DIVERSITY AND POPULATION DYNAMICS OF HELMINTH PARASITES IN FISH OF WETLAND ECOSYSTEM

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<u>SUMMARY</u>

A wetland is an area of land that is saturated with water, either permanently or seasonally, and typically supports vegetation adapted to wet conditions. Wetlands play a crucial role in the environment, providing various ecological services and supporting a high level of biodiversity (Garg *et al.,* 1998; Fraser and Keddy 2005). They are transitional zones between aquatic and terrestrial ecosystems, and their unique characteristics make them important for water purification, flood control, nutrient cycling, and habitat for numerous species. Wetlands can take various forms, including marshes, swamps, bogs, fens, and estuaries (coastal wetlands), each with distinct characteristics based on factors such as water flow, vegetation types, and the surrounding landscape, and are highly productive ecosystems and serve as nurseries for many fresh water and marine species. Despite their importance, wetlands are often threatened by human activities such as drainage, agriculture, and urban development. Conservation efforts are essential to protect and preserve these valuable ecosystems.

The diversity and population dynamics of helminth parasites in fish within wetland ecosystems are crucial aspects of aquatic ecology and fish health. Helminths are a diverse group of parasitic worms that include nematodes, trematodes, and cestodes. They can have significant impacts on fish populations, affecting their growth, reproduction, and overall fitness. Different species of fish may harbour various helminth species, each with its own life cycle and ecological niche. The characteristics of the wetland ecosystem, including water quality, temperature, and substrate, can influence the diversity of helminth parasites.

Helminth infections can negatively affect fish health by causing tissue damage, reducing growth rates, and impairing reproductive success. Some parasites may alter the behaviour of their host to increase the likelihood of transmission to the next host in their life cycle, influencing the overall ecology of the wetland. **Regularmonitoring** of fish populations and their helminth parasites can provide valuable information for the management and conservation of wetland ecosystems. The presence and abundance of helminth parasites can serve as indicators of the overall health of the wetland ecosystem. Parasite abundance and transmission are often influenced by water quality parameters such as temperature, pH, and oxygen levels. Factors affecting the immune system, such as stress, can impact the dynamics

of infection. Studying the population dynamics of helminth parasites in fish within a wetland ecosystem requires a multidisciplinary approach, combining field surveys, laboratory experiments, and mathematical modelling. Monitoring changes in environmental conditions, host populations, and parasite life cycles is essential for understanding the factors influencing helminth prevalence and intensity in fish populations. Additionally, such studies contribute valuable insights into the overall health and ecological balance of wetland ecosystems.

Fish plays an essential role in ensuring food security and contributing to the economy for a significant portion of the global population. It serves as a valuable source of high-quality protein with low-fat content. Additionally, it stands out as the most economically accessible animal protein for individuals with limited financial resources, as highlighted (Belton and Thirsted, 2014). The abundance of fish species in freshwater wetland ecosystems holds significant socio-economic importance for the communities residing in their vicinity. Efforts have been made to conduct surveys on the fish fauna present in these habitats, aiming to assist in the development of strategies for their sustainable production and efficient utilization (Renjith Kumar *et al.,* 2011).

Young fish are generally more susceptible to the tail region which is most prone to metacercarial infection and should, therefore, be avoided for human consumption (Gupta and Gupta, 2014). It was also suggested that acanthocephalans are an injurious roup of parasites, frequently found in the gut of freshwater Channidae fishes (Gupta and Gupta, 2014; Gupta *et al.,* 2015).The parasitic fauna associated with *Channa punctata* of family Channidae may vary due to excessive use of inorganic fertilizers and pesticides in cultivated lands, discharge of industrial effluents, inadequate waste disposal, etc. which can indirectly cause changes in the aquatic environment.

Cestodes prevail as the primary endohelminths in elasmobranches and exhibit limited noteworthy variations in bony fishes, specifically within Caryophyllidea, Proteocephalidea, and Pseudophyllidea (Cribb *et al.,* 2002). Various researchers across the globe have investigated cestodiasis in freshwater fishes, as evidenced by studies conducted (Ash *et al.,* 2015; Brabec *et al.,* 2015; and Koiri and Roy, 2017).

Nematodes are recognized as highly detrimental parasites for fish and are deemed the most lethal among helminths (Yasmin and Bilqees, 2007). A comprehensive study on parasitic nematodes in freshwater fishes of Europe contributes contemporary insights into the taxonomy, biology, and ecology of these parasites (Moravec, 1995).

Acanthocephala commonly referred to as spiny or thorny-headed worms have specialized adaptations for survival within the digestive tract of fish. They possess spines on a retractable proboscis at the front end, allowing them to anchor to the tissues of the digestive tract. Numerous studies have explored the prevalence, seasonal variations, and population dynamics of different acanthocephalans infesting fishes across various regions globally, as documented in a wealth of literature (Sheema *et al.,* 2015).

Wetlands possess significant economic, ecological, and cultural importance, serving as essential resources for various human needs such as food, fodder, and construction materials. The ecological functions of wetlands encompass crucial roles in maintaining water quality, hydrology, flood control, carbon cycle regulation, climate stability, water cycle management, shore stabilization, and groundwater recharge. Evaluating and quantifying these values economically can be challenging. Nevertheless, wetlands in India are currently encountering substantial anthropogenic pressures, as highlighted (Prasad *et al.,* 2002). In Uttar Pradesh, threats stemming from human activities, including excessive fish cultivation, soil excavation, overgrazing in proximity to wetland areas, and the use of pesticides and non-biological fertilizers, have been extensively studied (Kanaujia *et al.,* 2014; Kumar *et al.,* 2015).

Fish parasitology is a rapidly developing field of aquatic science. This is due to the growing importance of aquaculture, concerns about pollution effects on fish health, and a generally increasing interest in environmental biology (Moller and Anders, 1986). Consequently, infection-associated changes in the behaviour (Rohde, 1993).Increased temperature causes multiple changes in the physiology of fish such as growth, behavior, abundance, diversity, etc. of both their host and non-host organisms eliminating parasites and also affecting fish-host immunity suppression (Macnab and Barber, 2011; Lohmus and Bjorklund, 2015; Brunner and Eizaguirre, 2016; Dittmar *et al.,* 2014). The factors can hamper the transmission success of parasites, such as the removal of parasite infective stages by natural enemies due to temperature (Lafferty *et al.,* 2008; Kuris *et al.,* 2008) as a result eliminating parasitic larvae, thus, significantly reducing infection transmission in many aquatic organisms (Johnson et al. 2010; Welsh et al. 2014; Gopko et al. 2017). Feeding rates of poikilothermic organisms are strongly temperature-dependent like most metabolic processes (Schmidt-Nielsen, 1997). The presence of filter-feeder organisms eliminating infective parasite stages has a stronger effect on infection transmission at higher temperatures (Goedknegt *et al.*, 2015).

