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Research Article

Endoparasitic infections and host-parasite interactions of cyprinid fish in Murat River: A focus on *Rhabdochona denudata* and *Neoechinorhynchus zabensis*

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Abstract: This study investigated the endoparasitic fauna of four cyprinid fish species (*Cyprinion macrostomum, Capoeta umbla, Chondrostoma regium,* and *Squalius cephalus*) inhabiting the Murat River, with a focus on the ecological distribution and variation of two identified parasites: *Rhabdochona denudata* (Nematoda) and *Neoechinorhynchus zabensis* (Acanthocephala). *R. denudata* was found in both *C. macrostomum* and *C. umbla*, while *N. zabensis* was exclusively detected in *C. umbla*. No endoparasitic infections were observed in *C. regium* or *S. cephalus*. Morphological examination confirmed taxonomic characteristics of both parasites, including prostomal teeth and sexual dimorphism in *R. denudata*, and proboscis structures and unique lemniscus nuclei in *N. zabensis*. Prevalence and infestation density of *R. denudata* were higher in *C. umbla* than in *C. macrostomum*. Seasonal variations in infestation levels were observed, with the highest prevalence of *R. denudata* recorded in autumn and *N. zabensis* in spring and summer; however, these differences were not statistically significant. A significant positive correlation was found between host size and *R. denudata* infestation levels, with larger hosts exhibiting higher infestation rates. These findings provide insights into host-parasite interactions and contribute to the understanding of parasitic diversity and ecology in freshwater ecosystems.

Keywords: *R. denudata, N. zabensis,* Cyprinid fish, Freshwater parasitology, Host-parasite interaction, Murat River.

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1. INTRODUCTION

The growing global population increases pressure on food security, making it essential to optimize both plant and animal food resources. Fish, as a rich source of high-quality protein, have been an important part of human nutrition since prehistoric times (Turan et al., 2006).

Türkiye, with its rich freshwater biodiversity and geographic advantages, has not yet reached its full potential in fish production. Despite this, fish consumption remains below the global average, signaling the need for effective strategies to enhance production and ensure sustainability.

One major challenge to fish production is internal parasites, which weaken fish health by making them more susceptible to diseases and impairing their growth and reproduction (Altunel, 1981; Grabda, 1991).

This study aims to identify the parasitic fauna of selected cyprinid fish species in the Murat River. By documenting these endoparasites, the research seeks to provide valuable insights for improving fish health management and supporting sustainable fisheries practices.

2. PREVIOUS STUDIES

A literature review conducted for the examined fish species reveals numerous studies both in Turkey and abroad. It has been observed that the intensity of research varies according to the regions where the fish species are found, showing various differences across different areas.

For the fish species *C. macrostomum*, aside from two studies conducted in Turkey (Saygı & Bardakçı, 1990; Koyun et al., 2015), no other parasitological studies were found in the literature. The mentioned studies indicates the presence of *Rhabdochona* sp. and *Piscicola geometra*. More studies have been conducted on this fish species in the Dicle-Fırat River Systems in the regions of Iran and Iraq, where 40 different parasite species have been reported. A significant portion of the recorded monogeneans was taken from a control list published by Mhaisen & Abdul-Ameer (2019). However, since many of the sources cited were Arabic-language theses, their verification was not possible. Additionally, it is noteworthy that five different parasitic species from the genus *Rhabdochona* have been reported for this species.

Studies on *C. umbla* have mostly focused on the Murat-Fırat basins. A total of 19 different parasite species have been identified in these regions (Aksoy 1996; Örün et al. 2003; Aksoy et al. 2006; Dörücü & İspir, 2005; Dörücü et al. 2008; Koyun 2011a, 2011b, 2012; Gül et al. 2014; Kavak & Şeker, 2017). The diversity of parasites varies by region. For example, two species of monogenean trematodes were found in the Murat River, while five species were identified in Lake Hazar. Koyun (2011a, 2011b, 2012) identified five parasite species in *C. umbla* from the Murat River, including *N. zabensis* as a endoparasite in the intestine, while Gül et al. (2014) reported *Diplostomum* sp. as a endoparasite in the eyes, in the same river.

