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A Brief Study on Diversity of Helminth Parasites in Selected Fish

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ABSTRACT
The current study aims to investigate the species composition, helminth identification, and seasonal variations in the prevalence, abundance, and mean intensity of helminths in Bihar's common edible fish. In order to identify and characterize helminth parasites, light and scanning electron microscopy studies were conducted, along with gene sequencing for three widely used molecular markers: mitochondrial cytochrome c-oxidase subunit 1 (mtCO1), 18S (or small subunit-SSU), and nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2). The American Society of Parasitologists' developed procedures were used to determine the quantitative descriptors such as prevalence, abundance, and mean intensity.
Key words : Helminth identification, microscopy studies

INTRODUCTION

For most people on the planet, fish is essential to both the economic and food security. It is the most reasonably priced animal protein that the underprivileged may obtain, and it is also a low-fat, high-protein diet. Fisheries and aquaculture provide a living for almost 250 million people, either directly or indirectly. When it comes to ornamental fish, it's also a recreational and pet fish. The last several decades have seen a lot of growth and expansion in the fishing and aquaculture industries, which has brought attention to the issues that parasites bring and their significance for fish health, productivity, behavior modification, and cleanliness. Globally, fish infections with parasites are quite widespread and damage the hosts' mechanical, physical, and reproductive systems. The majority of fish are heavily infected with parasites, which reduces their nutritional value and may even be fatal. Furthermore, some zoonotic helminth infections can only infect fish and subsequently spread to humans. Helminths inflict microscopic lesions on the lining of the host's tissues, which can restrict immunological function as a defense against host immunity. This raises the possibility of secondary microbial infection. Because parasite infection of fish causes significant economic losses, either directly or indirectly, it poses a serious threat to aquaculture in tropical and subtropical regions. In addition to the economic damage, there is also a health risk to people. Human zoonotic infections are caused by numerous major helminths and are contracted from fish by eating raw or partially cooked fish.

Accurate identification is essential to comprehending the biology, variety, and epidemiology of a parasite. As scientific expertise grows, so does the necessity for precise organism identification and naming. One of the reasons for the crisis of new infectious diseases is thought to be the absence of thorough taxonomic explanations of parasites. The combination of biosystematics and phylogenetic studies improves our knowledge of the life cycle, dynamics of transmission, and prognostic framework. For the previous 250 years, taxonomic research has been conducted using an organism's morphological characteristics. While morphological identification is undoubtedly the most basic and direct method of identifying an organism, many people with comparable physical characteristics may have completely distinct genetic makeups (Friedheim, 2016). This is where the identification process becomes crucial with the molecular method.

The molecular approach to systematics has become increasingly popular with advances in genetic and evolutionary studies. Molecular techniques, such DNA-based PCR methods, have shown promise in distinguishing closely related helminth parasites and identifying parasites up to the species level. To address taxonomic concerns and distinguish closely related parasitic species, the nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2), 18S, and mitochondrial gene cytochrome c oxidase subunit 1 (mtCO1) have all been widely utilized in this context. These genes have become the go-to source for information on taxonomy, population genetics, species identification, and the evolutionary relationships of different helminth parasite species, such as trematode, nematode, and cestode, due to their rapid rate of evolution. The ability to infer ITS2's secondary structure from primary sequence data is another benefit of employing it; it is known to yield additional information that can be helpful in distinguishing closely related species. Plants, fungi, and parasitic groups such as cestodes and trematodes have all been effectively distinguished using this method. Therefore, the generally recognized way for identifying parasites is the integrated strategy that combines both morphological and molecular research.