Previous studies report Changes in fish vulnerability to infection caused by temperature increase could be mediated by fish behavior, during higher temperatures fish may increase their motor and ventilation activity that potentially increasing exposure rate, thus increasing the parasite's chances to penetrate the host skin and gills (Mikheev *et al.,* 2014). Previous studies report that the infections might become more detrimental for hosts during higher temperature conditions because of enhanced parasite growth (Macnab and Barber, 2011), and that sex differentiation is temperature-dependent in tilapia, *Oreochromis niloticus* (Baroiller *et al.*, 1995).

Fish diversity within the freshwater wetland ecosystem has great socio-economic importance for the people living around it (Renjithkumar *et al.,* 2011; Johnson *et al.*, 2012). Understanding the diversity and population dynamics of helminth parasites in fish within wetland ecosystems is essential for developing effective conservation and management strategies to maintain the ecological balance and health of these environments. Research in this field contributes to our broader understanding of aquatic ecosystems and the interactions between different ecosystem components. In this context, Uttar Pradesh state has vast potential for aquatic bioresources and offers a considerable scope of inland fisheries development and aquaculture. The objective of the present work is to provide a better knowledge and understanding of the fish diversity in wetlands at the Lucknow, Sultanpur, and Allahabad districts of Uttar-Pradesh, India, which can be utilized in the future as a tool for conservation and planning of the aquatic environment.

The objectives of the proposed investigations were to study the prevalence and population dynamics of helminth parasites of certain freshwater fishes in a wetland ecosystem through morphological identification of helminth parasites with the help of light scanning electron microscopy, and molecular characterization. Further to study the diversity of helminth parasites of freshwater fishes in wetland ecosystems of different regions of Uttar Pradesh. Finally, to study the effect of different abiotic environmental factors and seasonal variations on helminth parasites of freshwater fishes.

The present research work deals with the analysis of seasonal variation and population dynamics along with the morphological and molecular characterization of parasites from wetland fishes and the effect of abiotic factors on them. Uttar Pradesh is situated in the northern region of India, covering 2,40,92 square kilometers (23°52'N & 31°28' N and **77°**3' & 84°39' E). There are over 100 wetlands, and 10 of them have been recognized as Ramsar sites. The total area covered by wetlands in the state is roughly 1.5 million hectares. The present work involved different water bodies of the following districts Allahabad (Prayagraj), Sultanpur, and Lucknow.

The chosen research locations were identified as three distinct sites, Kallipaschim wetland near SGPGI (26° 44' 16" N & 80° 57' 18" E) at Lucknow, Saraiya wetland (26°7' 28'' N & 82°20' 33'' E) at Sultanpur and Phaphamau wetland (25° 30' 36'' N & 81° 51' 58'' E) at Allahabad (Prayagraj) district. The work was accomplished throughout one and a half years, from July 2021 to December 2022.

Fishes were carried out with the help of local fishers using gill nets, cast nets, and drag nets including hooks and lines (Bose *et al.*, 2013) and brought to the Enviro-Parasitology and Molecular Lab, Department of Zoology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. Fish were identified based on morphometric characters and standard taxonomic keys for fishes (Talwar and Jhingaran, 1991).Two different fishes were selected namely- *Channa punctata* (Channiformes) and *Heteropneustes fossilis* (Siluriformes).

For the study purpose, the total number of dissected fish of both fish species from every single sampling site for the complete duration of the study is 5940 during the year 2020 to 2022.Fish hosts were dissected and isolation of helminth parasites was from different parts of the host body like an alimentary canal, gastrointestinal tract, stomach, liver, etc. kept in 0.9% saline, aqueous Bouin's and Zenker's fixative according to (Stoyanov *et al.*, 2017). The collected parasites including cestodes and nematodes were quantified, documented, and processed for further identification according to standard techniques (Eiras *et al.*, 2006).The parasites were then examined under a light microscope (Evos imaging light microscope).The identification of parasites based on their morphological characteristics was carried out following established standards in the scientific literature (Yamaguti, 1959, 1961, 1963, 1971) and the comprehensive Keys to the Trematoda (Volumes 1-3) authored by (Gibson *et al.,* 2002; Jones *et al.,* 2005, and Bray *et al.,* 2008). Additionally, the CIH Keys to the Cestode Parasites of Vertebrates, as compiled by (Khalil *et al.,* 1994), and the CIH Keys to Nematodes (Nos. I-X), curated by (Anderson *et al.*, 1974-1983), were consulted to ensure a thorough and accurate identification process.

The collected parasites underwent thorough washing in PBS and were then fixed in 100% ethanol before being preserved at -20°C for subsequent molecular analysis. Among the helminth parasites that could not be identified at the species level based on morphology were *Parascaris sp. Tanqua sp, Accacoelium sp., Senga sp., Polyonchobothrium sp., Lytocestus sp., Lucknowia sp., Clinostomum sp., Euclinostomum sp., Morishitium sp., Lecithaster sp.* and*Pallisentis sp.,* were selected for genetic study using the markers 18S 28S, ITS, ITS2, and COI and they were successfully amplified.

The DNA was isolated by using a Qiagen Dneasy blood and tissue kit and the isolated DNA's quality was evaluated on 1.0 % agarose and utilized for conducting PCR amplification. The genetic markers employed in this procedure consist of the rDNA 18S, 28S, ITS2, and mtCO1 regions.Electrophoresis was performed by the protocol described by Sambrook (Sambrook, Fritsch, *et al.*, 1989). To determine DNA size, gels of different agarose strengths were utilized, each matched to specific ranges of DNA base pairs (bp).DNA samples, along with DNA molecular weight markers/ladder, were loaded into the gel using 6X DNA loading dye. Electrophoresis was conducted at a rate of 5 V/cm. Following electrophoresis, the gel was examined under transmitted UV light (302-312 nm) using a transilluminator (Fotodyne Inc. USA) and then photographed. After the amplification process, the PCR products were purified using a Qiagen PCR product purification Kit. Further, the purified PCR Products were sent to Biosciences Bengaluru. The quality of the resulting sequences was evaluated using sequence scanner software v2. And submitted to NCBI-GenBank, where accession numbers were obtained.The sequences obtained, in addition to sequences from other relevant helminth species, were obtained from GenBank for analysis. Various characterization tools and software programs, listed below, were employed for the analysis of these sequences.The Sequence Scanner software version 2.0 is applicable for visualizing, printing, and exporting sequence data produced by the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer.