Studies on *C. regium* in Turkey have primarily focused on various reservoirs, including the Almus, Karakaya, and Keban Reservoirs, as well as the Murat River. While reports on endoparasites are limited, several studies have identified endohelminths in the reservoirs, including *Bothriocephalus acheilognathi*, a cestode species, recorded from the Almus, Karakaya, and Keban Reservoirs (Örün et al., 2003; Dörücü & İspir, 2005, Özgül, 2008). *Ligula intestinalis* and *Neoechinorhynchus rutili* have been found in two distinct locations (Keskin & Erakan, 1987; Örün et al., 2003). However, in the Murat River, previous studies have focused on ectoparasites, with no reports of endohelminths. Koyun (2011c) identified *Dactylogyrus elegantis* and *Dactylogyrus vistulae*, while Gül et al. (2014) documented *Diplostomum* sp. The absence of endohelminths in the Murat River may provide valuable ecological insights.

The fish species *S. cephalus* has been the focus of numerous studies across Turkey due to its wide distribution, with 19 different parasite species identified in research conducted across 20 regions. The diversity of parasite species varies by region, and some species, such as *L. intestinalis*, have been recorded in up to eight different locations. In the Murat River and surrounding areas, endoparasites such as *Pomphorhynchus* sp. (Murat River, Aslan Çelik & Oğuz, 2021), *Diplostomum* sp. (Keban Dam, Dörücü & İspir, 2005) have been reported.

Several studies have documented the parasitic fauna of cyprinid fish in Turkish rivers, providing valuable data for comparison. For instance, in the Aras River, *Leuciscus cephalus* (Syn. *S. cephalus*) was found to host *Pomphorhynchus* sp., whereas no parasites were detected in this species from the Murat River (Aslan Çelik & Oğuz, 2021).

Capoeta capoeta hosted various parasites in both the Aras and Murat Rivers. In the Aras River, *Caryophyllaeus laticeps*, *Pomphorhynchus* sp., *Neoechinorhynchus* sp., and *R. denudata* were recorded. In contrast, in the Murat River, only *R. denudata* and *Neoechinorhynchus* sp. were detected, while *C. laticeps* and *Pomphorhynchus* sp. were absent (Aslan Çelik & Oğuz, 2021).

These findings highlight differences in parasitic diversity across habitats and underline the importance of further research to understand ecological factors influencing parasite-host dynamics.

3. MATERIAL AND METHOD

3.1. Materials

This study examined four significant cyprinid fish species in the region: *C. macrostomum* (Heckel, 1843), *C. regium* (Heckel, 1843), *C. umbla* (Heckel, 1843), and *S. cephalus* (Linnaeus, 1758).

3.2. General Information About the Study Area

The Murat River is a major tributary of the Euphrates River in the Eastern Anatolia Region. It originates north of Lake Van, starting from Diyadin in Ağrı Province and formed by the confluence of streams from Aladağ and Mount Muratbaşı (Wikipedia, 2020). The basin area is 40,000 km² and the river stretches for 722 km.

Sampling was conducted at various stations, as it was not possible to find the same fish species throughout the year at all stations. Therefore, different sampling sites were selected for field studies. The sampling stations included the Murat River, which flows through the town of Genç, and the Göynük Stream, which flows into the Murat River.

3.3. Water Quality Parameters in the Murat River

Throughout the study, water temperature, dissolved oxygen levels, and pH values were measured in the Murat River (Table 1). An examination of the annual data reveals significant seasonal variations in temperature, whereas oxygen levels and pH values exhibited relatively little fluctuation throughout the year. Notably, during the summer months, when water temperature reached its highest levels, dissolved oxygen and pH values were recorded at lower levels.

