

Article

Ascaridoid Nematodes Infection in Anadromous Fish *Coilia nasus* from Yangtze River

Qingjie Zhou ^{1,2}, Lijun Wang ^{1,2}, Bingwen Xi ^{1,2,*} , Congping Ying ^{1,2} and Kai Liu ^{1,2,*}¹ Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China; 2021113004@stu.njau.edu.cn (Q.Z.)² Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China

* Correspondence: xibw@ffrc.cn (B.X.); liuk@ffrc.cn (K.L.)

Abstract: The longjaw tapertail anchovy *Coilia nasus*, which migrates from ocean to freshwater for spawning in spring, is an important anadromous fish with ecological and cultural significance. To determine parasite infection in anadromous *C. nasus*, a total of 103 fish from the Yangtze River were collected and examined in 2021 and 2022. The overall infection prevalence of nematodes in *C. nasus* was 100%, with a mean intensity of 13.81 ± 16.45 . The mean intensity of nematode infections in 2022 was significantly higher than that observed in 2021 across all sampling sites ($p < 0.05$). Nematodes were widely detected in the mesentery, pyloric cecum, stomach, and liver, among which the mesentery accounted for the highest proportion, reaching up to 53.52%. A total of eight ascaridoid nematodes belonging to the family Anisakidae and Raphidascarididae were identified by using morphological characters and molecular biological techniques, including two species of *Anisakis*, five species of *Hysterothylacium*, and one species of *Raphidascaris*. *A. pegreffii* was found as the predominant species, accounting for 48.65% of all identified parasitic nematodes in liver, while *Raphidascaris* sp. was the most common nematode in the mesentery, pyloric cecum, and stomach, reaching up to 39.81%, 36.21%, and 74.36%, respectively. The present study systematically investigated the parasitic status and community structure of the nematode in *C. nasus* during its migration in the Yangtze River. This research provides a foundation for studying the impact of nematode parasitism on the reproductive migration and population recruitment of *C. nasus*, and offers valuable insights for biomarker screening and nematode identification in *C. nasus*.



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Keywords: *Coilia nasus*; parasite; nematode; *Anisakis*

1. Introduction

Fish parasitic ascaridoid nematodes, such as anisakid and raphidascaridid, are commonly encountered parasites in wild and cultured fish stocks [1–4]. The life cycle of ascaridoids involves marine mammals, birds, reptiles, amphibians, predatory fish, and elasmobranchs that serve as definitive hosts, and crustaceans, fish, squid, birds, and sea-snakes as intermediate hosts [5–8]. The growing impact of parasites on fish health and the economic significance of parasites in aquaculture and fisheries have enhanced the need for studies on fish/parasite relationships [9,10]. Meanwhile, the nematodes in the super-family Ascaridoidea have gained global recognition because of their significant impact on human health as the causative agents of anisakidosis, a fish-borne parasitic zoonosis via accidental ingestion of raw or under-cooked fish infected by the family Anisakidae [1,11–13].

The longjaw tapertail anchovy *Coilia nasus* Temminck & Schlegel, 1846 is a small to medium-sized anadromous fish belonging to the order Clupeiformes, and is widely distributed from the coastal waters of China, Korea, and Japan to the interconnected freshwater tributaries [14,15]. The lower and middle reaches of the Yangtze River is the most important channel for the anadromous migration of *C. nasus*. The migration is a preparatory process for spawning, and wellknown to be a season-dependent and temporally based reproductive activity [16]. Adult *C. nasus* has been reported to migrate upstream

from early February to the end of April, and spawn in the middle and lower reaches of the Yangtze River and connected lakes [14,17,18]. Unfortunately, in recent decades, stocks of *C. nasus* in the Yangtze River have experienced a significant decline due to anthropogenic influences such as excessive fishing activities, extensive hydraulic construction obstructing migration routes, and degradation of their natural habitats [19–21]. Recently, parasite infection in *Coilia* spp. has attracted much attention [22–26]. Xu et al. [22] found that *Anisakis simplex* and *Epiclavella chinensis* only parasitized the anadromous *C. nasus* in the Yangtze River. Therefore, *A. simplex* was considered as a “biological indicator” of the anadromous *C. nasus* in the Yangtze River. Li et al. [23] used the parasites as indicators of fish migratory movements. They pointed out that the monogenean *Heteromazocraes lingmueni* and the acanthocephalan *Acanthosentis cheni* are suitable biological tags for the *C. nasus* population migrating from the middle and lower reaches to the estuary of the Yangtze River, while the nematodes *Contracaecum* sp. and *A. simplex* are suitable biological tags for population migrating from the coast and estuary to the Yangtze River. The parasitic infection alters host behavior, damages tissue, affects ingestion, decreases weight, hampers reproduction, and can even cause death in some cases [10,27]. Thus, the parasites seriously threatened the health of *C. nasus*. In this report, *C. nasus* caught from the Shanghai and Anqing section of the Yangtze River from 2021 to 2022 were investigated to analyze the parasitic status and community structure of nematodes in *C. nasus*.