STUDY AREA AND COLLECTION OF HOSTS

The data on morphological features from fish samples were collected and statistically analysed using the programme SPSS version 16.0. The capital and largest city of the Indian state of Bihar is Patna, formerly known as Pataliputra. With a population of 2.35 million as of 2018, Patna was the 19th-largest city in India, according to the United Nations. Its urban agglomeration, which is nearly 2.5 million strong and spans 250 square kilometers (97 square miles), is the 18th largest in India. The Patna High Court is also located in Patna. Small-scale inland fishing is a key source of income for landless people in regions like Bihar that are landlocked. This industry is making a significant contribution to food and nutrition security. Growth in this area hasn't met expectations, despite the availability of water on hand and the persistent efforts of the state government in the shape of laws and programs. Climate change, overfishing, tainted natural water sources, and the exploitation of fishermen by strong personalities and certain government officials all pose challenges to small-scale inland fisheries. This market was found to be less competitive when compared to large-scale fisheries. 40% of all fish species are found in the inland ecosystem, while 75% of the world's aquaculture products are fin fish (FAO). According to the NBFGR's report, India is home to 2508 native fin fish that are divided into 928 genera and 237 families across 39 orders. Over the past 65 years, the production of inland fish in India has increased dramatically, rising from 2.18 LT to 37.50 LT. Compared to the global average of 20.0 kg per person per year, India has a per capita availability of fish of 10.0 kg. Only after the successful introduction of induced breeding technology in 1957, along with subsequent advancements in hatching system (from glass jar hatchery to commercial circular hatchery), introduction of New Species, system-based enhancement in open water(Pen & cage) fish production, Genetics improvement, and other factors, have Indian culture fisheries made rapid progress. In addition to culture fisheries, research at CIFRI on numerous river systems indicates a clear change in the composition of fish catches. The Hilsa fisheries of the past have entirely failed in Ganga, and the population of Indian majar carp has decreased to worrying levels. The various fishes with lower economic value have supplanted all the other species, including IMC and large catfish.

MORPHOMETRIC CHARACTERIZATION OF FISH

Morphometric Characters: All measurements and meristic countswere taken on the left side of the fish and by same person to minimize artificial error.

Body: The following body measurements of a fish were taken into account:

- **Total length (AH):** The greatest straight length between the anterior- most projecting part of the head (starting from any longer- one jaw) to the posteriormost tip of the elongated caudal fin lobe (upper or lower including single most filamentous prolonged fin ray, if any).
- **Standard Length (AG):** the straight distance from the anterior-most projecting part of the head to the end of vertebral column over the caudal peduncle.
- **Maximum body width (ii):** the vertical measurement from the ventral body side ofthe body to the greatest height on dorsal side in a straight line. It is generally taken between the origins of dorsal fin base to the vertical distance below on ventral side.
- **Minimum body width (iii):** the vertical distance measured especially near caudal peduncle region where the body has minimum width. It can also refer as minimum depth or least height of caudal peduncle.

- **Length of caudal peduncle (G):** the oblique measurement from the posteriormost part of anal fin base to the end of the vertebral column of the body over the caudal peduncle.
- **Pre-dorsal distance (AE):** the straight measurement from the anterior-most part ofthe head or tip of the snout or upper lip to the base of anterior- most part of dorsal fin ray.
- **Post-dorsal distance (FG):** The anterior most straight line measurement from the anterior structural base of dorsal fin to the end of vertebral column of the body overthe caudal peduncle.
- **Pre-anal distance (AI):** the straight line distance on the ventral side of the anterior- most part of the head (mid-point of lower jaw) to anus.
- **Post-anal distance (IG):** The straight line distance on ventral side starting from anusup-to end of vertebral column over caudal peduncle.
- **Distance between pectoral and pelvic fins:** The oblique measurement from the posterior most part of the pectoral fin base to the anterior most part of the pelvic finbase.
- **Distance between pelvic and anal fins:** The oblique measurement from the posterior most part of the pelvic fin base to the anterior-most part of the anal fin base.

HEAD: various measurements in the different parts of head are:

- **Head- Length (AD):** Straight line measurement from the snout tip to the posterior most point on the opercular membrane (on the upper angle of gill opening) including anyfleshy membrane of gill cover.
- **Head Depth (i):** The perpendicular distance measured vertically downward from the occipital crest to the ventral contour of the head or the breast just opposite to it.
- **Head width:** It is the straight distance across the head in a ventral position (if the opercles are dilated, they are forced into a reasonably normal position). **Pre-orbitaldistance (AL):** The distance between anterior- most point on the upper lip to the anterior margin of the eye-orbit.
- **Eye diameter (LM):** The distance or diameter across the bony rim of the eye ball.
- **Post- orbital distance (MD):** The greatest distance from the posterior edge of eye- orbit to the free edge of the operculum.
- **Inter-orbital distance:** The minimum distance between the bony rims if the inner margins of two eyes.
- **Length of barbells:** The measurements of each barbells viz. rostral or nasal,maxillary and mandibular present in the respective regions of the mouth from its base to the tip.