Biological processes hinge on complex arrays of biochemical reactions, shaped by physical elements like temperature. The temperature at the sampling site was measured using a YK-2005b instrument from Lutron, produced in Taiwan, and documented in degrees Celsius at the time of sample collection (°C). The measurement of pH was conducted on-site during sample collection using a YK-2005b device from Lutron, manufactured in Taiwan.

Water salinity is determined through the assessment of total dissolved solids (those that dissolve in water). The measurement of Total Dissolved Solids (T.D.S.) was conducted using a Multi Probe ITS-701 directly at the sampling site, and the results were expressed in milligrams per liter (mg/L).

Electrical conductivity is a measure of how well a substance or solution can conduct electrical charge. E.C. is contingent upon factors such as the presence of ions, their overall concentrations, ion mobility, valency, relative concentration, and temperature. Using a YK-2005b device by Lutron, manufactured in Taiwan, electronic conductivity was immediately measured at the collection site during sample collection and recorded in mhos/cm at 25°C.

The quantity of calcium carbonate (water hardness) present in water establishes its alkalinity, serving as an indicator of the water's capacity to neutralize acids. Alkalinity is assessed through a titration method employing two indicators: Phenolphthalein and Methyl orange. Phenolphthalein transitions from pink to colorless at a specific pH. Methyl orange operates under a pH of 4.5 (indicative of acidic conditions), with sulfuric acid serving as the titrant. The significance of alkalinity in evaluating the suitability of water for irrigation surpasses the importance of alkaline earth metal concentrations.

Water hardness is characterized by the presence of calcium (Ca) and magnesium (Mg) in the forms of bicarbonates, sulfates, chlorides, and nitrates. The total hardness of a substance is determined by the collective levels of its alkaline and metal cation concentrations.

Sulphate is a naturally present anion (SO_4^{2-}) that is present in various water sources, with elevated concentrations often observed in arid and semi-arid regions where natural waters have high salt content. Water with elevated concentrations may have an unpleasant taste, and sulfate has the potential to lead to digestive problems. At 25°C, the Lambda 25 UV/VIS spectrophotometer developed by Perkin Elmer is utilized to identify the most stable form of sulfur in water.

Nitrate plays a crucial role as a plant nutrient but may have adverse effects on freshwater productivity, as highlighted by Wetzel in 1983. Different forms of nitrogen, such as NO, ammonia-N, Nitrate-N, and nitrite-N, are present in water. Nitrate in water comes from agriculture, and phenol-di-sulphonic acid procedures were used to analyze it. Perkin Elmer UV/VIS spectrophotometer model Lambda 25.

Chloride is present in varying concentrations across all water bodies, yet substantial amounts may be contributed by industrial waste effluents. The method for measuring chloride ions includes several steps. The titration results in the precipitation of chloride ions as white silver chloride.

Dissolved oxygen functions as a pollution indicator, providing insights into the quality of water bodies or wastewater. Its significance lies in supporting the health of aquatic life, as it is taken in by diverse marine organisms found in both the water column and sediments, including animals, plants, algae, and bacteria. Water temperature affects the amount of DO in lakes and rivers; colder water has more DO than warmer water, and saturated occurs at a higher rate in the winter. Maximum DO 14.6 mg/L at 0ºC, the Temperature increases the solubility of oxygen decreases means DO of the water bodies decreases like at 0ºC 14.6, at 5ºC-12.8, at 10ºC- 11.3, at 15ºC-10.2 and 25ºC (room temperature) 8.4 mg/L, this shows the decrease of DO as the increase of temperature. In the case of Dissolved Oxygen (DO) levels ranging between 7-8 mg/L, the water quality is considered good. A DO level of 4 mg/L is identified as a critical threshold below which fish cannot survive, indicating poor water quality. When DO drops below 4 mg/L, the water quality is deemed low, and if it falls below 2 mg/L, it is categorized as a septic condition or a zone of low DO, commonly referred to as septic DO.

Dissolved Oxygen (DO) levels were assessed on-site using a modified Winkler's method. To estimate DO, water samples were collected in 300mL glass Stopper BOD Bottles. In these bottles, 1 ml of MnSO4 and 1 mL of alkaline K.I. were added, initiating a process where the solution was shaken and left until a precipitate formed. Subsequently, 2 ml of H2SO4 was added and vigorously shaken to dissolve the residue. A few drops of starch indicator were incorporated into 50 mL of this solution. The solution underwent titration against Sodium Thiosulphate solution (0.025N) until the initial blue color transformed into colorlessness.

Biochemical Oxygen Demand (B.O.D.) is the quantity of oxygen needed by bacteria to fully decompose or break down organic matter, resulting in a stabilized end product. B.O.D. serves as a parameter for assessing the strength of loading. The B.O.D. test is also referred to as the Bio-assay test.

The COD (Chemical Oxygen Demand) method is employed to determine the oxygen equivalent of organic matter in a sample that has undergone oxidation with a potent oxidant. COD serves as a parameter for assessing pollution loading. Data was analyzed and significant differences were estimated with the help of SPSS software version -26.The data will be analyzed for the following parameters (Margolis 1982).

Microscopy has become an increasingly valuable investigative tool in helminth studies, enhancing our comprehension of intricate life cycles and host-parasite dynamics. Its application in parasitology extends beyond taxonomy, encompassing diverse aspects such as functional analysis, epidemiology, and disease control (Halton, 2004). Scanning electron microscopy (SEM), in particular, plays a crucial role in elucidating the tegumental characteristics of parasites. This proves significant in conducting tests related to drug efficacy and drug resistance (Roy *et al.,* 2012; Giri and Roy, 2014). Historically, identifying helminth parasites has been reliant on light microscopy, but advancements in technology have introduced SEM as an additional method for morphological identification (Roy and Tandon, 1993).

The scanning electron microscope (SEM) facilitates a thorough examination of the surface structure of helminths, encompassing regions such as the head and tail. This includes detailed observations of features like the buccal cavity, teeth, lips, cuticular ridges, ornamentations, spines, papillae, alae, vulva, bursa, and spicules in the case of nematodes. For cestodes, SEM allows for the scrutiny of the scolex, bothria, hook arrangement, and the nature of segmentation, while in trematodes, it aids in examining oral suckers, acetabulum, holdfast (metacercariae), haptors, sensillary organs, and more (Tandon and Roy, 2002; Halton, 2004).

As advancements in genetic and evolutionary studies continue, molecular methods within systematic have gained widespread adoption. Techniques like DNA-based PCR have proven invaluable in identifying parasites at the species level and distinguishing closely related helminth parasites (Chaudhary *et al.,* 2016). Notably, molecular markers such as nuclear ribosomal internal transcribed spacer 2 (rDNA-ITS2), 18S, and mitochondrial gene cytochrome c oxidase subunit 1 (mtCO1) have been extensively utilized to address taxonomic challenges and differentiate closely related parasitic species (Locke *et al.,* 2015). Due to their rapid evolutionary rates, these genes have become preferred loci for investigating taxonomy, population genetics, species identification, and phylogenetic relationships in various helminth parasite species, including trematodes, nematodes, and cestodes.