2. Materials and Methods

2.1. Sample Collection

All procedures involved in the handling and treatment conducted as follows were given official approval by the animal ethics committee of the Chinese Academy of Fishery Sciences, and fish collection was approved by the Department of Agriculture and Rural Affairs of Shanghai and Anhui Province with scientific fish collection license. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (Authorization Number: 20221223).

Fish were caught at the Yangtze River estuary (Shanghai section) and the lower reach of Yangtze River (over 580 km to estuary, Anqing section) with gill net between May and July in 2021 and 2022, and immediately transported to laboratory on ice. A total of 103 *C. nasus* were examined for parasites, and fish body length and sex were recorded. Fish were dissected and the internal organs (abdominal cavity, mesentery, pyloric cecum, stomach, and liver) (Figure 1) were checked under stereomicroscope (Nikon SMZ18). Nematodes isolated from each organ were washed in saline and then fixed with 100% ethanol. In addition, specimens of *C. nasus* and nematode were all deposited at Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences. Specimen were observed under light microscopy and identified according to the morphological characters of head, tail tip, and excretory and digestive systems.



Figure 1. The nematodes in the liver of *C. nasus*, indicated with arrows.

2.2. Molecular Identification

A total of 250 nematodes isolated from *C. nasus* at the Shanghai (16 individuals) and Anqing (10 individuals) sections of the Yangtze River, which were sampled in 2022, were all used for molecular identification. Genomic DNA from individuals was extracted using a Column Genomic DNA Isolation Kit (Sangon, Shanghai, China) according to the manufacturer's instructions. The ribosomal ITS1-5.8S-ITS2 region was amplified using the primers NC2-ITS-F (TTAGTTTCTTTTCCTCCGCT) and NC5-ITS-R (GTAGGTGAAC-CTGCGGAAGGATCATT) [28] and the predicted PCR production was about 1000 bp. PCR reaction (50 μ L) includes 25 μ L 2 \times Taq Master Mix (Dye Plus) (Vazyme, Nanjing, China), 2 μ L of each primer, 4 μ L DNA template, and 17 μ L ddH₂O, and the thermal profile was set as 95 °C, 5 min (initial denaturation) followed by 35 cycles of 95 °C, 30 s (denaturation), 56 °C, 30 s (annealing), 72 °C, 60 s (extension), and a final extension of 72 °C for 10 min. PCR products were purified and sequenced at Sangon Biotech (Shanghai, China). The obtained DNA sequencing chromatograms were checked in Chromas 2.6.6 and assembled with SeqMan (LaserGene package 11.0), aligned in Geneious (5.6) [29]. Sequence similarity was searched against the GenBank database using the Basic Local Alignment Search Tool (BLAST).

Phylogenetic trees were conducted with Bayesian inference (BI) based on the obtained sequences. The best nucleotide substitution model (K2P) was estimated with Modelfinder according to Bayesian information criterion (BIC). BI analyses were conducted using the software MrBayes ver. 3.1.2 with parameter settings nruns = 4, rates = gamma, and ngen = 100,000.

2.3. Data Processing

2.3.1. Terms and Indicators

The terms and indicators were employed here according to Bush et al. [30] and Wu et al. [31], such as Margalef richness index = $(N - 1)/\ln(N)$, where N is the number of species;

Aggregate Index: $GI = (DI - 1)/(ni - 1)$, where ni is the total number of worm species i;

Infection Index: $Z = ni \times (Pi/N^2)$, where Pi is the number of hosts infected by worm i and N is the total number of worms;

Distribution type and test: $DI = \text{variance}(S^2)/\text{mean value}(X)$. If $DI > 1$, it is aggregated distribution. If $DI = 1$, it is random distribution. If $DI < 1$, it is uniform distribution. Use $d = \sqrt{2X^2} - \sqrt{2N - 3}$ to test the consistency of distribution types. If $d < -1.96$, accept the uniform distribution. If $d > 1.96$, accept the aggregated distribution. If $d < 1.96$, accept the random distribution.