Table 1. Temperature, dissolved oxygen, and pH levels measured in the Murat River

Annual water parameters	Min	Max	Mean±SD
Temperature (°C)	6.32	24.08	14.42±6.06
Oxygen (mg/L)	7.76	8.42	8.06±0.24
pH	7.38	8.60	8.14±0.40

Monthly water parameters	Temperature (°C)	Oxygen (mg/L)	pH
January	6.32	8.36	8.48
February	6.94	8.07	8.35
March	13.14	8.42	8.60
April	13.76	7.98	8.34
May	16.52	7.76	8.24
June	19.88	8.01	7.60
July	23.50	7.84	7.38
August	24.08	7.81	7.59
September	16.36	7.96	8.15
October	13.90	7.87	8.11
November	11.90	8.34	8.21
December	6.70	8.30	8.57

3.4. Methods

3.4.1. Sample Collection

Fish samples were collected using seine nets and gill nets, ensuring live specimens were kept in fish cages for transport to the laboratory. Fish were transferred from the field to the laboratory using holding tanks, provided with oxygen supplementation to ensure their survival during transit, and were dissected within 24 hours. The total, fork, and standard lengths of the captured fish were recorded in millimeters (mm), and their weights were recorded in grams (g).

3.4.2. Parasite Investigation

Endoparasites were examined by opening the internal organs anteriorly from the anal opening without fragmentation. The internal organs were first subjected to a macroscopic examination, and then the gastrointestinal tract (esophagus, stomach, and intestines) was placed in petri dishes containing physiological saline. The intestines were opened with needles and scissors and were examined under a binocular stereo microscope. The identified parasites were collected using needles, brushes, and pipettes, cleaned of tissue remnants, and allowed to settle.

For *N. zabensis*, specimens were processed without any additional treatment and were photographed under a light microscope. For R. denudata, samples were fixed in 10% formalin for description and measurement, then stored in 70% ethanol.

3.4.3. Identification of Parasite Specimens

Species identification of the recorded parasites was performed using resources from Bykhovskaya & Pavlovskaya (1962), and Amin et al. (2003). Specimens were examined using an Olympus stereo microscope and a light microscope, with significant morphological features photographed. Measurements of the important parts of the parasites were made using the “Am Scope” software with different calibrations for each microscope in micrometers (µm) and millimeters (mm). Over 2000 high-resolution images and videos were taken during the study, resulting in the creation of a digital database instead of permanent preparations or fixed samples.

3.4.4. Bio-statistics

Statistical analysis was performed using the SPSS 25.0 software. Mann-Whitney U tests were applied for pairwise comparisons, and Kruskal-Wallis variance analysis was used for groups with more than two samples to identify significant differences. To account for outliers, median tests were also utilized as suggested by Siegel (1959).

Fish sizes were categorized according to classification rules that best represent each fish species (Sümbüloğlu & Sümbüloğlu, 2012), resulting in a total of four classes for easier analysis and adequate information on the distribution.

3. RESULTS

This study aimed to identify endoparasites in four cyprinid fish species from the Murat River: *C. macrostomum*, *C. umbla, C. regium,*and *S. cephalus*. Among the two parasitic species encountered, *R. denudata* was found in both *C. macrostomum* and *C. umbla*, while *N. zabensis* was exclusively detected in *C. umbla*. No parasitic infections were observed in *S. cephalus* or *C. regium*.

The results section outlines the findings regarding the morphological characteristics, distribution, and statistical analysis of the two identified endoparasites, *R. denudata* and N. zabensis, parasitizing *C. macrostomum* and *C. umbla*. This section also discusses the distribution patterns of these parasites across host species, as well as their seasonal variation, providing insights into the factors influencing parasitic infestation levels.

4.1. *Rhabdochona denudata* (Dujardin, 1845)

4.1.1. Morphological and Diagnostic Features

The genus *Rhabdochona* is characterized by distinct morphological features, including a slender, elongated body. A key taxonomic marker for this genus is the number and structure of prostomal teeth, which play a critical role in species identification (Moravec, 2010).

In males, the tail is conical and bent inward, exhibiting multiple preanal and 3-6 pairs of postanal papillae (Figure 1).

Females have a straight, elongated tail, with the vulva located in the middle of the body. The eggs are elliptical. Characteristic structures in females include vulva, vagina, tube, ovary, and uterus, with numerous eggs present in the ovaries (Figure 2).

