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Infestation and histopathology of *Clinostomum* sp. (Digenea: Clinostomidae) in snakeskin fish (*Trichopodus pectoralis* Regan, 1910) in Tempe Lake, South Sulawesi, Indonesia

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Abstract. *Ika, Anshary H, Sriwulan. 2024. Infestation and histopathology of* Clinostomum *sp. (Digenea: Clinostomidae) in snakeskin fish (*Trichopodus pectoralis *Regan, 1910) in Tempe Lake, South Sulawesi, Indonesia. Biodiversitas 25: 3263-3272.* Fish diseases caused by parasites seriously threaten the aquaculture industry and wild fish populations in natural water. This research aims to identify and characterize the digenetic trematode *Clinostomum* sp. based on morphology and molecular characteristics and analyze the level of infestation and histopathological changes of the endoparasite infestation in snakeskin fish across three lakes (Tempe Lake, Lampulung and Latamperu Lakes) in South Sulawesi, Indonesia. A total of 300 snakeskin fish were collected from a survey conducted from July 2023 to January 2024 for parasite examination. The morphological identification of parasites, a meticulous process, was carried out to distinguish digenean species that infect snakeskin fish populations, ensuring the accuracy of our findings. The results showed that *Clinostomum* sp., was found infesting snakeskin fish across all three lakes within Wajo District, and the identity of the parasite was further confirmed with Polymerase Chain Reaction (PCR) and sequencing on the Internal Transcribed Spacer (ITS)-5.8S region. The prevalence and mean intensity of the parasite infestation varied among the three locations surveyed, though they are not different statistically; Lampulung Lake showed a prevalence of 34%, Latamperu Lake at 28%, and Tempe Lake at 21%. The mean intensity of infestation in Lampulung Lake at 10±10.70, Latamperu Lake at 09±12.82, and Tempe Lake at 06±6.22. Histopathological analysis showed changes within the intestinal tissues of snakeskin fish associated with the infestation of *Clinostomum* sp., including goblet cells, hemorrhage, and cellular infiltration. This study demonstrated a consistent presence of *Clinostomum* sp. across all lakes, with relatively high prevalence and mean intensity of infestation. The histopathological analysis showed mild tissue alterations in infested fish. However, the high prevalence of infestation of the zoonotic digenetic trematode raises concerns about the public health impacts for local communities who consume raw or undercooked fish.

Keywords: Digenetic trematode, endoparasite, Internal Transcribed Spacer, ITS, morphology, Tempe Lake

INTRODUCTION

The snakeskin fish (*Trichopodus pectoralis* Regan, 1910) is economically important as a source of protein and has high economic value (Gustiano et al. 2022). Meanwhile, various fish species inhabit Tempe Lake; it is influenced by agricultural land, residential areas, and some rivers flowing into the lakes. Tempe Lake is surrounded by lakes such as Lampulung Lake and Latamperu Lake. However, fish can be hosted by various species of parasites that are not only pathogenic to fish but can also incidentally infect humans after consuming infested or undercooked fish, leading to a disease called pharyngitis and laryngitis (Tavares-Dias et al. 2023; Ermakova et al. 2024). The metacercaria stage in fish can inhibit growth or change the appearance of fish, thereby reducing market value (Reshid et al. 2015) or changing the behavior of infested fish, leading to easy capture by predators (Nicola et al. 2020).

Clinostomum sp. is a trematode endoparasite that mainly infests fish, lives in freshwater as well as estuaries throughout the world, and has an indirect life cycle; the adult stage is generally found in the buccal cavity and esophagus of fish-eating birds as definitive hosts (Caffara et al. 2014; Reshid et al. 2015). Snails are the first intermediate hosts that store sporocysts, while fish, reptiles, and amphibians are the second intermediate hosts storing metacercaria stages (Tavares-Dias et al. 2023). In general, anthropogenic activities may be the source of organic pollution in lake ecosystems, significantly impacting the existence of parasites due to changes in the populations of aquatic organisms that serve as first or second intermediate hosts. For instance, parasite *Clinostomum complanatum* (Rudolphi, 1814) Braun, 1899 thrives in environments with shallow water depths and multiple species of mollusks, fish, and birds to complete the life cycle (Menconi et al. 2020). This study also shows that water quality plays a significant role in the life cycle of parasites. Seasonal fluctuations also disrupt the life cycle of parasites such as *Clinostomum* sp. (Aghlmandi et al. 2018). Moreover, persistent deterioration of water quality due to high levels of organic matter and pesticides entering the lake can lead to a decline in water quality, causing fish and other benthic organisms to disappear (Reid et al. 2019).

Fish pathogens, which often infest fish and seriously impact aquaculture facilities and wild fish populations, are a severe concern. Specifically, parasites have a specific niche within the fish's body, with ectoparasites residing on the surface, while endoparasites inhabit the internal organs (Anshary 2016). The abundance and presence of parasites are critical indicators of environmental health, as they are susceptible to changes in water quality and habitat conditions (Sures et al. 2017). This sensitivity profoundly impacts the parasite itself and the entire aquatic ecosystem, including the habitat of various organisms (Silva et al. 2022).

Parasite infestations result in histological changes in the fish tissue. They can cause damage to muscle tissue and internal organs, slow growth and decreased reproductive efficiency, bleeding on the body surface, and mass mortality (Bera et al. 2021). Several recent studies show that identifying digenetic trematodes based only on morphological approaches is prone to identification errors, so molecular data must also support the identification of digenetic trematodes (Caffara et al. 2011; Won et al. 2020). In the South Sulawesi region, there is a lack of information on the species of fish parasites that inhabit natural water sources, particularly in Tempe Lake and the surrounding lakes. To address the gap, this study aimed to determine the identity of *Clinostomum* sp. based on morphology and molecular characteristics, level of infestation, and histopathological changes of the endoparasites that infest snakeskin fish (*T. pectoralis*) across three lakes located in South Sulawesi, Indonesia. Understanding the morphology, level of infestation, and histopathological changes of *Clinostomum* sp. in snakeskin fish is crucial to developing effective management strategies to mitigate the impact of parasites on fish populations and the ecosystem. This study contributes to the existing knowledge of fish parasites in the region. The current results emphasize the importance of monitoring and managing aquatic ecosystems to maintain the health and sustainability of fish populations and the impact of parasites on public health.