2.3.2. Statistical Analysis

Statistical analyses were performed in SPSS 26 and Excel (Ver. 2401), and the graph was generated using Origin2021. All infection intensity data were expressed as mean \pm sd and analyzed using nonparametric test (Mann–Whitney test). Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Nematode Infection in *C. nasus*

The body length of longjaw tapertail anchovy *C. nasus* examined in this study ranged from 23 cm to 35 cm, with an average of 27.71 ± 2.36 cm. Nematode infection in *C. nasus* samples collected at Shanghai and Anqing in 2021 and 2022 were all 100% (Table 1).

Table 1. Biological characteristics of anadromous *Coilia nasus* migrating to the Yangtze River.

Site	Year	N	Body Length (cm)	Female to Male Ratio	Prevalence (%)	Mean Abundance
Shanghai section	2021	30	26.93 ± 2.26	4.00:1	100	6.97 ± 5.26
Anqing section	2021	30	27.40 ± 2.28	3.29:1	100	9.40 ± 11.78
Shanghai section	2022	23	28.16 ± 3.12	4.75:1	100	21.35 ± 21.29
Anqing section	2022	20	29.50 ± 2.64	5.67:1	100	22.00 ± 20.72

A total of 1422 nematodes were detected in 103 *C. nasus*, with a mean intensity of 13.81 ± 16.45 . The mean intensity of *C. nasus* that were sampled in 2021 and 2022 was 8.18 ± 9.12 and 21.63 ± 20.80 , respectively. The mean intensity was higher at all sampling sites in 2022 than that in 2021, and there was a significant difference ($p < 0.05$) in mean intensity at the same sampling site in 2022 compared to 2021 (Figure 2). The highest infection intensity was detected in a female *C. nasus*, which was approximately 30.10 cm in body length, with a total of 87 nematodes. Taking gender differences into account, the results indicated a significant difference in nematode infection intensities between males and females in 2021 ($p < 0.05$), whereas such sex-related characteristics were not determined in 2022 (Figure 3). In addition, the number of individuals with an intensity of 1–10 was the highest in this study (Figure 4). Finally, a not strict trend of nematodes with an increase in body length in two years was observed (Figure 5).

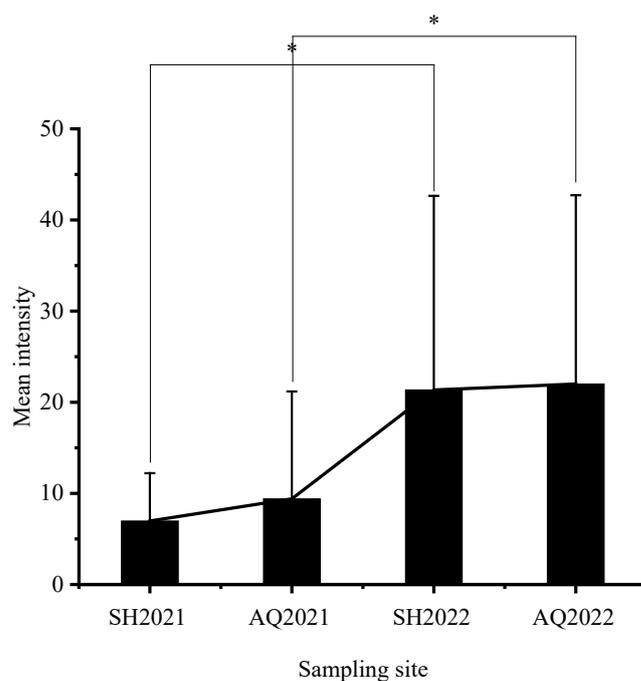


Figure 2. The variations in the mean intensity of nematodes in *C. nasus* at each sampling site across different years. Abbreviations: SH2021—Shanghai section of the Yangtze River (2021), AQ2021—Anqing section of the Yangtze River (2021), SH2022—Shanghai section of the Yangtze River (2022), AQ2022—Anqing section of the Yangtze River (2022). The numbers enclosed in parentheses indicate sampling date. Bars represent standard deviation. The '*' represents statistically significant disparities ($p < 0.05$).