FINS: Fins are very characteristically designed organ in fishes as they help the fish in swimming very swiftly. These are primarily of four types: dorsal fin, analfin, caudal fin and paired pectoral and pelvic or ventral fins. Following measurements are taken on these fins:

- **Length of dorsal fin base (EF):** The maximum distance measured along the lengthof body in a straight line between anterior most points of junction with the body.
- **Depth or height of dorsal fin (ab):** The maximum length of the longest fin raystarting from its base to its tip even if the other ray does not reach this point.
- **Length of anal fin base (KI):** The maximum distance measured along the length ofthe body on ventral side starting from the anterior most base of anal fin ray to its posterior most base of anal fin ray.
- **Depth or height of anal fin (cd):** The greatest distance measured of the longest analfin ray from its base up-to its anterior most tip even if other rays does not reaches there.
- **Length of pectoral and pelvic fins (BJ & gh):** The maximum measurement of thelongest fin ray of pectoral or pelvic fin from its base or origin on the body to its extreme tip.
- **Length of caudal fin (GH):** The maximum lengths of the longest ray of caudal finmeasured from the base of caudal fin or end of caudal peduncle up-to its extreme tip.

MERISTIC COUNTS: These characters are good and reliable source of identification and classification of fish species. Following meristic counts have beenundertaken for the present study:

SCALE COUNT: Scales provide the strong evidence about the fishes and itsnumber is very specific in each genus.

- **Lateral line scales (XY):** It is the number of scale present along the lateral line startingfrom the end of operculum edge up to the base of caudal fin or end of caudal peduncle.Even if the lateral line is not present, the scales are counted in the lateral line series alongits flank in the same manner.
- **Scales count between lateral line and dorsal fin origin (iv):** The transverse scale count in oblique manner between the dorsal fin origin upto the lateral line on the flank(not included lateral line scale).
- **Scales count between lateral line and pelvic fin base (v):** The transverse scale countin oblique backward manner between the lateral line upto the base of any pelvic fin ray (not including the scales nearest to pelvic fin is counted as half when included).

FIN RAY COUNT: Fin rays in the bony fishes have long and filamentous elongations. These are classified into three types:

- **Soft Ray:** These are thin, flexible and mostly branched. These are represented by naturalnumbers (1, 2, 3, etc).
- **Hard Ray:** They are formed by number of soft rays united to form a hard cartilaginous ray which is rigid and may or may not have serrations on its inner edge while ending into a fine tip. The hard ray present at the margins of caudal fin lobes is referred as thePrincipal ray. These can be cited as lower-case roman numerals (i, ii, iii, etc).
- **Spinous Ray:** These are made up of bony tissue and are harder and stronger than hardrays. These are very common in catfishes and perches. These spinelike rays may or may not be covered by membranous sheath. They have serrations or denticles on the edges and are represented by uppercase roman numerals (I, II, III, etc).

Table-1
Morphometric Characters

AE – Pre dorsal distance	FG – Post dorsal distance
AH – Total length	AG – Standard length
AD – Head length	AL – Pre orbital distance
MD – Post orbital distance	BJ – Length to pectoral fin
AI – Pre anal distance	IG – Post anal distance
KI – Length of anal fin	EF – Length of dorsal fin
GH – Length of caudal fin	LG – Length of caudal peduncle
XY – Lateral line scale	LM – eye diameter
i – head depth	ii – maximum body width
iii – minimum body width	iv – scales above lateral line
v – scales below lateral line	ab – depth of dorsal fin
cd – depth of anal fin	gh – length of pelvic fin