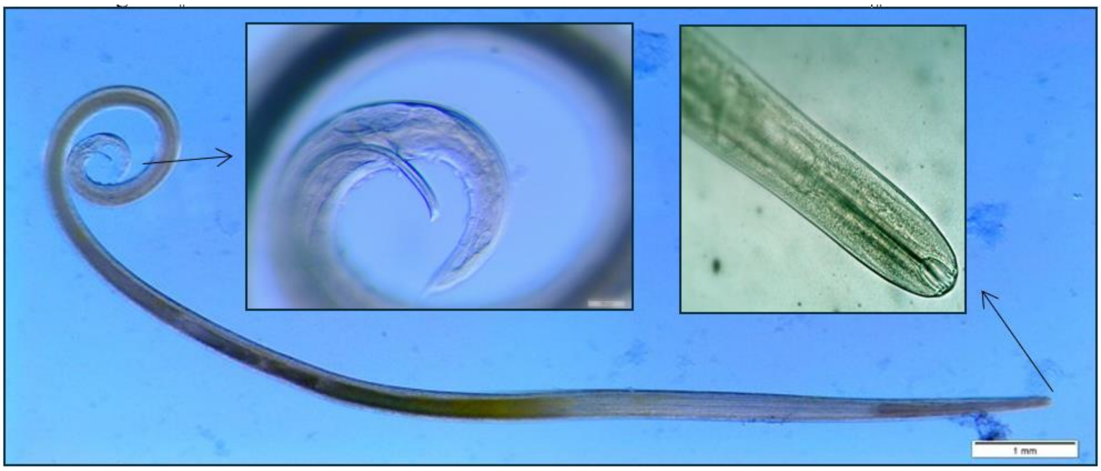

Figure 1. *Rhabdochona denudata* (Male)

