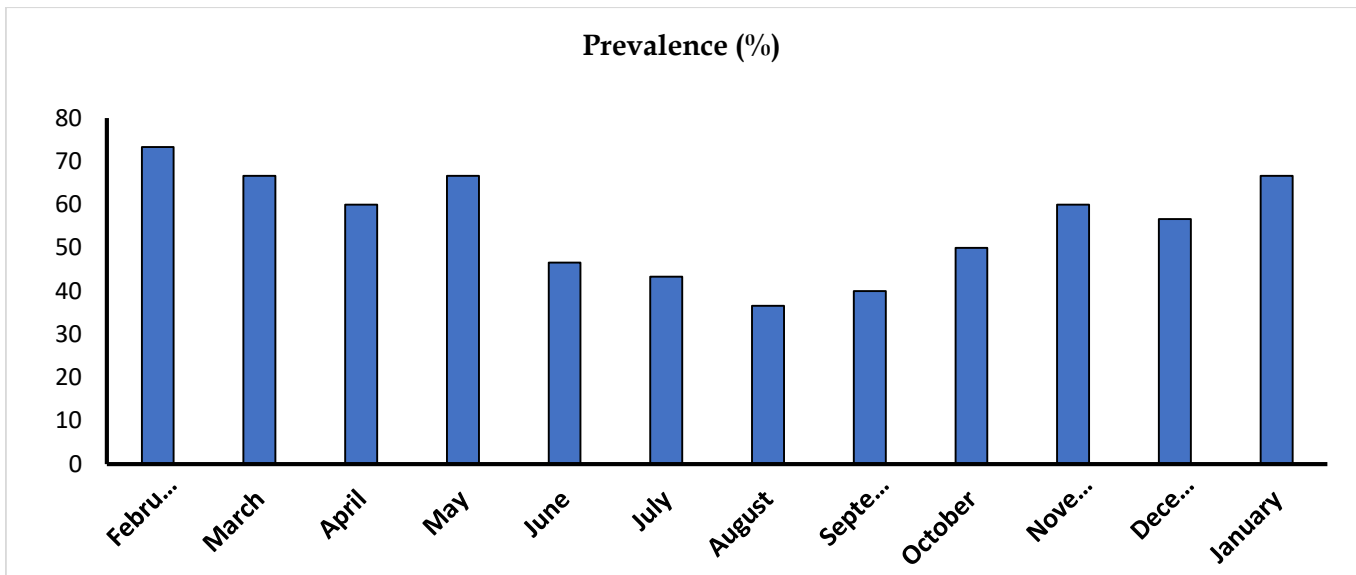


Fig 9. Graph showing the highest prevalence of parasites in pre-monsoon and the lowest prevalence of parasites in monsoon in *C. punctata* based on the season