The integration of morphological and molecular studies is widely embraced for precise identification, distinguishing intra-specific variations and cryptic species (Sharma *et al.,* 2016a; Janssen *et al.,* 2017). The amplified sequences of *Parascaris sp., Tanqua sp., Senga sp. Polyonchobothrium sp., Lytocestus sp., Lucknowia sp., Clinostomum sp., Euclinostomum sp., Accacoelium sp., Morishitium sp., Lecithaster sp.and Pallisentis sp.* were recorded and assigned distinctive accession numbers upon submission to the NCBI GenBank. Initially, the submitted sequences were aligned with those obtained from GenBank, and the generated sequences, along with others from diverse species (representing each country or locality), essential for the current study, were retrieved for analysis and comparison.

In the alignment of the COI gene sequences, no gaps were found, but several mismatches were evident. Notably, only one mismatch was observed between the *Parascaris equorum* and the isolate under investigation. Among the twelve helminth parasites subjected to molecular characterization, it is revealed that for *Clinostomum sp.* comparison the multiple sequence alignment (MSA) and similarity index for the gene region CO1 and ITS2 showed that CO1 gives a better result. In India, the *Clinostomum* species of common occurrence is represented by *C. complanatum* (Shareef and Abidi, 2012; Rizvi *et al.*, 2012) which is distinctly different in morphological and molecular features from the one we studied. Molecular analysis of different parameters in the present study clearly indicates that the species under study belongs to *Clinostomum briene*.

The 18S sequence generated from *Senga sp.* was first checked in BLAST, NCBI and it matched with the taxa Senga where the result follows in accordance with the classification of Bothriocephalidae based on morphological traits (Kuchta *et al.,* 2008). The molecular analysis of the sequence query showed an interesting result. It was observed that the inter-specific variation among Senga is very less which means they are very similar to one another. The similarity index matrix showed that our *Senga sp.* is identical to *S. lucknowensis* of Vietnamese isolate (99.8%) and well as S. vishakapatnamensis (99.7%) with a variation between them in just 0.1. Moreover, in the phylogenetic tree, all the three species were clustered together in one clade with bpp value. The slight difference could be because of the geographical variation. It has clearly indicated from all the molecular information that the species of our study belongs to *Senga lucknowensis*.

The population dynamics of parasites in fish within wetland ecosystems can be influenced by various ecological factors. Understanding these dynamics is crucial for assessing the health of both the fish populations and the overall wetland ecosystem. Wetlands provide a unique environment with varying water quality, temperature, and nutrient levels. Changes in these environmental factors can influence the abundance and distribution of parasites. For example, certain parasites may thrive in warmer water conditions or specific pH ranges. The abundance and health of the fish host population play a significant role in parasite dynamics. Factors such as fish density, age structure, and reproductive patterns can affect the prevalence and intensity of parasitic infections.

The results of Multiple Linear Regression (MLR) by backward method in SPSS applied on the effect of hydrobiological parameters on *Senga* sp. are showed in Table 4.4.1. It is found that the water temperature, EC and phosphate parameters were significant at 5% level and hence these water quality parameters play a significant role in the infection prevalence of *Senga* sp. in Lucknow.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and maximum frequency (max. freq.) of p values of particular hydrobiological parameter, EC (last model p value-0.007 and max. freq.3) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Senga sp.* in *Channa punctata* at wetland site of Lucknow.

The water temperature and TDS parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Euclinostomum sp.* in Lucknow. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and maximum frequency of p values of particular hydrobiological parameter, TDS (last model p value-0.017 and max. freq.7) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Euclinostomum sp.* in *Channa punctata* at wetland site of Lucknow. The water temperature, EC and chloride parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Lecithaster sp*. in Lucknow. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max.freq. of p values of particular hydrobiological parameter, Water temperature (last model p value-0.000 and max. freq.8) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lecithaster sp.* in *Channa punctata* at wetland site of Lucknow.

The TDS, EC, phosphate, chloride, DO and BOD parameters were significant at 5% level showed (Table 4.4.4). Hence, these water quality parameters play a significant role in the infection prevalence of *Pallisentis* sp. in Lucknow.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, TDS (last model p value-0.001 and max. freq.6) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Pallisentis* sp.in *Channa punctata* at wetland site of Lucknow.The pH, EC, alkalinity and phosphate parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Parascaris* sp. in Sultanpur.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Alkalinity (last model p value-0.001 and max. freq.6) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Parascaris* sp. in*Channa punctata* at wetland site of Sultanpur.

The pH, sulphate and phosphate parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Tanqua sp.* in Sultanpur. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Phosphate (last model p value-0.033 and max. freq. 9) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Tanqua sp.* in *Channa punctata* at wetland site of Sultanpur. The water temperature, TDS, alkalinity, sulphate and phosphate parameters were significant at 5% level (Table 4.4.7). Hence, these water quality parameters play a significant role in the infection prevalence of *Polyonchobothrium sp.* parasite in Sultanpur.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Phosphate (last model p value-0.023 and max. freq. 5) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Polyonchobothrium sp.* in *Channa punctata* at wetland site of Sultanpur. The pH, TH, sulphate and BOD parameters were significant at 5% level (Table 4.4.8). Hence, these water quality parameters play a significant role in the infection prevalence of *Clinostomum sp.* in Sultanpur.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, BOD (last model p value-0.042 and max. freq. 5) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Clinostomum sp.* in *Channa punctata* at wetland site of Sultanpur.

The pH, alkalinity, TH, sulphate, phosphate, nitrate, chloride, DO, BOD and COD parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Euclinostomum sp.* in Sultanpur. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, pH and BOD (last model p value-0.003 and 0.004 respectively and max. freq. 4) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Euclinostomum sp.* in *Channa punctata* at wetland site of Sultanpur. The TH and sulphate parameters were significant at 5% level (Table 4.4.10). Hence, these water quality parameters play a significant role in the infection prevalence of *Pallisentis sp*. in Prayagraj. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, TH (last model p value-0.004 and max. freq. 9) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Pallisentis nandai Channa punctata* at wetland site of Prayagraj.