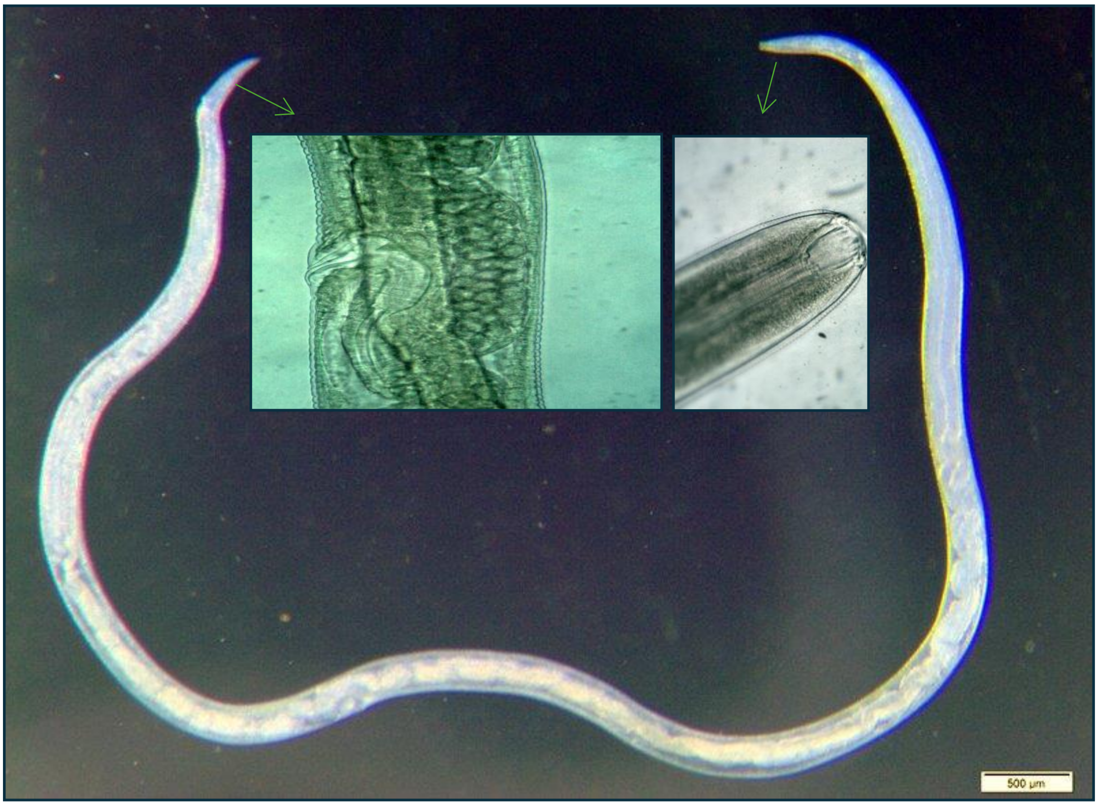

Figure 2. *Rhabdochona denudata* (Female)

4.1.2. Distribution According to Host Species

The nematode *R. denudata* was found to infect both *C. macrostomum* (9/91; prevalence: 9.9%) and *C. umbla* (27/109; prevalence: 24.8%). Infestation density was higher in *C. umbla* (2.4±0.6) compared to *C. macrostomum* (1.4±0.2). However, the Mann-Whitney U test (U=120.5, p=0.966) revealed no statistically significant difference in infestation levels between the two host species. This suggests that while prevalence and density may vary slightly, the distribution pattern of *R. denudata* remains consistent between the two fish species (Table 1).

Table 1. Distribution of *R. denudata* according to host species and statistical analysis

Host	NIF (n)	P (%)	ID (Count)	Mean Rank	**Test Statistics a,b** ***R. denudata*-Infected Fish**	
C. macrostomum **(N=91)**	9	9,9	1,4±0,2	18,4	Mann-Withney U	120,5
					Z	-0,42
C. umbla **(N=109)**	27	24,8	2,4±0,6	18,5	Asymp. Sig.	0,966
					a. Mann-Withney U test	
Total (N=200)	36	18,0	2,5±1,1		b. Grouping Var.: Host	

NIF (n): Number of Infected Fish, P: Prevelance (%), ID (Count): Infestation Density

4.1.3. Seasonal Distribution

The Kruskal-Wallis test (H=4.749, p=0.191) indicates no statistically significant seasonal difference in *R. denudata* infestation levels among the studied host fish. Prevalence was highest in autumn (25.0%), followed by winter (19.2%), spring (16.9%), and summer (11.1%). In contrast, infestation density peaked in spring (3.4±1.6) and was lowest in summer (1.0±0.0). These findings suggest that while seasonal variations in prevalence and density exist, the differences are not statistically significant, pointing to a relatively stable pattern of infestation across seasons (Table 2).

Table 2. Seasonal Distribution of *R. denudata* infestation and statistical analysis

Seasons	NIS (n)	P (%)	ID (Count)	Mean Rank	Test Statistics a.b *R. denudata*- Seasonal Distribution	
Spring (N=59)	10	16.9	3.4±1.6	19.4	Kruskal-Wallis H	4.749
Summer (N=45)	5	11.1	1.0±0.0	11.5	Df	3
Autumn (N=44)	11	25.0	1.9±0.3	22.0	Asymptotic Sig.(2-sided test)	0.191
Winter (N=52)	10	19.2	1.7±0.4	17.3	a. Kruskal Wallis Test	
Total (N=200)	36	18.0	2.1±2.9		b. Grouping Var.: Seasons	

NIF (n): Number of Infected Fish, P: Prevelance (%), ID (Count): Infestation Density.

4.1.4. Distribution by Size

The Kruskal-Wallis test (H=12.992, p=0.005) reveals a statistically significant difference in *R. denudata* infestation levels among different size classes of host fish. Larger hosts, particularly those in the 4th size group, exhibited the highest infestation rates, with a prevalence of 26.7% and an average infestation density of 3.6±1.3. In contrast, smaller hosts in the 1st size group showed the lowest infestation levels, with a prevalence of 17.5% and an average density of 1.1±0.1. These results indicate a clear trend of higher infestation in larger fish, suggesting that host size is a significant factor influencing the intensity of *R. denudata* infestation.