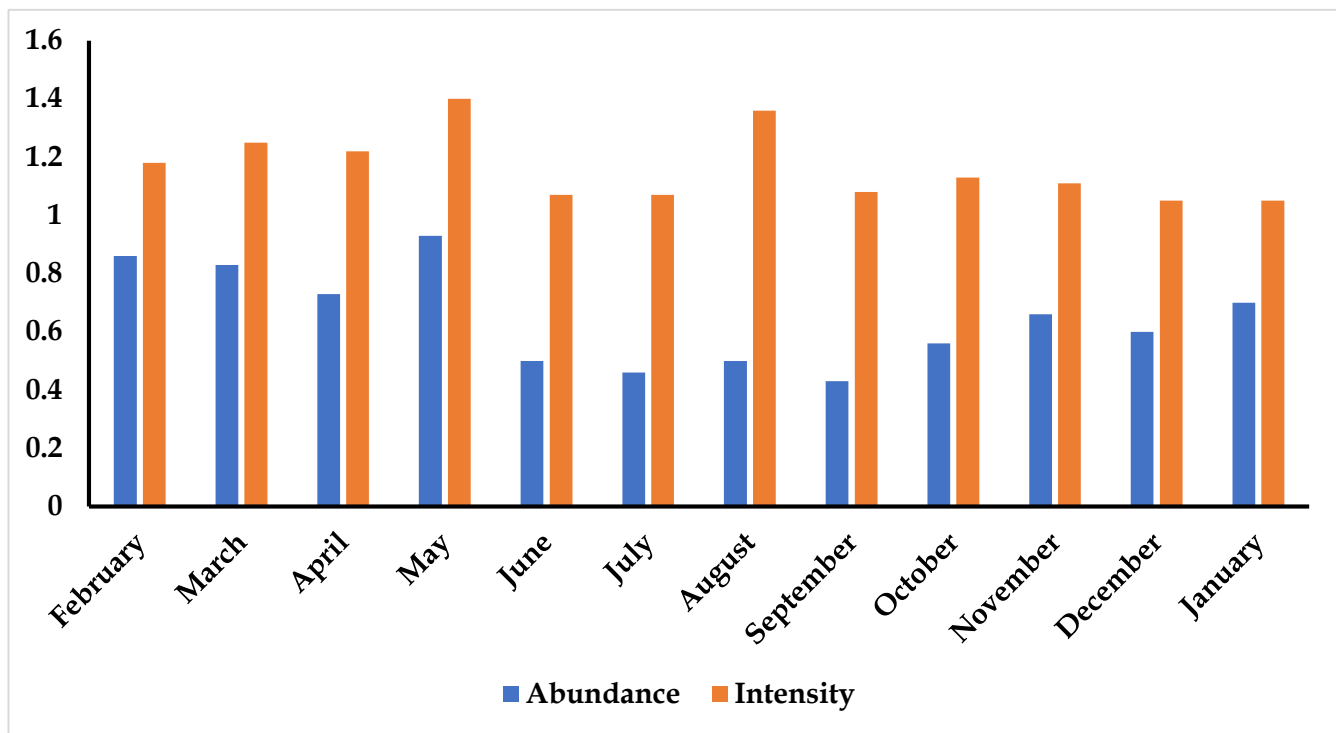
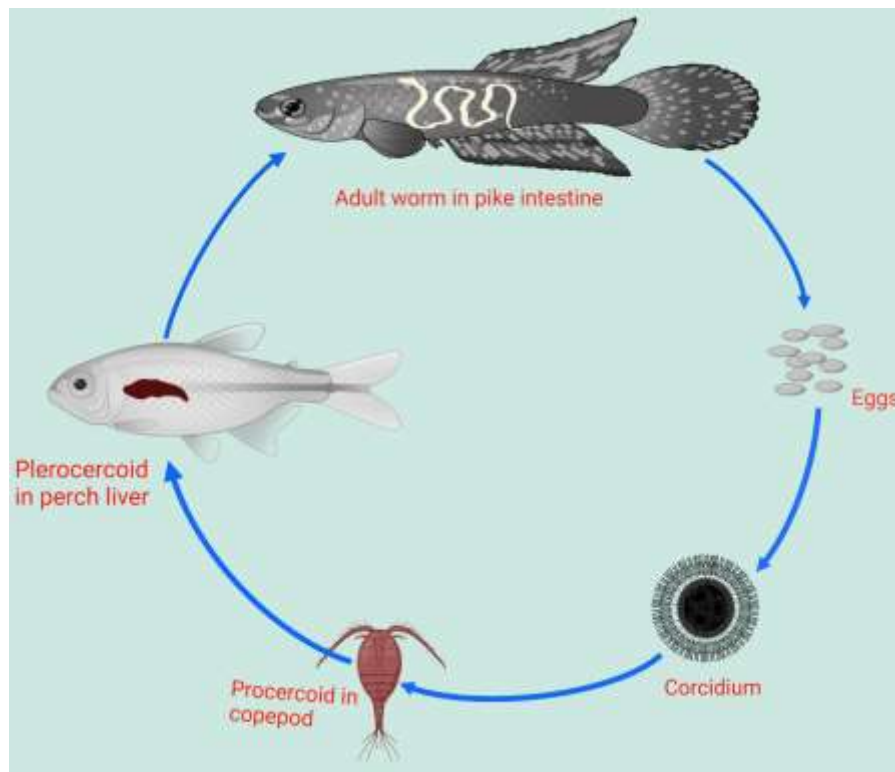


Fig 10. Graph showing the highest abundance of the parasite in pre-monsoon and lowest abundance of the parasite in monsoon and the highest intensity of parasites in pre-monsoon and the lowest intensity of parasites in monsoon based on the season.