MATERIALS AND METHODS

Ethical approval

This study, with protocol number [254/UN4.14.1/TP. 01.02/2024], was approved by the Ethics Committee on Animal Use of the Faculty of Public Health, Universitas Hasanuddin, Makassar, Indonesia.

Fish sampling

Fish sampling was conducted monthly from July 2023 to January 2024, and then sample analyses were performed at the Laboratory of Parasites and Fish Diseases, Universityas Hasanuddin. The survey method was used with sampling at three lake points: Tempe Lake (4°6'39,46212"S 120°0'55,782"E), Lampulung Lake (4°8'48,32988"S 120°3'40.6141"E) and Latamperu Lake (4°6'59.82012"S 120°7'34.54212"E). A map of the study location is presented in Figures 1 and 2. Characteristics of location 1 (Tempe Lake) include proximity to a river and agricultural land, a depth of 3-4 m, dense vegetation characterized by water hyacinth and water spinach at the sampling point, and the presence of intermediate hosts such as fish-eating birds, snails, and fish. Characteristics of location 2 (Lampulung Lake) include proximity to residential areas, a water depth of about 1 m, dense vegetation with water hyacinth and lotus plants dominated at the sampling point, and muddy soil bottom. Intermediate hosts such as fish-eating birds, snails, and fish occupied the location. The characteristics of location 3 (Latamperu Lake) include proximity to agricultural land (rice fields), a water depth of about 2-3 m, dense vegetation characterized by shy princess flower plants at the point sampling, and intermediate hosts such as fish-eating birds, snails, and fish. Therefore, the environmental conditions of the three lakes are favorable for the completion of the digenetic trematode life cycle.

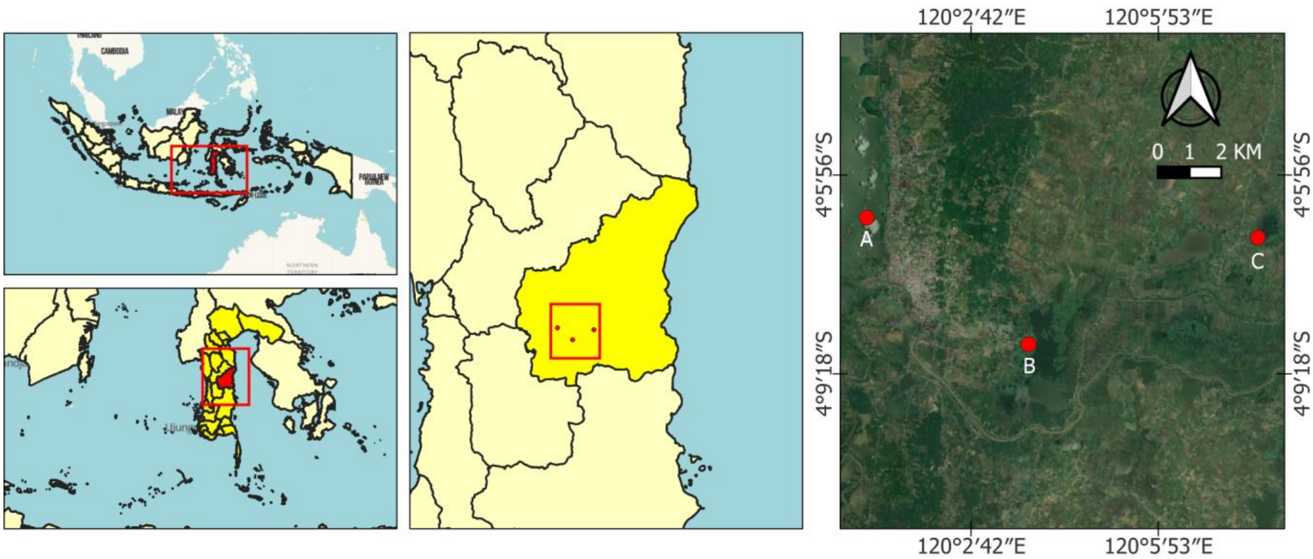

Figure 1. Map of the sampling location in Wajo District, South Sulawesi, Indonesia: A. Tempe Lake; B. Lampulung Lake; C. Latamperu Lake

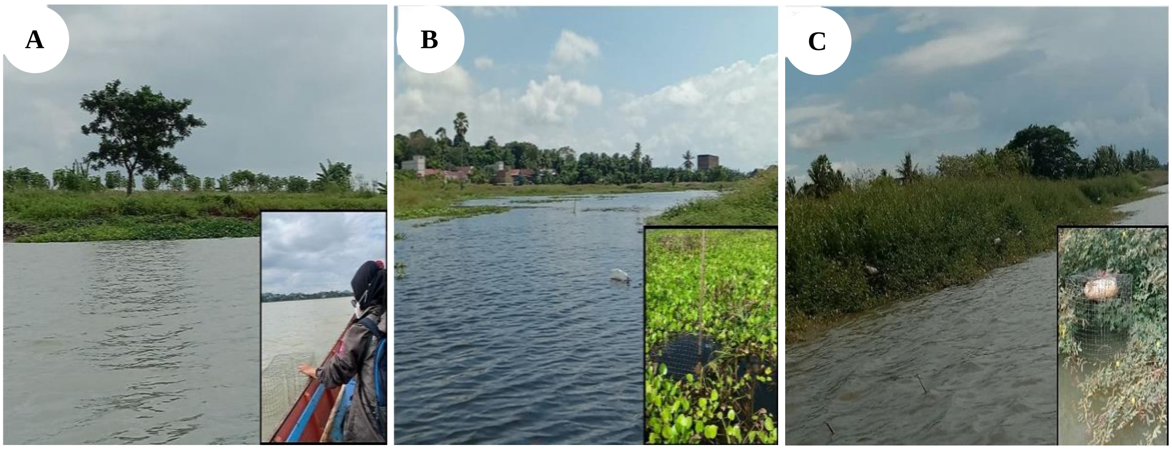

Figure 2. Sampling sites of snakefish in Wajo District, South Sulawesi, Indonesia. A. Tempe Lake, B. Lampulung Lake, and C. Latamperu Lake