Table 3. Distribution by size of *R. denudata* infestation and statistical analysis

Size Groups	NIS (n)	P (%)	ID (Count)	Mean Rank	Test Statistics a,b *R. denudata* – Host Size	
1. Group (N=40)	7	17,5	1,1±0,1	13,7	Kruskal-Wallis H	12,992
2. Group (N=64)	11	17,2	1,3±2,0	14,8	Df	3
3. Group (N=51)	6	11,8	2,0±1,0	15,4	Asymp. Sig.	0,005
4. Group (N=45)	12	26,7	3,6±1,3	26,3	a. Kruskal Wallis Test	
Total (N=200)	36	18,0	2,1±2,9		b. Grouping Var.: Host Size	

NIF (n): Number of Infected Fish, P: Prevelance (%), ID (Count): Infestation Density.

4.2. *Neoechinorhynchus zabensis* Amin, Abdullah and Mhaisen, 2003

4.2.1. Morphological and Diagnostic Features

N. zabensis can be distinguished by body size, proboscis, proboscis hooks, the number of large nuclei in the lemniscus, and the position of the female genital organs. The large nuclei within the lemniscus, fragmented by para-vaginal muscles, are a unique distinguishing feature (Amin et al. 2003). This species exhibits sexual dimorphism, characterized by a cylindrical body surrounded by a thick wall in the dorso-ventral direction, containing large cells within the hypodermal tissue. There are 2-3 nuclei ventrally and 8-10 dorsally (Figure 3-C,E). The proboscis is approximately equal in width and length, surrounded by rooted hooks. The hooks in the first row are highly developed, while those in the second and third rows are less developed and shorter. The middle hooks are almost the same length as those in the third row but are thinner.