The water temperature, pH, alkalinity, phosphate and DO parameters were significant at 5% level (Table 4.4.11). Hence, these water quality parameters play a significant role in the infection prevalence of *Polyonchobothrium sp.*in Prayagraj. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and maximum frequency of p values of particular hydrobiological parameter, Water temperature and alkalinity (last model p value-0.001 and 0.004 respectively and max. freq. 6) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Polyonchobothrium sp.* in *Channa punctata* at wetland site of Prayagraj. The TDS, alkalinity, TH and chloride parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Accacoelium sp.* in Prayagraj.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, TH and chloride (last model p value-0.002 and 0.029 respectively and max. freq. 7) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Accacoelium sp.* in *Channa punctata* at wetland site of Prayagraj.

Only one parameter is significant at 5% level. Hence, DO play a significant role in the infection prevalence of *Morishitium sp.* in Prayagraj. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, DO (last model p value-0.014and max. freq. 2) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Morishitium sp.* in *Channa punctata* at wetland site of Prayagraj. The water temperatures, phosphate, DO and BOD parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Pallisentis sp.* in Prayagraj. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Water temperature and BOD (last model p value-0.000 and 0.001 respectivelyand max. freq. 9) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Pallisentis sp.* in *Channa punctata* at wetland site of Prayagraj.

The water temperature, TDS, nitrate, DO, BOD and COD parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Lytocestus sp.* in Lucknow. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Water temperature (last model p value-0.000 and max. freq. 8) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lytocestus sp.* in *Heteropneustes fossilis* at wetland site of Lucknow. The water temperature and phosphate parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Lucknowia sp.* in Lucknow.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Water temperature (last model p value-0.000 and max. freq. 10) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lucknowia fossilisi* in *Heteropneustes fossilis* at wetland site of Lucknow.

The water temperature, TDS, nitrate, DO, BOD and COD parameters were significant at 5% level. Hence, these water quality parameters play a significant role in the infection prevalence of *Lytocestus* sp. in Sultanpur.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, water temperature (last model p value-0.000, and max. freq. 6) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lytocestus* sp. in *Heteropneustes fossilis* at wetland site of Sultanpur. The water temperature, phosphate and nitrate parameters were significant at 5% level (Table 4.4.18) and hence these water quality parameters play a significant role in the infection prevalence of *Lucknowia sp.* in Sultanpur. Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, Water temperature (last model p value-0.000 and max. freq. 11) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lucknowia* sp. in *Heteropneustes fossilis* at wetland site of Sultanpur.

The pH, TDS, alkalinity, EC, TH, nitrate and DO parameters were significant at 5% level, these water quality parameters play a significant role in the infection prevalence of *Lytocestus* sp. in Prayagraj.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, pH, EC and nitrate (last model p value-0.000, 0.002 and 0.001 respectively and max. freq. 6) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lytocestus* sp. in *Heteropneustes fossilis* at wetland site of Prayagraj.

The EC, TH, sulphate and phosphate parameters were significant at 5% level, these water quality parameters play a significant role in the infection prevalence of *Lucknowia* sp. in Prayagraj.Among all the models of MLR, considering the significant p value ($p<0.05$) of last model and max. freq. of p values of particular hydrobiological parameter, TH (last model p value-0.001 and max. freq. 9) is the most effective hydrobiological parameter when compared to other water parameters on the infection prevalence of *Lucknowia sp.* in *Heteropneustes fossilis* at wetland site of Prayagraj.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Lucknow for two years. The PCA produced four and five axes that cumulatively explained 96.77% and 87.46% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only eleven parameters were retained for the year 2020-21 and ten for 2021-22, which were water temperature, TDS, EC, alkalinity, sulphate, phosphate, nitrate, chloride, DO, BOD, COD (2020-21) and temperature, pH, TH, sulphate, phosphate, nitrate, chloride, DO, BOD, COD (2021-22).

For the year 2020-21, the first axis had high loadings of TDS, sulphate, phosphate, chloride, BOD, the second axis had high loadings of water temperature, EC, Alkalinity, DO, the third axis had high loadings of nitrate, COD and the fourth axis had no high loadings. However, for the year 2021-22, the first axis had high loadings of water temperature, sulphate, the second axis had high loadings of TH, phosphate, DO, BOD, the third axis had high loadings of nitrate, COD, the fourth axis had high loadings of pH only and the fifth axis had high loadings of chloride only.

Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *C. punctata* at Lucknow site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Sultanpur for two years are presented in Table 4.4.22. The PCA produced four and third axes that cumulatively explained 97.21% and 86.10% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only eleven parameters were retained for the year 2020-21 and eleven for 2021-22, which were water temperature, EC, alkalinity, sulphate, phosphate, chloride, BOD, pH, TDS, TH and COD (2020-21) and alkalinity, TH, sulphate, phosphate, nitrate, DO, BOD, TDS, EC, chloride and COD (2021-22).

For the year 2020-21, the first axis had high loadings of water temperature, EC, alkalinity, sulphate, phosphate, chloride, BOD and the second axis had high loadings of water temperature, pH, TDS, TH and COD. However, for the year 2021-22, the first axis had high loadings of alkalinity, TH, sulphate, phosphate, nitrate, DO, BOD, TDS, EC, chloride, the second axis had no high loadings, and the third axis had high loadings of TDS, EC, chloride and COD. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *C. punctata* at Sultanpur site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Prayagraj for two years are presented in Table 4.4.23. The PCA produced four and three axes that cumulatively explained 94.65% and 79.22% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only twelve parameters were retained for the year 2020-21 and eight for 2021-22, which were water temperature, pH, EC, alkalinity, Total hardness (TH), sulphate, COD, TDS, phosphate, DO, chloride, nitrate (2020-21) and water temperature, Alkalinity, sulphate, phosphate, nitrate, DO, chloride (2021-22).

For the year 2020-21, the first axis had high loadings of Total hardness, alkalinity, water temperature, EC, pH, COD, sulphate, BOD, the second axis had high loadings of Chloride, TDS and phosphate, the third axis had high loadings of nitrate, DO and the fourth axis had high loadings of nitrate. However, for the year 2021-22, the first axis had high loadings of phosphate, water temperature, alkalinity and sulphate, the second axis had high loadings of DO, nitrate, the third axis had high loadings of chloride and EC only. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *C. punctata* at Prayagraj site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Lucknow for two years are presented in Table 4.4.24. The PCA produced third and fifth axes that cumulatively explained 92.74% and 87.20% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only eleven parameters were retained for the year 2020-21 and nine for 2021-22, which were water temperature, pH, TDS, TH, sulphate, phosphate, COD, alkalinity, DO, nitrate and BOD (2020-21) and water temperature, TH, phosphate, DO, COD, pH, EC, BOD, chloride (2021-22).