Samples were obtained from fishermen who catch fish using a trap (local language; Jebba). Trap is a rectangular wire netting with has entrances that guide fish into a confined space. Fishermen normally place traps for certain period of time in areas where fish are congregate in around aquatic vegetation or in shallower waters where fish are forage. We selected fishermen based on their extensive experience and actively engaged in fishing activities during the study period, familiar with the lake's seasonal variations, fish behavior, and optimal fishing methods. Fish were packed using plastic bags, aerated, placed in a cool box, and transported alive to a laboratory. Upon arrival, the fish samples were acclimatized in a bucket and examined for digenetic trematode and other endoparasites. Fish were dissected and gently pull out the digestive tract, liver, and other internal organs and examined for presence of *Clinostomum* sp. and other endoparasite helminths. Parasites found were pick out from the organs using fine forceps and place them in a petri dish containing freshwater. Then, the trematodes were cleaned and preserved in 70% alcohol for further examination. Parasites were individually placed in a bottle for further identification at the Laboratory of Fish Parasite and Disease. The water quality measured was pH using pH meter, temperature with thermometer, and Dissolved Oxygen (DO) with titration method in the laboratory. Water pH and temperature were measured in situ, while DO samples were prepared in DO bottle and then fixed it. DO was measured by titration using Winkler method in the laboratory. The measurements were carried out when fish sampling was undertaken.

Parasite examination and measurement

A total of 300 snakeskin fish were collected from three distinct lakes located in Wajo District, South Sulawesi, with 100 fish obtained from each lake with the fish length ranged from 10-16 cm in Tempe Lake, 11-17 cm in Lampulung Lake and 11-16 cm in Latamperu Lake (Table 2). Samples were anesthetized either using a eugenol solution or by piercing the nerves with a needle and then placed on a tray. Subsequently, the total length and weight were measured.

The presence of endoparasites was observed on the liver and intestines. The fish was dissected, stomach was cut, then the intestines and liver were taken out and separated using tweezers and surgical scissors. The organs were placed on a glass slide, to which several drops of tap water were added. Followed this, the parasites were observed under a microscope. Parasites were isolated, placed on a petri dish, and cleaned from debris, followed by either direct observation under stereo- (Olympus SZ61) and compound microscope (Olympus CX43) or staining using carmine dye solution and then observed under a compound microscope with 4×10 magnification. Furthermore, parasites were morphologically identified, and some measurement of the parasite characteristics was used for further identification. Morphological characteristics referred to Bera et al. (2021) and digital image measurements for the specimens were obtained with the Dino capture camera 2.0 (Dino-Eye) installed in a computer device.

DNA extraction, polymerase chain reaction, and sequencing

Some parasites were stored in a 1.5 mL microfuge tube containing 70% alcohol for molecular confirmation of the parasite identity. Parasite DNA was individually extracted using QiaAmp Mini Kit (Qiagen) following the protocols of the kit and then stored at -20°C before further analyses. Polymerase Chain Reaction (PCR) was performed to amplify the parasite DNA on ITS-5.8S region using a pair of primer forward BD1 (5'-GTCGTAACAAGGTTTCCGTA-3') and reverse BD2 (5'-TATGCTAAATTCGGGT-3'). The total volume for the PCR reaction was 20 uL, with a master mix concentration of 0.3 µm for each primer and 1x for KOD enzyme (Toyobo); then, nuclease-free water was added to reach the reaction volume. PCR conditions were set for pre-denaturation at 95°C 3 min, and 35 cycles of (denaturation at 94°C 1 min, annealing at 58°C 1 min, extension at 72°C 2 min), and final extension at 72°C 5 min. Electrophoresis was performed on the PCR product on 1.5% agarose gel, stained with GelRed (Biotium), and visualized on UV Transilluminator. PCR product showed a clear band was prepared and sent to a private company named 1st BASE Malaysia for sequencing through PT. Genetika Science Indonesia. Sequence results were analyzed and compared with the chromatogram of the sequence. Alignment was performed on BioEdit and the phylogenetic tree using the maximum likelihood model was constructed using the software MEGA X (Kumar et al. 2018).

Histopathology preparations

The intestines of snakeskin fish infested with parasites were cut and put into a bottle sample containing 10% buffered formalin to preserve the tissue for 48 h. Then samples were stored in 70% ethanol before being pursued to histological processing procedures. The samples were placed in tissue cassettes and dehydrated in alcohol series of 70, 80, 90, and 95% for 24 h each, and then placed in 100% alcohol I, II, and III for 24 h to achieve complete dehydration. Subsequently, the samples were placed in xylol I for 1 h, xylol II for 30 min, and xylol III for 30 min. The samples were soaked in a paraffin block and the position of the samples was adjusted. The cutting process used a microtome with a thickness of 5 µm.

The H&E (Hematoxylin-Eosin) staining solution was used for staining the samples after the deparaffinization process by soaking the samples in a solution of xylol I and xylol II for 30 min each. This was followed by soaking the sample in 100, 95, 85, and 70% alcohol for 30 min each. The sample was soaked in distilled water and then put in a hematoxylin staining solution for 10 min, dripped with distilled water, and then soaked again in eosin solution for 20 min. Subsequently, the sample was placed into graded alcohol of 70, 80, 90, 95, and 100% for 1 h each and xylol solution for 30 min. The sample was mounted in Canada Balsam and covered with a coverslip. The observation was then carried out under a compound microscope.