vertebrate taxa [5]. The newly discovered data further imply that stabilization was a sequential process that moved from non-proglottid, non-segmented forms (Caryophyllidea), to proglottid, non-segmented cestodes (Spathebothriidea), and eventually to proglottid, segmented forms (upper

Eucestoda) [57]. Species-rich Bothriocephalidea is another family from the Indian subcontinent. There are 35 species of bothriocephalus and *Senga* has been described from the region of Indo-malayan requiring a critical revision [12]. Phylogenetic classification systems were shown that the prediction of the



**Figure-11. Cycle showing the life cycle of cestode.**

organism relationship and place in the biosphere has shown the most effective framework [58]. *Senga triaenophorus* has a three-host life cycle, with proceroid development in the first intermediate host and plerocercoid development in the muscle and cavity of the body in the second intermediate host, which is a teleost [59] (Fig 11).

The species of the genus *Senga* has been first isolated from fish labyrinthiform and cypriniform in South East Asia. It has been reported by S. Bernardi Dolfusin in 1934, as *Bettasplendens*, a Siamese fighting fish, in a French aquarium. In 1934, Dollfus erected the genus *Senga*, using *S. besnardii* from *Betta splendens* as the type species. In this study, the species *Senga* is distinct from all other previously identified species of *Senga* in the shape of the scolex, the number of hooks, and the proglottid's anatomy. It is similar to *S. lucknowensis* Johri (1956). The *Senga* species measure 202-208 in length. The body is somewhat spindle-shaped, tapering to the narrow scolex at the anterior end and also slightly tapering towards the posterior end. *Senga's* scolex, composed of an armed apical disc and two shallow bothria, is typical of the genus *Senga*. The scolex is pear-shaped and ranges in length from 1.32- 1.78. The bothrium is oval-shaped and somewhat elongated. It extends to about three-fourths the length of the scolex, and may

even sometimes overhang the lower margin of the scolex. The apical disc bears a crown of marginal hooks which are distributed in two semi-circles (Fig 5). There are 41- 45 large and rudimentary hooks. They measure 0.065 – 0.074 in maximum length of large hooks and 0.029- 0.043 in maximum length of rudimentary hooks (Table 2). In molecular study, the species *Senga* showed maximum identity from the cestode species *S. lucknowensis*. The similarity index matrix exposed with the sequence of our species is 100% matching to *S. lucknowensis* of Vietnamese isolate. The variation is almost negligible. From various places, the following species were later introduced to this genus. *S. lucknowensis* (1956), *S. khami* (1971), *S. aurangabadensis* [60], and *S. maharashtrii*, all vary from *S. khami* in the shape of the scolex (triangular vs rectangular) and hooks number (36 vs 55-57). *S. Godavari* [60] was reported from *Mastacembelus armatus* from Nanded, Maharashtra in India, in this study, the scolex is triangular vs pear-shaped, the hooks are circular vs semi-circular, the oocyte is spherical vs oval, and the vitellaris is follicular vs granular. *S. paithanensis* [61] was found in *Mastacembelus armatus* from Paithan, Maharashtra, India, rostellar hooks number (36 vs 54), testes number (45-50 vs 130-135), and the vaginal location (36 vs 54) were all higher in this study. *S. maharashtrii* [62] was reported from fish *Mastacembelus armatus* at

Daryapur, Maharashtra in India, in this study the shape of the scolex (triangular vs oval), the testes number is 45-50 vs 80-90 and the genital pore position is in the anterior half of the segment vs in the posterior half of the segment. *S. chauhani* [63] was reported from *C. punctatus* from Jamshedpur, Bihar in India, in this study, the scolex's shape is triangular vs oval, the hooks number is (36 vs 40-44), the testes number is 45-50 vs 300-310. *S. armatusae* [64], the number of the hooks is 36-40, testes are distributed in two lateral fields vs. densely distributed. *S. tappi* [65] was reported from *Mastacembelus armatus* from Shripur Dist., Dhule, Maharashtra in India, in this study, hooks number (36 vs 40), the testes number is 45-50 vs 285-295. *S. visakhapatnamensis* [66] was reported from *Ophiocephalus punctatus* from Vishakhapatnam in Andhra Pradesh, India, the scolex was circular, with 46-52 hooks, 50-55 testes, with vitellaria lobulated, and was described from *Ophiocephalus punctatus* in India.