Statistical analysis of morphological measurements
Morphometric Characters

- Standard Length,
- Pre-Dorsal Distance,
- Post-Dorsal Distance,
- Length of Dorsal Fin,
- Depth of Dorsal Fin,
- Length of Anal Fin,
- Depth of Anal Fin,
- Pre-AnalDistance,
- Length of Pectoral Fin,
- Length of Pelvic Fin,
- Length of Caudal Fin,
- Length of Caudal Peduncle,
- Minimum Body Width,
- Maximum Body Width,
- Distance Between Pectoral and Pelvic Fin,
- Distance Between Pelvic and Anal Fin,
- Head Depth,
- Pre-OrbitalDistance,
- Post-Orbital Distance,
- Eye Diameter and
- Inter-Orbital Distance

The data on morphological features from fish samples were collected and statistically analysed using the programme SPSS version 16.0. The capital and largest city of the Indian state of Bihar is Patna, formerly known as Pataliputra. With a population of 2.35 million as of 2018, Patna was the 19th-largest city in India, according to the United Nations. Its urban agglomeration, which is nearly 2.5 million strong and spans 250 square kilometers (97 square miles), is the 18th largest in India. The Patna High Court is also located in Patna. Small-scale inland fishing is a key source of income for landless people in regions like Bihar that are landlocked. This industry is making a significant contribution to food and nutrition security. Growth in this area hasn't met expectations, despite the availability of water on hand and the persistent efforts of the state government in the shape of laws and programs. Climate change, overfishing, tainted natural water sources, and the exploitation of fishermen by strong personalities and certain government officials all pose challenges to small-scale inland fisheries. This market was found to be less competitive when compared to large-scale fisheries. 40% of all fish species are found in the inland ecosystem (WCMC, 1992), while 75% of the world's aquaculture products are fin fish (FAO). According to the NBFGR's report, India is home to 2508 native fin fish that are divided into 928 genera and 237 families across 39 orders. Over the past 65 years, the production of inland fish in India has increased dramatically, rising from 2.18 LT (1950-51) to 37.50 LT (2005-06). Compared to the global average of 20.0 kg per person per year, India has a per capita availability of fish of 10.0 kg. Only after the successful introduction of induced breeding technology in 1957, along with subsequent advancements in hatching system (from glass jar hatchery to commercial circular hatchery), introduction of New Species, system-based enhancement in open water(Pen & cage) fish production, Genetics improvement, and other factors, have Indian culture fisheries made rapid progress. In addition to culture fisheries, research at CIFRI on numerous river systems indicates a clear change in the composition of fish catches (Sinha et al., 1998). The Hilsa fisheries of the past have entirely failed in Ganga, and the population of Indian majar carp has decreased to worrying levels. The various fishes with lower economic value have supplanted all the other species, including IMC and large catfish.

One of the prerequisites for conducting such research is to have a thorough understanding of the state's fish population. In order to do so, the sample size of the gathered fish populations must be large enough to allow for accurate morphometric analysis comparisons. Comparative morphometric analysis was performed on 30 samples from the collecting site. For this study, 21 morphometric characters (standard length, pre-dorsal distance, post-dorsal distance, length of dorsal fin, depth of dorsal fin, length of anal fin, depth of anal fin, pre-anal distance, length of pectoral fin, length of pelvic fin, length of caudal fin, length of caudal peduncle, minimum body width, maximum body width, distance between pectoral and pelvic fin. The parameters set out by Jayaram (1999) were used to define the various fish measures to be taken. Original readings were used to generate graphs and calculate regression equations ($Y = a + bX$) along with their correlation coefficients after recording all of the above-mentioned morphometric measures (r). The dependent characters have been computed in the proportion of various independent characters to measure the range difference, the minimum and maximum range for the computation of plastic and non-plastic characters. If the correlation coefficient is negative, the dependent character will drop with each unit increase in the independent character. If the correlation coefficient is positive, the dependent character will grow with each unit increase in the independent character. If it is positive, the dependent character will increase in proportion to the increase in independent character per unit. The correlation coefficient is usually positive in the fishes under investigation, although the degree of association varies. There are four types of correlation: high ($r = 0.90$-1.00), good ($r = 0.75$-0.90), moderate ($r = 0.50$-0.75), and bad ($r = 0.25$-0.50).