For the year 2020-21, the first axis had high loadings of water temperature, pH, TDS, TH, sulphate, phosphate, COD, the second axis had high loadings of Alkalinity, DO, the third axis had high loadings of nitrate, COD. However, for the year 2021-22, the first axis had high loadings of water temperature only, the second axis had high loadings of TH, phosphate, DO, COD, the third axis had high loadings of pH, EC, the fourth axis had high loadings of BOD only and the fifth axis had high loadings of chloride only. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *Heteropneustes fossilis* at Lucknow site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Sultanpur for two years are presented in Table 4.4.25. The PCA produced third and fifth axes that cumulatively explained 92.11% and 87.32% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only twelve parameters were retained for the year 2020-21 and nine for 2021-22, which were water temperature, pH, EC, alkalinity, DO, TDS, sulphate, phosphate, chloride, COD, nitrate and BOD (2020-21) and water temperature, TH, phosphate, DO, COD, pH, EC, BOD and chloride (2021-22).

For the year 2020-21, the first axis had high loadings of water temperature, pH, EC, alkalinity, DO, the second axis had high loadings of TDS, sulphate, phosphate, chloride, COD, the third axis had high loadings of nitrate and DO only. However, for the year 2021-22, the first axis had high loadings of water temperature, the second axis had high loadings of TH, phosphate, DO, COD, the third axis had high loadings of pH and EC, and the fourth and fifth axis had high loadings of BOD and chloride respectively. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *Heteropneustes fossilis* at Prayagraj site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on prevalence of four parasites of Prayagraj for two years are presented in Table 4.4.26. The PCA produced fourth and second axes that cumulatively explained 94.40% and 74.18% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only eleven parameters were retained for the year 2020-21 and eight for 2021-22, which were water temperature, pH, alkalinity, TH, TDS, phosphate, chloride, COD, BOD, nitrate and DO (2020-21) and water temperature, alkalinity, sulphate, phosphate, COD, TDS, EC,TH (2021-22).

For the year 2020-21, the first axis had high loadings of water temperature, pH, TDS, TH, sulphate, phosphate, COD, the second axis had high loadings of TDS, phosphate, chloride and COD, the third axis had high loadings BOD only, the fourth axis had high loadings of nitrate and DO. However, for the year 2021-22, the first axis had high loadings of water temperature, alkalinity, sulphate, phosphate, COD; the second axis had high loadings of TDS, EC and TH only. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *Heteropneustes fossilis* at Prayagraj site.

Principal component (PC) loadings from principal component analysis (PCA) of water quality parameters on the prevalence of four parasites of Lucknow for two years are presented in Table 1. The PCA produced four and five axes that cumulatively explained 96.77% and 87.46% of water quality variations in the year 2020-21 and 2021-2022 respectively. From thirteen water quality parameters evaluated, only eleven parameters were retained for the year 2020-21 and ten for 2021-22, which were water temperature, TDS, EC, alkalinity, sulphate, phosphate, nitrate, chloride, DO, BOD, COD (2020-21) and temperature, pH, TH, sulphate, phosphate, nitrate, chloride, DO, BOD, COD (2021-22).

For the year 2020-21, the first axis had high loadings of TDS, sulphate, phosphate, chloride, BOD, the second axis had high loadings of water temperature, EC, Alkalinity, DO, the third axis had high loadings of nitrate, COD and the fourth axis had no high loadings. However, for the year 2021-22, the first axis had high loadings of water temperature, sulphate, the second axis had high loadings of TH, phosphate, DO, BOD, the third axis had high loadings of nitrate, COD, the fourth axis had high loadings of pH only and the fifth axis had high loadings of chloride only. Therefore, high loadings of these water quality parameters are the main reason for the prevalence of infection in *C. punctata* at Lucknow site.

Wetland habitat is a harbinger of parasite diversity in fishes and gives an idea of infection prevalence according to the physicochemical parameters and other abiotic factors. In the present study, the diversity of fishes in the wetland ecosystem was established. These fish were collected and analyzed during the time of six months (from July 2021 to December 2022). They were examined according to the body color of fishes, total and standard length of the body, head and snout length, body (depth) height, number of barbels, and fin and fin rays counted for identification purposes. In this survey, it was reported that murrels and catfishes from the wetland ecosystem in three districts of Uttar Pradesh. The bottom-dwelling fishes show the highest prevalence of helminth parasites, especially the cestodes, followed by trematodes and acanthocephalans.

Diversity indices are quantitative measurements used to analyze a biological community's diversity, taking into account aspects such as species richness and evenness. These indices provide a numerical representation of the diversity and distribution of different species in a given area. There are several diversity indices, each with their strengths and limitations such as Species Richness (S), Shannon-Wiener Diversity Index (H' or Shannon Index), Simpson's Diversity Index (D) and

Evenness (E). It complements richness and diversity indices by providing information on how evenly individuals are distributed among species.

In the present study, the ten parasite population dynamics from the fish *Channa punctatus* obtained from the three Wetland areas including Lucknow, Sultanpur, and Prayagraj revealed higher diversity indices and species richness and dominancy when compared to the population dynamics of the two parasites obtained from the other fish *Heteroptneustes fossilis*. Particularly, the Simpson (1-D) index for parasites obtained from *C. puntatus* of the Wetland near Lucknow was higher (0.69) when compared to Prayagraj (0.68) and Sultanpur (0.62) in comparison to the and districts during the experimental period. It is important to note that a higher D is more diverse. The Shannon_H indices reveal a higher H value for Wetland near Sultanpur (1.32) in comparison to the Lucknow (1.27) and Prayagraj (1.25) districts. The Evenness indices J are higher for Wetlands near Lucknow (0.89) than Prayagraj (0.87) and Sultanpur (0.63). However, the Evenness_e^H/S indices show a higher J value for Lucknow (0.8936) than Prayagraj (0.87) and Sultanpur (0.63).

While considering the fish *H. fossilis*, during the same experimental period, the Simpson (1-D) index for parasites obtained from the Wetland near Lucknow was slightly higher (0.50) and Prayagraj (0.50) was approximately similar in comparison to the Sultanpur (0.49) which give the impression of a slight decline in diversity of Sultanpur wetland. The Shannon_H indices reveal a higher H value for Wetlands near Sultanpur (0.69) and Prayagraj (0.69) in comparison to the Lucknow (0.68) district. There was a close similarity between Sultanpur and Prayagraj H index. The Evenness indices J reveal a maximum evenness for Wetlands near Prayagraj (1) when compared to Sultanpur (0.99) and Lucknow (0.99). It can be established by all these diversity indices or these three measures, that the Lucknow wetlands are more diverse, even though the other two wetlands, i.e., Sultanpur and Prayagraj have identical species richness. This may be attributed to the environmental, climatic, and geographical specifications such as land or soil type and water flow system, and physio-chemical properties of the wetland sampling sites. As Lucknow is situated in the middle of the Indus-Gangetic Plain region of Uttar Pradesh, under the foothills of the Himalayan belt, lying just near the Terrai regions, its wetlands provide a cradle of a diverse group of organisms and parasites are no different from this concept.