Data analysis

The findings of our study, which include the presentation of parasite morphology and measurements, comparison with other described *Clinostomum* sp., analysis of water quality parameters, and descriptive analysis of histopathological changes in organs infested by parasites, are of significant importance. The statistical analysis of parasite infestation levels using non-parametric tests in the SPSS 25.0 statistical package adds further weight to our findings. Parasitological terms used followed by Bush et al. (1997): prevalence is the percentage of fish infected with certain parasites, mean intensity is the average number of certain parasites found in infected fish, while mean abundance is the average number of certain parasites found in fish examined (infested or not infested). Additionally, a Spearman rank correlation coefficient was carried out to determine the relationship between the parasite mean abundance and host body condition factors, fish length and weight, which further underscores the significance of our study.

RESULTS AND DISCUSSION

Clinical signs of infested fish

The clinical symptoms of snakeskin fish infested with the parasite *Clinostomum* sp. were pale color, bulging belly, and inactive (weak) when caught (Figure 3.B). Healthy fish are characterized by blackish green and bright color, normal body shape with no visible wounds, condition of the fins intact as well as active when swimming or caught (Figure 3.A). The *Clinostomum* sp. were isolated from infested tissue (Figure 3.C), cleaned and preserved in 70% alcohol (Figure 3.D), and stained with carmine staining solution (Figure 3.E).

Morphology and molecular identification of parasites

Based on morphological observations, parasites infesting snakeskin fish at Tempe Lake, Lampulung Lake, and Latamperu Lake were identified as *Clinostomum* sp., a metacercarial stage of digenetic trematode found attached to the outer side of the intestinal organs of fish samples (Figures 4.A-C).

Clinostomum sp. metacercaria was found in the intestinal organs of fish surveyed in the three locations. The characteristics of *Clinostomum* sp. are that it has a worm-like body shape, bluntly rounded at the anterior and posterior ends, as well as a yellowish body color. *Clinostomum* sp. metacercaria was measured with 5.08-5.26 mm length and 1.46-1.75 mm width. It has an oral sucker and a ventral sucker, with spines on the anterior body. The middle part of the body has a uterus that extends between the ventral sucker and the testes. In the posterior part, there are two testes (anterior and posterior testis), the cirrus sac, the ovary between the two testes, and an intestinal caecum (intestine).

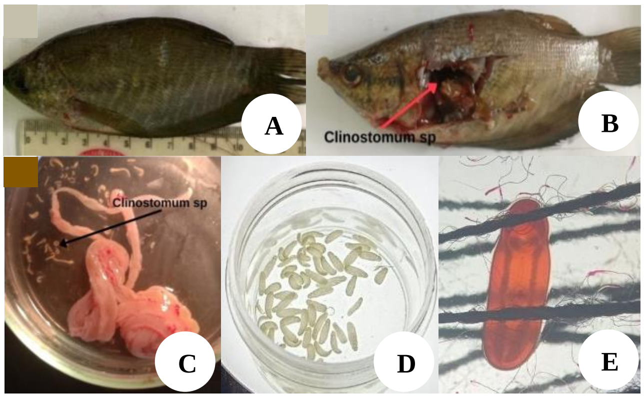

Figure 3. A. Healthy snakeskin fish; B. Snakeskin fish infested with *Clinostomum* sp.; C. *Clinostomum* sp. detached from the intestine; D. *Clinostomum* sp. preserved in 70% alcohol; E. *Clinostomum* sp. stained in carmine solution

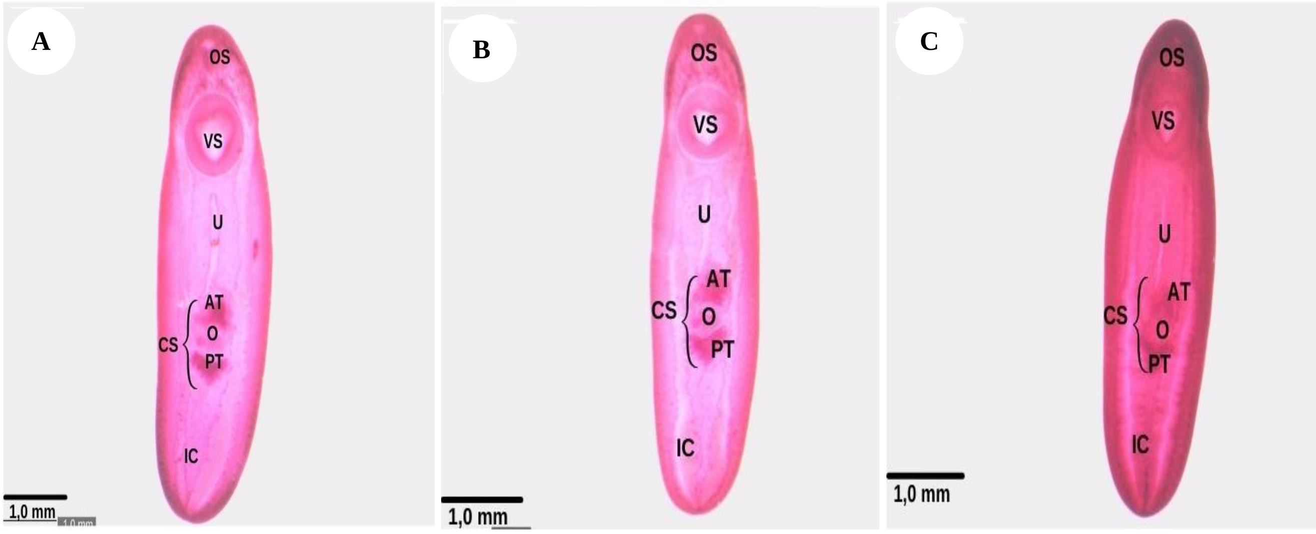

Figure 4. Metacercaria of *Clinostomum* sp. infesting snakeskin fish in Tempe Lake and the surrounding lakes in Wajo District, South Sulawesi, Indonesia; OV: Oral Sucker, VS: Ventral Sucker, U: Uterus, AT: Anterior Testis, O: Ovary, PS: Posterior Testis, CS: Cirrus Sac, IC: Intestinal Caeca. A. Tempe Lake; B. Lampulung Lake; C. Latamperu Lake. Bar = 1.0 mm

Table 1. Measurements of *Clinostomum* sp.