All dependent characters in each fish stock were calculated as a percentage of their respective independent character (total length or head length) for this purpose. The entire specimen's data is then pooled and examined, including mean value, minimum and maximum range, and range difference. As proposed by Vladykov, the most significant analysis is the determination of non-plastic and plastic characters (1934). If the range difference is less than 5%, the character can be classified as non-plastic or genetically regulated, but if it is greater than 20%, the character will be classified as plastic or environmentally controlled. Intermediate characters are those that have a percentage of 5.01 percent to 19.99 percent.

Statistical analysis

The means of prevalence and intensity across various seasons were compared using Anova at the levels of 0.01 and 0.05. The levels of 0.01 and 0.05 were also used for the correlation analysis. Utilizing the SPSS 17 bundle of programs, statistical analysis of the data was performed.

Table-2

Quantitative phenotype traits based on morphometric characters and meristic counts used for differentiation analysis

Characters	**Acronyms**
MORPHOMETRIC ANALYSIS	
Total length	TL
Standard length	SL
Head length	HL
Head depth	HD
Pre-orbital distance	Pr-OD
Post-orbital distance	Po-OD
Eye diameter	ED
Inter orbital distance	IOD
Pre-dorsal distance	Pr DD
Post-dorsal distance	Po DD
Length of dordal fin	LDF
Depth of dorsal fin	DDF
Length of anal fin	LAF
Depth of anal fin	DAF
Pre-anal distance	Pr AD
Length of pectoral fin	LPF
Length of pelvic fin	LpF
Minimum body width	Min BW
Maximum body width	Max BW
Distance between pectoral & ventral fins	Dist. pec. & vent.
Distance between pelvic & anal fins	Dist. pel.& anal
Length of caudal fin	LCF
Length of caudal peduncle	LCP
MERISTIC ANALYSIS	
Lateral line scale count	LLSC
Scales above the lateral line	SALL
Scales below the lateral line	SBLL

Dorsal fin rays count	DFRC
Pelvic fin rays count	Pel FRC
Pectoral fin rays count	Pec FRC
Anal fin rays count	AFRC
Caudal fin rays count	CFRC

The measurements were obtained with the intention of including only fresh and well-preserved specimens in the current investigation. Specimens that were spoiled, misshapen, damaged, or infected were not accepted. Graphs are used to examine the relationship between independent characters such as total length (TL) and head length (HL) vs various dependent elements.

Several dependent morphometric characters are plotted in the same graph with regard to their independent character to reduce the number of graphs in the text, and each character is expressed in various colour symbols. When plotting a character on a graph between independent and dependent characters, care has been taken to ensure that the values do not overlap. The independent characters are plotted horizontally, whereas the dependent characters are plotted vertically. These sides are referred to as the graph's X (horizontal) and Y (vertical) arms. Regression equations for various dependent characters to their respective independent characters (total length and head length) have been derived based on these values, and matching regression lines have been created. There is some ambiguity in the dependent characters, such as eye diameter and so on. When these characters were plotted against their respective independent character, such as head length (HL), a straight line was detected between them in some samples. This uncertainty can be attributed to a measurement error or a broad skin flap around the orbital region that prevents proper recording of the exact dimensions of numerous morphometric features of the eyes.

QUANTITATIVE PHENOTYPIC ANALYSIS

The goal of this study was to look at the differences in morphomeristic properties of Tor putitora populations from the Indrayani River in Pune, Maharashtra. The mean values of morphometric measurements are compared, and there are no significant variations between the mean indices values, with the exception of ED/HL, which differs at a 5% level of significance (Table-3).

Table-3

Means And Standard Deviation Of Quantitative Phenotype Traits Based OnMorphometric Character Indices Used For Differentiation Analysis Of Fish Populations (MEAN±S.D.)