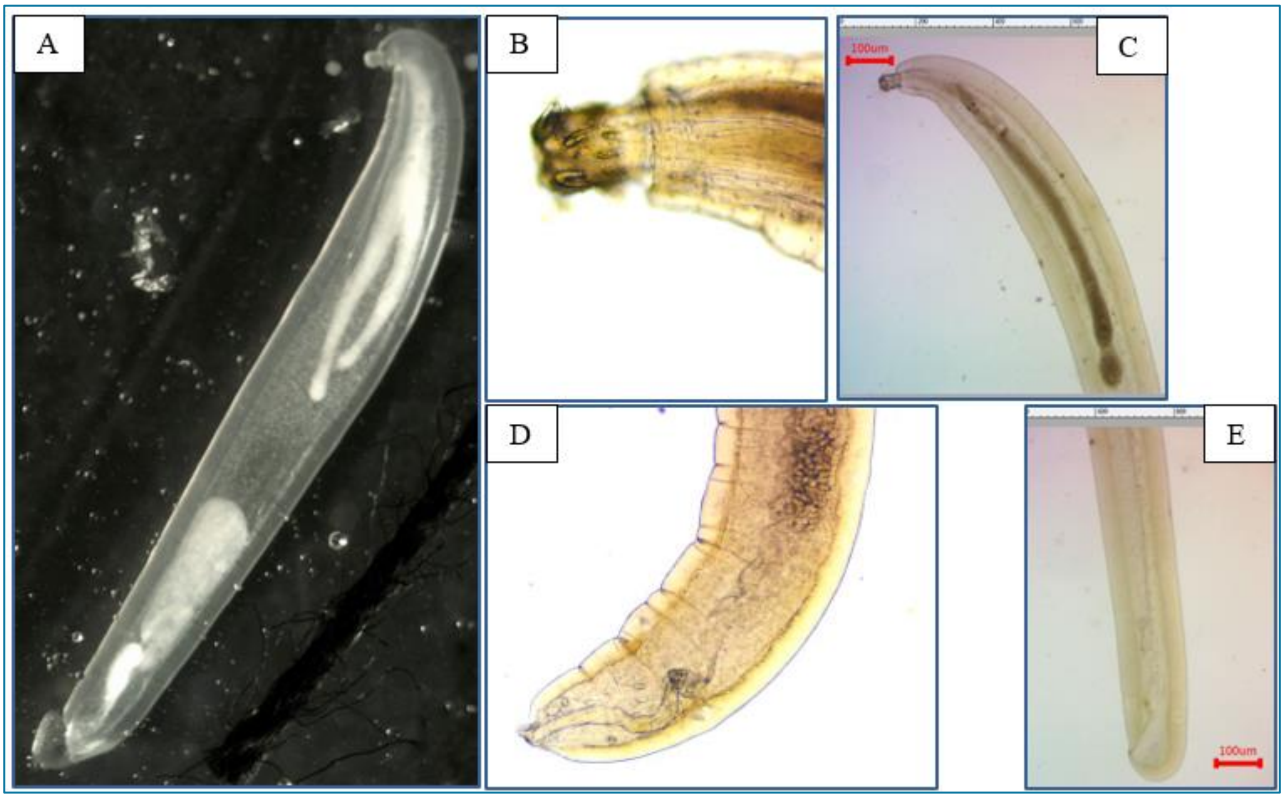

Figure 3. *N. zabensis* A: Total, B-D: Male, C-E: Female

The brain is oval and large, located just below the proboscis-sized extension. The proboscis receptacle is approximately six times the size of the proboscis. The receptacle extends into two separate strips, one long and the other shorter, moving away from the genital region (Amin et al., 2003) (Figure 3 A-E).

4.2.2. Distribution According to Host Species

N. zabensis was exclusively found in *C. umbla*, with a prevalence of 9.2%. A total of 12 parasites were recorded across 10 infected fish, resulting in an average infestation density of 1.2 ± 0.1 parasites per infected fish. Due to the limited sample size, only descriptive statistics are provided, and no statistical tests or further interpretations have been conducted. These results indicate that *N. zabensis* infested only *C. umbla*, with no occurrences in other host species.

4.2.3. Seasonal Distribution

The seasonal distribution of *N. zabensis* infestations among the sampled fish presents in Table 4. Prevalence was highest in spring (3.7%) and summer (3.6%), followed by winter (2.2%), while no infestations were recorded in autumn. Infestation density (ID) peaked in spring (1.25 ± 0.6) and winter (1.7 ± 0.4). However, the Kruskal-Wallis test revealed no statistically significant difference in infestation levels among the seasons (H = 1.031, df = 2, p > 0.05). These results suggest that seasonal variations do not significantly influence *N. zabensis* infestation rates.

Table 4. Seasonal Distribution of N. zabensis infestation and statistical analysis

Seasons	NIS (n)	P (%)	ID (Count)	Mean Rank	Test Statistics N. zabensis - Seasonal Distribution	a.b
Spring (N=39)	4	3.7	1.25±0.6	19.4	Kruskal-Wallis H	1.031
Summer (N=22)	3	3.6	1.0±0.0	11.5	Df	2
Autumn (N=22)	0	0	0	0	Asymptotic Sig.(2-sided test)	0.597
Winter (N=26)	3	2.2	1.7±0.4	0.33	a. Kruskal Wallis Test	
Total (N=109)	10	18.0	2.1±2.9		b. Grouping Var.: Seasons	

NIF (n): Number of Infected Fish, P: Prevelance (%), ID (Count): Infestation Density.

4.2.4. Distribution by Size

Table 6 summarizes the distribution of *N. zabensis* infestation across different host size groups and the results of statistical analysis. Prevalence was highest in the fourth size group (26.7%), followed by the first group (17.5%), the second group (17.2%), and the third group (11.8%). Infestation density (ID) also increased with host size, peaking in the fourth group (3.6 ± 1.3) compared to lower values in the smaller groups (e.g., 1.1 ± 0.1 in the first group). Despite these trends, the Kruskal-Wallis test showed no statistically significant differences in infestation levels among the size groups (H = 5.25, df = 3, p > 0.05). These results suggest that while infestation density appears to increase with host size, the variation is not statistically significant.