Characteristics	***Clinostomum* sp. Tempe Lake (n=20)**	***Clinostomum* sp. Lampulung Lake (n=20)**	***Clinostomum* sp. Latamperu Lake (n=20)**	***Clinostomum complanatum***
	Range (average±SD) (mm)	**Range (average±SD) (mm)**	**Range (average±SD) (mm)**	**(Bera et al. 2021) (range) (mm)**
Length	5.08±0.52 (4.05-6.24)	5.11±0.51 (4.26-5.41)	5.26±0.31 (4.75-5.72)	5.22-9.81
Width	1.46±0.18 (1.10-1.60)	1.55±0.20 (1.33-1.92)	1.75±0.15 (1.49-1.95)	1.32-1.99
Oral sucker length	0.05±0.01 (0.04-0.09)	0.05±0.01 (0.03-0.07)	0.05±0.01 (0.04-0.07)	0.22-0.31
Oral sucker width	0.11±0.03 (0.01-0.19)	0.05±0.01 (0.03-0.07)	0.10±0.01 (0.05-0.12)	-
Ventral sucker length	0.77±0.06 (0.65-0.88)	0.70±0.13 (0.50-0.87)	0.71±0.10 (0.56-0.88)	0.17-0.28
Ventral sucker width	0.77±0.09 (0.62-0.90)	0.76±0.16 (0.48-1.11)	0.65±0.07 (0.55-0.80)	0.32-0.67
Uterus length	0.93±0.13 (0.67-1.16)	1±0.15 (0.79-1.28)	1.05±0.15 (0.81-1.38)	-
Uterus width	0.13±0.20 (0.07-0.10)	0.12±0.04 (0.10-0.15)	0.11±0.04 (0.07-0.11)	-
Anterior testis length	0.30±0.06 (0.23-0. 55)	0.32±0.07 (0.26-0.36)	0.33±0.08 (0.28-0.40)	0.32-0.68
Anterior testis width	0.35±0.08 (0.23-0,55)	0.38±0.07 (0.36-0,44)	0.37±0.07 (0.28-0.48)	0.18-0.47
Ovary length	0.25±0.12 (0.19-0.42)	0.22±0.08 (0.16-0.41)	0.22±0.05 (0.18-0.25)	0.13-0.16
Ovary width	0.35±0.09 (0.20-0.46)	0.38±0.08 (0.38-0.50)	0.37±0.06 (0.26-0.39)	0.37-0.79
Cirrus sac length	1.05±0.13 (0.99-1.40)	1.03±0.23 (0.85 -1.27)	1.10±0.09 (0.99-1.29)	0.20-0.37
Cirrus sac width	0.53±0.09 (0.33-0.71)	0.60±0.08 (0.49-0.70)	0.58±0.10 (0.37-0.75)	0.11-0.16
Posterior testis length	0.30±0.06 (0.17-0.44)	0.28±0.06 (0.19-0.42)	0.30±0.05 (0.24-0.40)	0.20-0.37
Posterior testis width	0.37±0.08 (0.28-0.46)	0.41±0.10 (0.29-0.57)	0.38±0.09 (0.27-0.54)	0.45-0.51

Note: n: number of samples;±SD: standard deviation

Parasite *Clinostomum* sp. found across the three locations in Wajo District had approximately similar size with an average length of 5.08-5.26 mm and width of 1.46-1.75 mm (Table 1). Morphological observations showed close similarities in terms of body shape, the position of the oral and ventral suckers, intestinal caeca, and complex position, as well as genital (two testes, ovary, and cirrus sac) and on the edges of the body smooth, non-porous, similar to the *Clinostomum* sp. (Tansatit et al. 2014). The sample shares similarities with the *Clinostomum piscidium* Southwell & Prashad, 1918 parasite found in snakeskin fish in Thailand (Choudhary et al. 2022) and *C. complanatum* in *Trichogaster fasciata* Bloch & Schneider, 1801 fish in India (Bera et al. 2021). Furthermore, DNA amplification in the region of ITS-5.8S of the *Clinostomum* sp. found in the present study showed a very close distance with the *Clinostomum* sp., available in the GenBank, which has the accession number MT446431 (Table 3) reported from Australia. The phylogenetic tree constructed using the maximum likelihood method showed that the present sample was in the same clade as *Clinostomum* sp. recorded from snails in Australia (Shamsi et al. 2023), but a different clade with the valid species described worldwide, suggesting the present sample might be a new species (Figure 7).

Prevalence and mean intensity of *Clinostomum* sp. infestation

The length and weight, as well as the number of fish examined from the three lakes, are presented in Table 2. The fish samples obtained from the fishermen have similar sizes, with the average length ranging from 12.9 cm in Tempe Lake and 13.9 cm in Latamperu Lake. The prevalence of *Clinostomum* sp. in Lampulung Lake, Latamperu Lake, and Tempe Lake was 34, 28, and 21%, respectively. There were no statistical differences in the prevalence of *Clinostomum* sp. infestation based on the chi-square statistical test between the three locations surveyed (χ^2=4.2, P=0.121) (Table 2). The mean intensity of parasite infestation was highest in Lampulung Lake, with 10±10.7 SD parasite/fish, followed by Latamperu Lake and Tempe Lake, with a mean intensity of parasite infestation of 9±12.8 SD and 6±6.2 SD, respectively. The correlation of parasite mean abundance with fish length (P=0.05, r_s=-0.113) and weight (P=0.012, r_s=-0.146) showed significant different. However, correlation between parasite mean abundance and the factor of fish body condition showed no significant difference based on the Spearman rank correlation coefficient (P = 0.626, r_s =-0.028) (Figures 5 and 6).