S.No.	Morphometric characters in relation to total length	Means and standard deviation
1.	SL/TL	73.97±6.37 [a]
2.	Pr DD/TL	39.37±2.49 [a]
3.	Po DD/TL	60.75±7.07 [a]
4.	LDF/TL	20.50±1.57 [a]
5.	DDF/TL	16.15±0.83 [a]
6.	LAF/TL	12.61±0.85 [a]
7.	DAF/TL	10.09±0.71 [a]
8.	Pr AD/TL	62.91±5.48 [a]
9.	LPF/TL	10.70±0.82 [a]
10.	LpF/TL	8.47±0.58 [a]
11.	Min BW/TL	6.17±0.51 [a]
12.	Max BW/TL	12.42±0.52 [a]

13.	Dist. pec.&vent. /TL	13.02±1.16 [a]
14.	Dist. pel.& anal/TL	17.67±1.17 [a]
15.	LCF/TL	15.03±2.72 [a]
16.	LCP/TL	7.95±1.02 [a]

Mean values in the same row having the same letters do not differ significantly (P≤0.05).

Table-4

Morphometric characters in relation to head length

S.No.	Morphometric characters in relation tototal length	Means and standard deviation
1.	HD/HL	72.25±4.64 [a]
2.	Pr-OD//HL	35.84±2.44 [a]
3.	Po-OD/HL	45.74±3.82 [a]
4.	ED/HL	21.64±1.46 [c]
5.	IOD/HL	30.31±4.00a

Mean values in the same row having the same letters do not differ significantly (P≤0.05).

MORPHOMETRIC MEASUREMENTS OF SELECTED FISH BODY MEASUREMENT IN RELATION TO TOTAL LENGTH

The mean, correlation coefficient (r), and regression coefficients (values of a and b in regression equations) are calculated for various body measurements in relation to total length of Tor putitora, and the regression equation between an independent character (X) and various dependent characters (Y) is extrapolated. Minimum body width (r = 0.979), pre dorsal distance (r = 0.978), post dorsal distance (r = 0.976), and depth of anal fin (r = 0.976) are the most highly correlated body parameters in relation to total length, while pre-anal distance (r = 0.935), depth of dorsal fin (r = 0.941), and distance between pectoral and pelvic fins (r = 0.944) are the least correlated. Standard length (r = 0.958), dorsal fin length (r = 0.965), anal fin length (r = 0.969), pectoral fin length (r = 0.958), pelvic fin length (r = 0.952), maximum body weight (r = 0.973), distance between pelvic and anal fin (r = 0.963), length of caudal fin (r = 0.974), and length of caudal peduncle (r = 0.963).

HEAD MEASUREMENTS IN RELATION TO HEAD LENGTH

In relation to head length, body measurements like inter-orbital distance (r = 0.991), eye diameter (r = 0.978), and head depth (r = 0.975) are highly associated, while post orbital distance (r = 0.943) is the least correlated. The parameter pre-orbital distance (r = 0.964) is extrapolated from the range of maximum and lowest correlation coefficient values and regression coefficients (values of 'a' and 'b' in regression equations) between an independent character (X) and several dependent characters (Y). Table-3 lists all of the body and head measurements taken during the current investigation among Seer stream population that fall within the high correlation coefficient range (0.90-1.00).

Table-5

Values of different morphometric characters

Parameters	Regressionequation Y=a+bX	Mean	SD	Range difference	Correlation coefficient (r)
In Percentage of Total length					
Standard length	Y=-0.286+0.752X	73.61	1.29	4.66	0.958**
Predorsal length	Y=-1.732+0.688X	40.20	0.49	2.3	0.978**
Post dorsal length	Y=0.417+0.378X	59.18	1.03	3.51	0.976**
Length of dorsal fin	Y=-0.171+0.205X	19.58	0.33	1.33	0.965**
Depth of dorsal fin	Y=-1.627+0.224X	13.43	0.68	2.17	0.941**