In the present study, the identification and characterization of isolated parasites from two different host fishes of three wetland sites across Uttar Pradesh was conducted. Among the helminth parasites studied, a total of 12 species (Table 1) were identified and isolated of which two nematodes were reported from *Channa punctata,* out of which *Parascaris equorum* also reported in hosts of the *Equus* species (Han *et al.,* 2022)..

Tanqua tiara was first identified in the Saraiya Wetland of Sultanpur District, Uttar Pradesh, is classified as a nematode species, as detailed in the findings by (Bogale *et al.,* 2020). The characteristics of its shape and body structure closely resemble those of the previously observed *Tanqua tiara* found in *Channa striata* in Indonesia, as recorded (Murwantoko and Hayati, 2022). The current worm exhibits a close resemblance to all the recognized species within the *Senga* genus (Dollfus, 1934). Notably, *S. lucknowensis* was reported (Johri, 1956) from *Mastacembelus armatus* in India. Moreover, Fernando and Furtado in 1963 reported *S. malayana* from *Channa striata*. *Senga* genus with *Polyonchobothriumsp*, suggesting differences for the species (Tadros, 1968).

Lucknowia fossilisi exhibits unique characteristics distinguishing it from other *Lucknowia* species, including variations in body and scolex shape, as well as the length of the previtellaria. In contrast to the majority of caryophyllideans, *L. fossilis* tapeworms were seldom observed in the anterior section of the intestine in stinging catfish. Most tapeworms, in contrast, were commonly found in the middle and posterior thirds of the host intestine, as noted by (Ash *et al.,* 2011).

Lytocestus heteropneustii, is identified as a novel species of Caryophyllaeid Cestode (Tandon *et al.,* 2005). This cestode species is classified within the Lytocestidae family, according to the classification proposed by (Wardle and McLeod, 1952). Our investigation exhibits closer similarities with previously documented species of *Lytocestus heteropneustii*, as well as distinct variations from other species.

Clinostomum brieni were found in in *Channa punctata* fish from species that are widespread parasites found in avian, mollusc, amphibian, and fish hosts (Rosser *et al.,* 2017). It differs from the previously documented *Clinostomum* species in India, namely *Clinostomum complanatum*. Subsequently, the parasite species identified in our research work constitutes the initial report of its kind in India. Although there are no documented instances of *Clinostomum* infection in humans in India, the occurrence of such infections is widespread in several other Asian countries, as reported (Hara et al. in 2014).

Fish-borne trematode infections, especially those transmitted from animals to humans, have had adverse impacts on human health, with approximately 40 million cases reported, mainly in Asian nations (WHO, 2002). The increased incidence of parasite prevalence is linked to cultural customs like eating raw or undercooked fish. It is noteworthy that 19 cases have been reported in Japan alone (Hara *et al.,* 2014). Infections with *Clinostomum* have been linked to cases of pharyngitis and inflammation of the lacrimal duct in Thailand and Korea (Park *et al.,* 2009). Apart from its impact on human health, widespread infections with *Clinostomum* metacercariae, also referred to as the "yellow grub," can cause significant harm to freshwater fishes, which serve as intermediate hosts (Shareef and Abidi, 2012).

In the present study, *E. heterostomum* has been previously reported (Purivirojkul and Sumontha, 2013; Athokpam *et al.,* 2014; and Senapin *et al.,* 2014). However, a comparison of morphological data, specifically the description provided by (Purivirojkul and Sumontha, 2013), reveals that the specimens from the eastern isolates differ more significantly from the central morphometric variation of *E. heterostomum* than our specimens. Consequently, it is more plausible that our specimens correspond to *E. heterostomum* rather than the ones reported by (Purivirojkul and Sumontha, 2013; Athokpam *et al.,* 2014).

The reported *Accocoelium contortum* from snakehead fish wasmore closely related to previously reported *Accacoelium contortum* from the oropharyngeal chamber, including the gills, and oral cavity. The unique morphology of the tegumental papillae on the anterior part of the body is thoroughly examined, and the circum-oral papillae are reported for the first time. Moreover, the length of the peduncle of the ventral sucker in *A. contortum* seems variable and cannot be strictly characterized as either "short-penduncled" or "sessile," contrary to previous descriptions (Bray *et al.,* 1977; Gibson *et al.,* 1979).

The dimensions and morphology of the species described (Visconti *et al.,* 1988) closely resemble those of the specimens examined in this study. In Europe, instances of *Morishitium polonicum* infections in Turdidae birds have been reported in Poland (Sulgostowska and Czaplinska, 1987), and Spain (Jaume-Ramis and Pinya, 2018). In contrast to earlier reports, a singular species, *Lecithaster salmonis* (Yamaguti, 1934), and which was previously reported in the Pacific region. Morphologically, significant characteristics are found in *L. salmonis*, *Lecithaster confusus* (Odhner, 1905).

Adult and larval stages of the *Pallisentis sp.* were reported in *Channa punctata* and also reported in the same fish (Gautum *et al.,* 2017, Banerjee *et al.*, 2017).Our specimens were unequivocally identified as *Pallisentis. nandai,* as at first described (Sarkar, 1953). However, the characteristic feature was observed, particularly in the dimensions of proboscis hooks, receptacle, and lemnisci, when compared to the previously reported species (Amin *et al.,* 2000), was similar to examined in this study.

The current investigation reveals that among the helminth parasites transmitted through fish, *Clinostomum metacercariae* and *Parascaris sp.* have been identified as significant zoonotic trematode and nematode, respectively, prevalent in Uttar Pradesh. The study records the existence of twelve species of helminth parasites, and notably, three of them (*Tanqua tiaria, Morishitium polonicum*, and *Lecithaster salmoni*) are reported in Uttar Pradesh for the first time.

The scanning electron microscopy of helminths worms obtained from wetland fish revealed the presence of intact dome-shaped sensory papillae around the oral sucker and the tegumental spines spread over the body surface. The oral sucker was subterminal and somewhat smaller than the ventral sucker and the ventral sucker were located at the anterior one third level of body, having lots of dome-shaped sensory papillae on its rim. Tegumental spines were not seen, but dome-shaped sensory papillae were sparsely distributed on the whole ventral tegument. Numerous stratified spinous processes with a single tip were distributed on the whole dorsal tegumental surface.