Histopathology

Histopathological examination was conducted on the internal organs of four selected samples infested with *Clinostomum* sp. Histopathological changes observed on the intestinal organs of snakeskin fish infested with *Clinostomum* sp. include the presence of goblet cells, hemorrhage (bleeding), and inflammatory cell infiltration (Figure 8). The parasite attached outside the intestinal tissue, in which the hemorrhage was frequently observed.

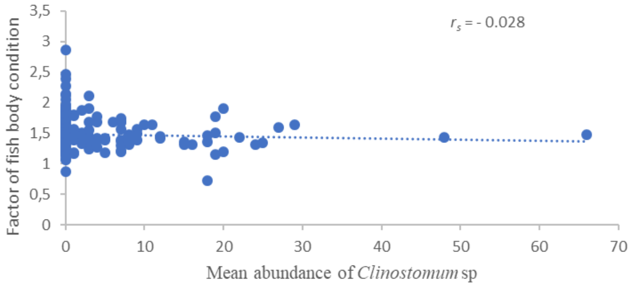

Figure 6. Correlation of the fish body condition and mean abundance of *Clinostomum* sp. (P=0.626, r_s=-0.028)

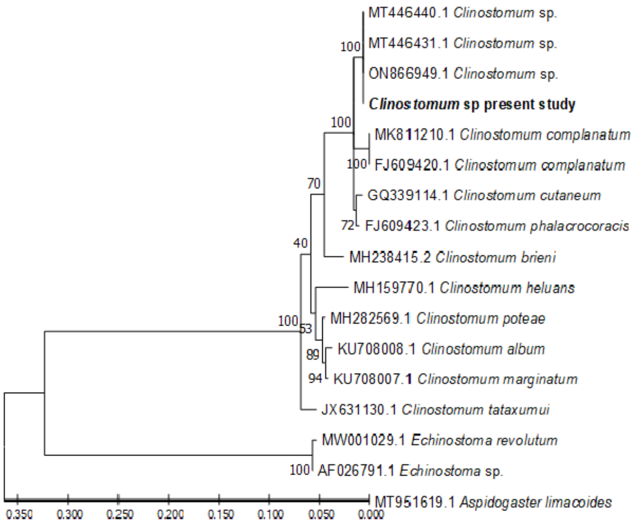

Figure 7. Phylogenetic tree of the present *Clinostomum* sp. based on ITS1-5.8S-ITS2 region. The maximum likelihood tree was constructed on software Mega X, using the Nearest Neighbor Interchange (NNI) method with the Tamura Nei Model and 1,000 bootstrap numbers with complete deletion

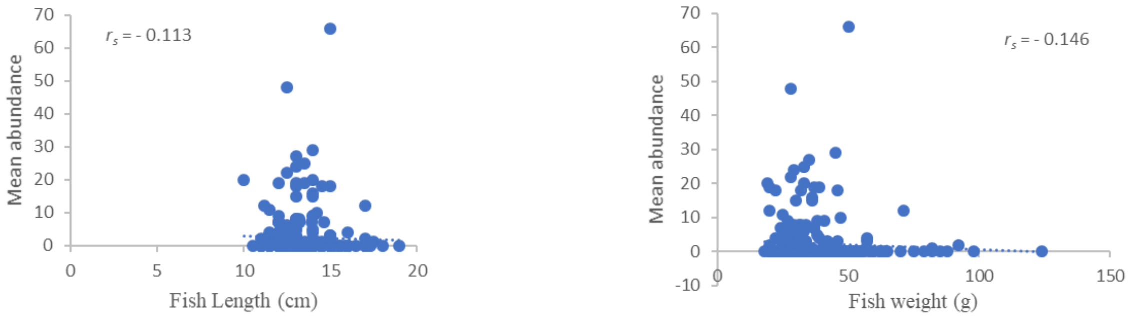

Figure 5. Correlation of the parasite mean abundance with the fish's total length (P=0.05, r_s=-0.113) and weight (P=0.012, r_s= -0.146)

Table 2. Fish length and weight and the level of parasite infestation of snakeskin fish at different locations

Location	Fish length (cm) (X±SD)	Fish weight (g) (X±SD)	No. of fish examined	No. of fish infested	No. of parasites	Prevalence (%)	Mean intensity (X±SD)
Tempe Lake	10-16 (12.9±1.2)	20-64 (31.6±8.3	100	21	135	21	6±6.2
Lampulung Lake	11-17 (13.5±1.63)	20-88 (40.4±18.4)	100	34	331	34	10±10.7
Latamperu Lake	11-16 (13.9±1.29)	22-79 (39.2±11.1)	100	28	247	28	9±12.8

Note: X: average); ±SD: standard deviation

Table 3. Pairwise distance of nucleotide sequences of *Clinostomum* spp. including present *Clinostomum* sp. based on ITS1-5.8S-ITS2 region

	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*Clinostomum* sp. present study												
2	MK811210 *Clinostomum complanatum*	0.046											
3	KU708008 *Clinostomum album*	0.083	0.057										
4	KU708007 *Clinostomum marginatum*	0.078	0.053	0.008									
5	MH282569 *Clinostomum poteae*	0.076	0.051	0.009	0.006								
6	MH159770 *Clinostomum heluans*	0.094	0.089	0.05	0.049	0.047							
7	JX631130 *Clinostomum tataxumui*	0.075	0.078	0.044	0.037	0.036	0.063						
8	MH238415 *Clinostomum brieni*	0.058	0.065	0.055	0.05	0.048	0.076	0.059					
9	GQ339114 *Clinostomum cutaneum*	0.04	0.017	0.056	0.052	0.051	0.091	0.077	0.058				
10	FJ609423 *Clinostomum phalacrocoracis*	0.036	0.017	0.053	0.049	0.048	0.086	0.073	0.055	0.01			
11	ON866949 *Clinostomum* sp.	0.016	0.023	0.061	0.056	0.054	0.093	0.075	0.058	0.018	0.016		
12	MT446431 *Clinostomum* sp.	0	0.029	0.074	0.068	0.065	0.094	0.081	0.059	0.022	0.019	0	0