Table 6. Distribution by size of *N. zabensis* infestation and statistical analysis

Size Groups	NIS (n)	P (%)	ID (Count)	Mean Rank	Test Statistics a.b *N. zabensis* – Host Size	
1. Group (N=39)	1	1.6	1.1±0.1	9.5	Kruskal-Wallis H	5.25
2. Group (N=22)	5	4.3	1.3±2.0	4.5	Df	3
3. Group (N=22)	3	2.8	2.0±1.0	6.2	Asymp. Sig.	0.154
4. Group (N=26)	1	1.5	3.6±1.3	4.5	a. Kruskal Wallis Test	
Total (N=109)	10	18.0	2.1±2.9		b. Grouping Var.: Host Size	

NIF (n): Number of Infected Fish, P: Prevelance (%), ID (Count): Infestation Density.

4. DISCUSSION AND CONCLUSION

This study investigated the endoparasitic fauna of four cyprinid fish species (*C. macrostomum*, *C. regium*, *C. umbla*, and *S. cephalus*) in the Murat River, identifying two endoparasite species: *R. denudata* and *N. zabensis*. The findings provide valuable insights into the ecological distribution, morphological characteristics, and infestation patterns of these parasites.

Host-Parasite Relationships
Among the four host species, *R. denudata* was detected in both *C. macrostomum* and *C. umbla*, while *N. zabensis* was exclusively found in *C. umbla*. No parasitic infections were observed in *C. regium* or *S. cephalus*. These results suggest host-specific preferences, with *N. zabensis* showing strict specificity to *C. umbla*. Such specificity aligns with previous reports highlighting acanthocephalan parasites' tendency to exhibit narrow host ranges. In contrast, the broader host range of *R. denudata* suggests its ability to adapt to different cyprinid hosts.

Distribution by Host Species
The higher prevalence and infestation density of *R. denudata* in *C. umbla* compared to *C. macrostomum* may reflect ecological or behavioral differences, such as feeding habits or habitat preferences, that influence exposure to infective stages. However, statistical analysis revealed no significant differences in infestation levels between the two host species, suggesting that *R. denudata* utilizes both hosts with similar efficiency.

Seasonal Variations
Seasonal trends in infestation levels were observed for both parasites. *R. denudata* exhibited the highest prevalence in autumn, while its infestation density peaked in spring. Similarly, *N. zabensis* showed higher prevalence in spring and summer. These patterns could be linked to environmental factors such as temperature, host behavior, and the life cycles of intermediate hosts. However, the lack of statistically significant seasonal differences for either parasite indicates that infestation levels remain relatively stable across seasons.

Host Size and Infestation
A significant positive correlation between host size and infestation levels was observed for R. denudata, with larger fish experiencing higher prevalence and infestation density. This trend likely reflects the prolonged exposure of older, larger fish to infective stages or their ability to support higher parasite loads. Although a similar trend was noted for N. zabensis, statistical analysis did not confirm significant size-related variation.

Morphological Observations
Morphological examination confirmed key diagnostic features of the two parasites, consistent with previous descriptions. *R. denudata* was characterized by its prostomal teeth and sexual dimorphism in tail morphology, while *N. zabensis* displayed distinctive proboscis and proboscis hook structures, along with a unique arrangement of large nuclei in the lemniscus. These morphological details contribute to the growing body of taxonomic knowledge and facilitate accurate species identification in future studies.

Conclusion
The study highlights the ecological and biological factors influencing the distribution and infestation patterns of *R. denudata* and *N. zabensis* in cyprinid fish from the Murat River. The findings emphasize the role of host species, size, and season in shaping parasitic dynamics while underscoring the value of detailed morphological analyses for accurate identification. Future research could explore additional environmental and ecological factors, including intermediate host availability and water quality, to further elucidate the drivers of parasitic distribution in aquatic ecosystems.

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Compliance with Ethical Standards:

This study was approved by the Ethics Committee of Bingöl University, confirming its compliance with research ethics.

Authors' Contributions:

NK: Manuscript design, Field sampling, Data collection, Statistical analysis, Writing – Original draft, Review & Editing.
MK: Conceptual guidance, Supervision.
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Statement on the Welfare of Animals

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study was approved by the Ethics Committee of Bingöl University (Approval No: 33117789/299/33221).

Data Availability Statements

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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