The 18S sequence from *Senga* sp. has been analyzed in BLAST, NCBI, and it was found to match the taxon *Senga*, showing that the discovery is congruent with the classification of Bothriocephalidae which is based on morphological characteristics. Our species' 18S molecular sequence research revealed that inter-specific variation among *Senga* is quite low, implying that they are very similar to one another.

The analyses of prevalence, abundance, and intensity of parasites have been done during one year survey from May 2020 to April 2021. The number of total fish sample was 360 of which 200 was infected with *S. lucknowensis* isolated from DVC Dam. The prevalence, abundance, and intensity of infestation have been analyzed based on the sex of the fish and season. Based on sex, the prevalence of infestation in males is higher than in females. The sex percentage found in present study consisted of more males than females occurred in sampled fish population from the location. In a similar manner, more cestode parasites were discovered in males than in females.

Whereas based on the season, (Table 6) shows the monthly data for infection prevalence, abundance, and intensity. The prevalence of *S. lucknowensis* parasite infection over the course of a year was generally found to range from 36.0% to 73%, with an average value of 66.6%. Pre-monsoon had the largest proportion of the parasite's prevalence and post-monsoon had the lowest percentage. It was found

that Pre-monsoon was the season with the highest rates of abundance and infection intensity, while post-monsoon had the lowest rates. The statistical study of the data demonstrated that the three seasons pre-monsoon, monsoon, and post-monsoon had significantly different infection prevalence rates. In natural fish host *C. punctata*, the prevalence of *S. lucknowensis* observed a significant response to the seasonal variation over the course of one year. In the majority of cases, it was found that this stringed parasite was most prevalent during the pre-monsoon.

The prevalence of infestation is higher in males than in females. It has been noted that the growth of parasites requires a combination of high temperatures, little rainfall, and adequate moisture [67]. Due to favorable environmental conditions from late winter until the end of summer, fish and other animals typically become infected with a lot of parasites.

In our investigation, the infection rate varied according to the season, with the highest levels of parasites often found in the pre-monsoon and monsoon seasons. Following the end of the host fishes' prime reproductive season, the cestodes reached their highest prevalence in the pre-monsoon in their hosts. Numerous authors have also observed that in the summer the prevalence of cestodes is higher and lower in the monsoon season [68]. The parasite infection showed seasonal fluctuations, with the summer months having the highest prevalence, according to [69] Genc. A decrease in water volume during dry seasons leads to imbalanced nutritional conditions, which, according to [70], makes fish vulnerable to diseases. In this regard, there are conflicting results. Some authors have seen more infection in male hosts than in female hosts [71], whereas other observers have noted that females are more susceptible [72, 73]. These variations could result from a number of variables, such as host species, infectious species, and geographic conditions.

The molecular and morphological characterization and identification of *S. lucknowensis* are also presented in this paper. This research also demonstrates the value and usability of molecular technologies in the identification and delineation of parasites of medical and veterinary interest. This is the first record of *S. lucknowensis* being found in Koderma reservoir, Jharkhand India, and it has been

recognized in the current study as *S. lucknowensis* up to the species level.

## Conclusions

Seasonal variations are evident in our current study's data from an epidemiological survey over one year, which may be attributed to changes in the environment. The variance in the incidence of the *S. lucknowensis* cestode could be attributed to habitat differences, aquatic copepod abundance, and the availability of intermediate hosts. Because of the parasite's potential zoonotic character, a continuous survey that lasts for about a year seems to be useful for developing parasite management measures that address both the transfer of larval forms and infection of the final host. As a result, it can help in reducing the number of intermediate hosts and subsequently the parasite infection. It can be concluded that the lack of water availability and accumulation of waste, the pre-monsoon climate appears to be favourable for the abundance of parasites. Care should be taken before pre-monsoon so that we can break the life cycle of these parasites and can save the fish species to being infected.

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## Conflicts of Interest

Authors declare that there is no conflict of interests regarding the publication of this paper.

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