Table 4. The water quality parameters on the lakes

Locations	Water quality parameters		
	Temperature (°C)	pH	Dissolved oxygen (mg/L)
Tempe Lake	29	7.30	4.37
Lampulung Lake	30	7.42	3.52
Latamperu Lake	30	7.23	3.20

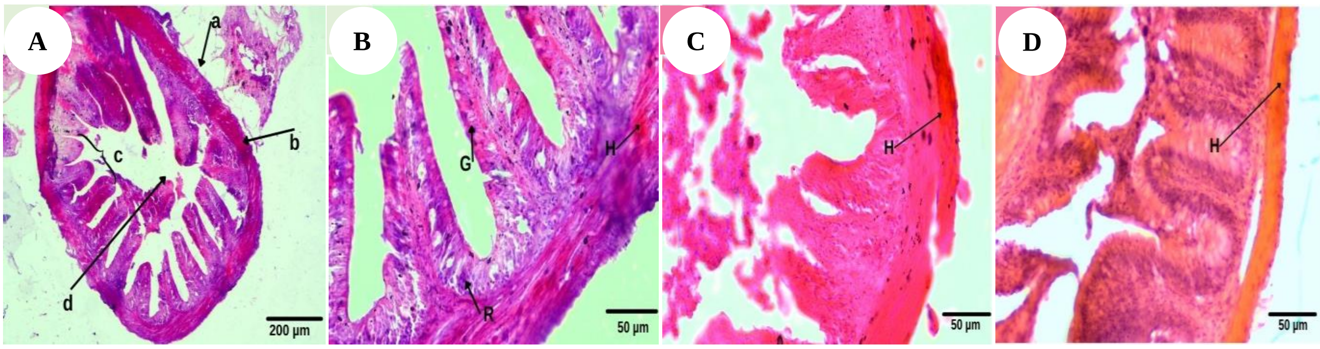

Figure 8. Histological profile of the intestine of snakeskin fish (A): serosa (a), submucosa (b), villi (c), lumen (d). Histopathological changes of the snake skin fish intestine from Tempe Lake (B), Lampulung Lake (C), and Latamperu Lake (D); goblet cell (G), hemorrhage (H), infiltration inflammation cells (R); Staining with H&E solution

Water quality

Table 4 presents the water quality parameters measured for Tempe Lake, Lampulung Lake, and Latamperu Lake: temperature, pH, and dissolved oxygen. The measured values can still support the life of snakeskin fish and other macroinvertebrates in the lakes.

Discussion

Clinostomum sp. is a cosmopolitan helminth worm parasite that is known to have a complex life cycle involving several intermediate hosts, including gastropods serve as the first intermediate host and fish and amphibians as the second intermediate host. This parasite's life cycle is complex, and infestation of parasites has a significant impact on various hosts. It reaches adult stage after the parasite moves to fish-eating birds (Sutili et al. 2014). Adult parasites are found in the oral cavity and esophagus of fish-eating birds. Eggs released along with final host feces experience several stages of development, namely the miracidium, sporocyst/redia, cercaria, metacercaria, and adult (Zimmermann et al. 2016). In water, the eggs hatch and become a free-swimming miracidium larva; when in contact with snails, it penetrates the tissue of the gastropods and changes to sporocysts or redia. After maturing inside the snail, the larvae change into the cercariae stage and emerge from the snail as free-swimming larvae to actively search for fish as a second intermediate host. The parasite larvae penetrate the skin, muscles, gills, and body cavities of fish and become metacercaria. The parasite becomes adult in the final hosts, particularly in the fish-eating bird, and will be released into the oral cavity, pharynx, and esophagus (Caffara et al. 2014; Acosta et al. 2016).

The lake ecosystem allows for the life cycle of digenetic trematodes due to the availability of intermediate hosts and definitive hosts. Tempe Lake has dense vegetation from various species suitable for the development of snails as the first intermediate host. It is inhabited by various species of fish and fish-eating birds so that the digenetic trematodes' life cycle can be completed within the ecosystem. Furthermore, the lake is shallow, with abundant gastropods and various species of fish, as well as birds, making it a suitable ecosystem for the life cycle of *Clinostomum* sp. Characteristics of the sampling locations are relatively shallow and overgrown with dense plant vegetation, providing snakeskin fish a good habitat for spawning, shelter, and foraging. Tempe Lake ecosystem, which has aquatic plant vegetation, acts as a food source for aquatic organisms (feeding ground), a place for fish to lay eggs (spawning ground), spawn (nursery ground), and shelter (Nasir and Nur 2018). Snakeskin fish live in permanently or seasonally flooded swamps, which have shallow paddles, muddy and composed of several aquatic plant vegetation (Rais et al. 2020). Water enters the lake carrying many nutrients from rivers and land, creating an ideal habitat for fish and other aquatic biota, such as macroinvertebrates (Imroatushshoolikhah and Laelasar 2017).