Length of anal fin	Y=-3.617+0.324X	12.34	1.25	3.59	0.969**
Depth of anal fin	Y=-2.567+0.278X	13.58	0.91	3.36	0.976**
Pre-anal distance	Y=-2.606+0.335X	61.59	2.25	6.92	0.935**
Length of pectoral fin	Y=-3.441+0.313X	12.24	1.23	4.3	0.958**
Length of pelvic fin	Y=-3.821+0.333X	12.14	1.44	4.66	0.952**
Minimum body width	Y=-1.489+0.130X	5.26	0.51	1.85	0.979**
Maximum body width	Y=-1.521+0.134X	15.72	0.34	1.7	0.973**
Distance between pectoral & pelvic	Y=-3.124+0.358X	18.46	1.31	4.42	0.944**
Distance between pelvic & anal	Y=-4.110+0.431X	20.34	1.49	4.1	0.963**
Length of caudal fin	Y=-3.383+0.392X	20.48	1.23	4.04	0.974**
Length of caudal peduncle	Y=-3.732+0.269X	6.24	1.27	4.18	0.963**

In %age of head length

Parameters	**Regressionequation Y=a+bX**	**Mean**	**SD**	**Range difference**	**Correlation coefficient (r)**
Head depth	Y=1.517+0.057X	76.22	6.06	19.88	0.975**
Pre-orbital distance	Y=0.598+0.868X	67.28	4.79	16.68	0.964**
Post-orbital distance	Y=0.02+0.510X	51.68	2.82	8.89	0.943**
Eye diameter	Y=0.108+0.220X	25.59	0.82	3.37	0.978**
Inter- orbital distance	Y=-1.205+1.091X	69.81	6.09	18.94	0.991**

** $p < 0.05$ level of significance.

CONCLUSION

Twenty distinct species of helminth parasites were found in the fish species under investigation, according to a parasitological survey. This comprises four species of trematodes (Astiotrema reniferum, Clinostomum philippinense, Phyllodistomum sp. and Posthodiplostomum sp.), six species of cestodes (Lytocestus attenuatus, L. indicus, L. filiformis, L. longicollis, Djombangia penetrans, and Anisakis sp.), and four species of nematodes (Camallanus anabantis, Neocamallanus singhi, Paraquimperia manipurensis, Paracamallanus ophiocephali, Procamallanus sp. and Anisakis sp.), six species of cestodes, and six species of cestodes.

Although the nematode parasites Paracamallanus and Camallanus have some physical similarities, their differences are significant enough to classify them in different genera. There are just four species of Paramquimperia known to exist in the globe. Paraquimperia manipurensis is one of such species. P. manipurensis's lip structure was found to differ somewhat from that of previously known species from this area using scanning electron microscopy analysis. It is therefore rewritten here. There were morphological similarities between the species of the genera Lytocestus and Djombangia and the previously identified species. Nevertheless, morphological identification of the Lytocestidae larvae retrieved during the sample was not possible. Therefore, these larval cestodes were effectively amplified using genetic markers, particularly CO1, ITS2, and 18S, along with four other helminth parasites that could not be recognized to the species level. The amplicons underwent sequencing, were entered into Genbank, and were assigned accession numbers. The two metacercariae's CO1 and ITS2 sequencing analysis showed that they belonged to Phyllodistomum sp. and Clinostomum philippinense, respectively. Lytocestus indicus, Senga lucknowensis, and Pallisentis sp. are the species to which the two cestodes and one acanthocephalan belong, according to the 18S analysis of the samples. Furthermore, Clinostomum philippinense and Phyllodistomum sp. ITS2 secondary morphometrics showed the recognizable four-helix model with branches, loops, and bulges.

The prevalence, abundance, and mean severity of helminth infections were significantly correlated with meteorological parameters such as temperature, humidity, and rainfall. The temperature range of 24-27˚C was found to be advantageous for the infection of helminth parasites. In order to potentially effectively manage helminth infections, we propose that treatments be given throughout the monsoon and pre-monsoon seasons. The current study's findings will support the creation of all-encompassing disease management plans for the management of parasite diseases in fish as well as fish-borne zoonoses. Numerous approaches, such as treating fish diseases, removing snails from water bodies because they serve as intermediary hosts for many cercariae, and drying up ponds, have been proposed.

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