Scanning electron microscopic observation on Lytocestidae revealed considerable variation in the structure, dimensions, and density of these surface extensions. In adult *Senga lucknowensis*, scanning of the whole-body surface showed that the tegument is scaly where each segment has sub-scales arranged transversely and directed posteriorly. The differences and diversity in the structure or pattern of microtriches are indicative of diversity in function like absorption,define mechanism, attachment or locomotion (Kearn, 1988; Roy and Tandon, 2003). Surface fine topographical observation on *Clinostomum brieni* revealed the presence of an excretory pore surrounded by well-developed spines, thin thread-like structure bisecting the ventral sucker internally, antero-lateral spination of the tegument and presence of genital pore in the ventral surface of the body which were found to be absent and/or different in other species such as *C. cuteneum* and *C. complantum* (Abidi *et al.,* 1988; Gustinelli *et al.,* 2010; Ngamniyom *et al.,* 2012).

Out of the twelve species of helminth parasites, ten species of them were found in *Channa punctata* and two species were found in *Heteropneustes fossilis*. A parasitological survey of the mentioned fishes revealed the presence of twelve different types of helminth parasites. This includes two different species of nematodes (*Parascaris equorum* and *Tanqua tiara*), four species of cestodes (*Lytocestus heteropneustii, Lucknowia fossilisi, Senga lucknowensis,* and *Polyonchobothrium sp.*), four species of trematodes (*Clinostomum brieni, Euclinostomum heterostomum, Lecithaster salmoni,* and *Morishitium polonicum*) and one species of acanthocephalan (*Pallisentis nandai)*.

In the study of helminth, microscopy is being used more and more as a potent investigative tool. This has improved our understanding of the intricate life cycles and host-parasite connections. Its application in parasitology extends beyond taxonomy to include multidisciplinary research on parasites, including epidemiology, disease control, and functional studies (Halton, 2004). Accordingly, drug efficacy and drug resistance tests have shown the value of using scanning electron microscopy to comprehend the tegumental character of parasites (Roy *et al.,* 2012; Giri and Roy, 2014).Helminth parasite identification is typically done using a light microscope, however as technology has advanced, SEM has been employed as a complimentary tool for morphological identification (Roy and Tandon, 1993). Recently, molecular technologies combined with various microscopical methods have been employed and widely recognized for determining the true taxonomic classification of parasites (Ghatani *et al.,* 2014; Robles *et al.,* 2014). Thus, for the structural characterization of helminth parasites, SEM analysis remains one of the most important methods in helminthology.

Nematodes, among helminths, show variation in their morphology at the optical level; in addition, scanning electron microscopy (SEM) studies have demonstrated an enhanced and wide range of morphological variations, effectively removing observational restrictions. The surface electron microscope (SEM) facilitates a thorough understanding of the surface architecture of helminths, which includes the head and tail regions such as the buccal cavity, teeth, lips, cuticular ridges or ornamentations, spines papillae, alae, vulva, bursa, and spicules in the case of nematodes; scolex, bothria, hooks arrangement, the nature of segmentation in cestodes and oral suckers, acetabulum, holdfast (metacercariae), haptors, sensillary organs, etc. in trematodes (Tandon and Roy, 2002; Halton, 2004).

Numerous publications have demonstrated the use of SEM as a supplemental tool in the taxonomy of helminths, including fish parasites (In Camallanidae: Santos and Moravec, 2009; Chaudhary *et al.,* 2017). To complement the light microscopic study and determine the particular identity of the helminth parasites collected from edible fishes of Manipur, India, the present study used SEM to characterize the parasites. To demonstrate the differences amongst parasites in the same species, tegmental comparisons were done.

The evolutionary history of the isolated parasites was deduced through the Neighbor-Joining method (Saitou and Nei, 1987), and the optimal tree presents a broad range of relatedness and also relatedness. Bootstrap testing (1000 replicates) indicates the percentage of trees where associated taxa clustered together along branches (Eisenstein, 1985). Evolutionary distances, measured in base substitutions per site, were computed using the Maximum Composite Likelihood method (Tamura, 2004). The analysis comprised 11 nucleotide sequences, with codon positions covering 1st+2nd+3rd+Noncoding, and ambiguous positions were eliminated using pairwise deletion. The final dataset encompassed 1867 positions, and the analyses were executed in MEGA11 (Tamura, 2021).

The 18S sequence derived from *Senga sp.* was initially verified using BLAST on NCBI, aligning with the *Senga* taxa. The outcome aligns with the classification of Bothriocephalidae, as established through morphological traits (Kuchta *et al.,* 2008). The sequence examination revealed a crucial insight: *Senga sp.* demonstrates restricted inter-specific variation, emphasizing a substantial degree of similarity among them. The molecular data unequivocally establishes that the species under investigation is identified as *Senga lucknowensis*. To conduct sequence analysis and compare the 18S region of *Pallisentis sp.*, various genera within the Eoacanthocephala taxon were selected from BLAST results. This choice was made due to the limited availability of Pallisentis genus members in GenBank specifically for this gene. Consequently, sequences obtained from GenBank, in addition to the one generated in this study, were included for comprehensive analysis and phylogenetic inference the current research highlights the valuable application of molecular tools for in particular characterizing and identifying medically and veterinary important parasites.

The host-wise study of prevalence, abundance, and mean intensity was observed highest in pre-monsoon in the case of *C. punctata*, and in *H. fossilis*, prevalence was highest in monsoon, and abundance and mean intensity was highest in post-monsoon. Climatic factors like temperature, humidity, and rainfall showed a significant correlation with the prevalence, abundance, and mean intensity of helminth infections. The results of the present study will help in developing comprehensive disease management strategies to control fish-borne zoonoses and parasitic disease management in fish. Various methods have been suggested for disease management of fishes which include pond drying, snail removal from the water bodies as they are the intermediate hosts of many cercariae, giving treatments, etc. It is also clear from the current study that *Clinostomum* metacercariae, a fish-borne helminth parasite, maybe a zoonotic trematode that inhabits the wetlands of Uttar Pradesh, India.

In the wetland fish that were infected with *Pallisentis nandai* infection prevalence (%) with an increase in temperature, with the highest prevalence of 47.27 % at 22 ^{o}C during November 2021 and 52.73 % at 32^{o}C during May 2022. *Pallisentis sp.* showed the highest prevalence of 44.55 % at pH 8.84 of the wetland water during April 2022, while the prevalence decreased (21%) with a decrease in TDS at 480ppm during August and October 2021. There was a steep rise in infection prevalence of 44% with an increase in Electrical Conductivity (EC) at 370 mhos/cm during March 2021, while increased alkalinity of wetland water revealed a decreased infection prevalence of 35% during July 2021 and December 2021. Other physio-chemical parameters like sulphur, phosphate, chloride, BOD, and COD exhibited lower infection prevalence with their respective decrease. However, increased TH, Nitrate, and DO show decreased infection prevalence.

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