Clinostomum sp. requires snails as the first intermediate host, which are abundant in Tempe Lake. According to a previous report, snails function as intermediate hosts, including Lymnaea, Radix, Bulinus, Biomphalaria, Planorbella, and Helisoma (Tavares-Dias et al. 2023). In Tempe Lake, gastropods are the most dominant macroinvertebrate group, including those from the Lymnaea family (Imroatushshoolikhah et al. 2016). The prevalence of *Clinostomum* sp. infestation in the three locations ranged from 21 to 34%, while the mean intensity was between 6±6.2 to 10±10.7, showing the parasite infestation has already persisted in the lake. The high prevalence and mean intensity could be because sampling was conducted during the dry season when the water temperature was high. This condition stimulates snails to release cercaria. Fish forage or spawn around the vegetation, which is shallow and in close vicinity to the gastropods. The prevalence of Clinostomidae species may vary depending on biotic and abiotic environmental factors and may also be influenced by global warming. In the temperate area, during the summer, the larval stage of parasites is released from snails and can easily find an intermediate host. According to a previous study, a shallow habitat is an ideal place for definitive hosts such as herons, to catch infested fish (Fedorčák et al. 2019). Ecological and climatological factors play an important role in the emergence and spread of natural infestations of parasite *Clinostomum* sp. (Shareef and Abidi 2015). Menconi et al. (2020) reported that Endine Lake in Italy has favorable conditions for the life cycle of the *C. complanatum* parasite since the lake is shallow and occupied with several species of gastropods, as well as fish and birds. Shallow waters with a high abundance of gastropods and fish attract flocks of fish-eating birds, thereby completing the cycle of parasite *Clinostomum* sp. The high prevalence of digenetic trematodes infestation may be due to the time of emergence of cercariae in high temperatures from snail intermediate hosts. The variation in prevalence and mean intensity of the metacercariae of *Clinostomum* sp. can be attributed to the differences in the three habitats surveyed, abundance of aquatic snails, availability of the infective host (the intermediate host) and the aquatic piscivorous birds (Mahdy et al. 2023). Environmental temperature and availability of snails may be the important factors that cause the variation in the intensity (Khan et al. 2018). Anthropogenic pollution may influence snail distribution in lakes through a combination of direct toxic effects, habitat alteration, and changes in environmental conditions (Gawad 2018; Olkeba et al. 2020). The specific impact depends on the type and extent of pollution, as well as the sensitivity and adaptability of the snail species involved (Min et al. 2022; Olkeba et al. 2020). These factors may contribute to the existence and the level of infestation of trematodes, including the digenean *Clinostomum* sp., in freshwater ecosystems, which will be further studied.

Morphological characteristics of *Clinostomum* sp. include a worm-like body shape, bluntly rounded at the anterior and posterior ends, as well as a yellowish body color when alive. The metacercariae is 5.08-5.26 mm in length and 1.46-1.75 mm in width. There are oral and ventral suckers that have spines on the anterior body, and in the middle part, there is a uterine organ (womb) extending between the ventral sucker and the testes. Furthermore, in the posterior part, there are two testes (anterior and posterior), the cirrus sac, the ovary between the two testes, and an intestinal caecum (intestine). According to (Kabata 1985) *Clinostomum* parasite is dorsoventrally flat, unsegmented, and oval in body shape. Accurately identifying the various species is a challenge due to the very high similarity between *Clinostomum* metacercaria in fish. There are only slight differences in the morphological structure of parasites. Hence, identification based solely on morphology can lead to misidentification due to the wide variation in the phenotype of parasites of the same species. Amplification of DNA and sequencing on ITS-5.8S region showed in the phylogenetic tree that the present sample was in the same clade as *Clinostomum* sp. group, but a different clade with other known valid species. Pairwise distance analysis also showed the closest distance in the sample studied compared to those reported from snails in Australia. Other studies in Indonesia have reported that *Clinostomum* sp. in fish *Anabas testudineus* showed high similarity with the *C. complanatum* available on GenBank, based on molecular of ITS-1 region, with pairwise distances ranging from 0.016 to 0.018 (Riauwaty et al. 2011; Riauwaty et al. 2012).

This study did not show a significant correlation between the abundance of *Clinostomum* sp. in fish and the factor of body condition, however, the mean abundance of the digenean was different in total length, and fish weight, with smaller fish carrying a higher parasite burden than the larger fish. In this study Shareef and Abidi (2012) reported that *C. complanatum* infestation in *Channa punctatus* (Bloch, 1793) fish did not show a positive relationship between the parasite abundance and fish body condition.

Moreover, Wang et al. (2017) did not find a positive correlation between *C. complanatum* infestation in fish in Taiwan from several sampling locations.

Infestation of *Clinostomum* sp. may cause severe damage to the tissues of infested hosts. Histopathological observation of various hosts has shown pathological changes in infested organs. The pathological changes observed in this study did not seriously affect the host; hemorrhage, and the presence of goblet cells, while the host response observed was the infiltration of inflammatory cells in the infested areas. However, in another study, infestation of *C. complanatum* causes fibroplasia in host tissues, with the structure of connective tissues becoming encapsulated, vascularized, and mainly comprised of collagen fibers, fibroblasts, and fibrocytes (Gjurčević et al. 2022). Other study shows that *Clinostomum* sp. is embedded in the musculature of the buccal cavity, and infiltration of lymphocytic cells on the infested cells and oedema. The destruction of bony tissue and muscle fibers with intense inflammatory cell infiltration and necrosis has also been observed due to parasite infestation (Mahdy et al. 2021). Vascular congestion and infiltration of inflammatory cells, mainly lymphocytes, were also commonly observed in infested fish (Montes et al. 2020). This evidence shows that *Clinostomum* sp. infestation can cause severe damage to the host tissue in various species of fish, causing slow growth, changes in behavior, and yellow grub disease. This parasite is also a zoonotic potential that may infect humans when consumed raw or undercooked fish.

In conclusion, the parasite found in snakeskin fish was *Clinostomum* sp. based on morphological data and molecular analysis. The parasite is in a different clade from other known *Clinostomum* sp. species that have been reported, but has a closer distance from *Clinostomum* sp. reported from Australia. This parasite was found across the three lakes observed with relatively high prevalence and mean intensity. The condition of the lake ecosystem allowed the parasite to thrive due to the availability of intermediate and final hosts. This parasite causes mild damage to host tissue and has a high potential of infecting humans.

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