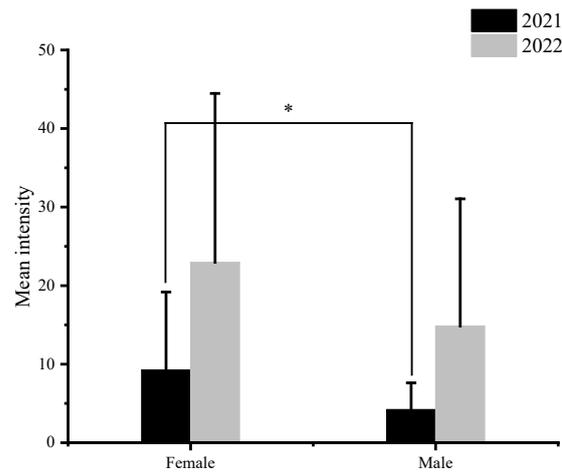


Figure 3. Relationship between the sex of *C. nasus* and the intensity of infection at various sampling time points. Bars represent standard deviation. The ‘*’ represents statistically significant disparities ($p < 0.05$).

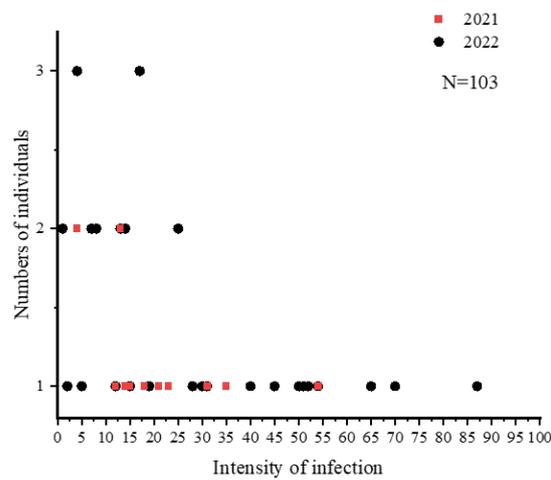


Figure 4. Number of individuals with different infection intensities of nematodes in *C. nasus* from two sampling years.

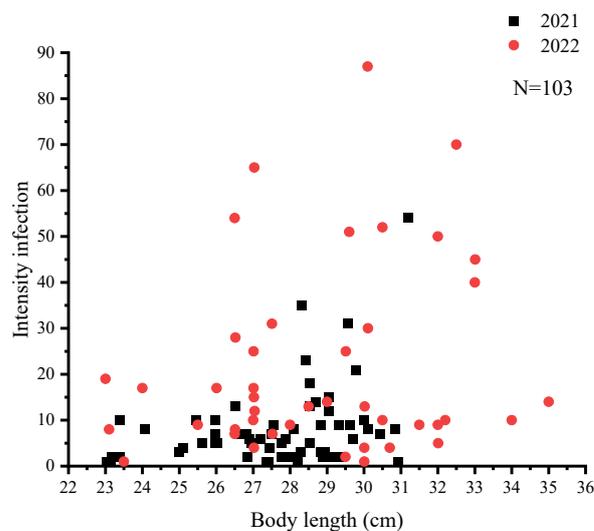


Figure 5. Relationship between the body length of *C. nasus* and the intensity of infection in two sampling years.

3.2. Infection Difference among Organs

In this study, the mesentery exhibited the highest proportion of nematodes among all tissues of *C. nasus*, with a total count of 761 nematodes, accounting for 53.52%. There were 470 nematodes in the pyloric cecum, accounting for 33.05%. In addition, the stomach and liver exhibited the lower parasitic level of nematodes, with a count of 100 and 91, accounting for 7.03% and 6.40%, respectively. The mean infection intensity of the mesentery and pyloric cecum was found to be significantly higher ($p < 0.05$) than that of other organs and tissues (Figure 6). The mesentery exhibited the highest percentage of nematode detection in *C. nasus* at various sampling locations and times, exceeding or almost 50% in all sampling locations except for the Shanghai section of Yangtze River in 2022. In addition, the stomach consistently displayed the lowest percentage, except for the Shanghai section of Yangtze River in 2022. Interestingly, no nematodes were detected in the stomach of *C. nasus* at the Anqing section of Yangtze River from 2022. Furthermore, a comparison between mean intensities of nematodes in tissues or organs from different years revealed that the mean intensity was higher in 2022 compared to 2021 (Figure 6).

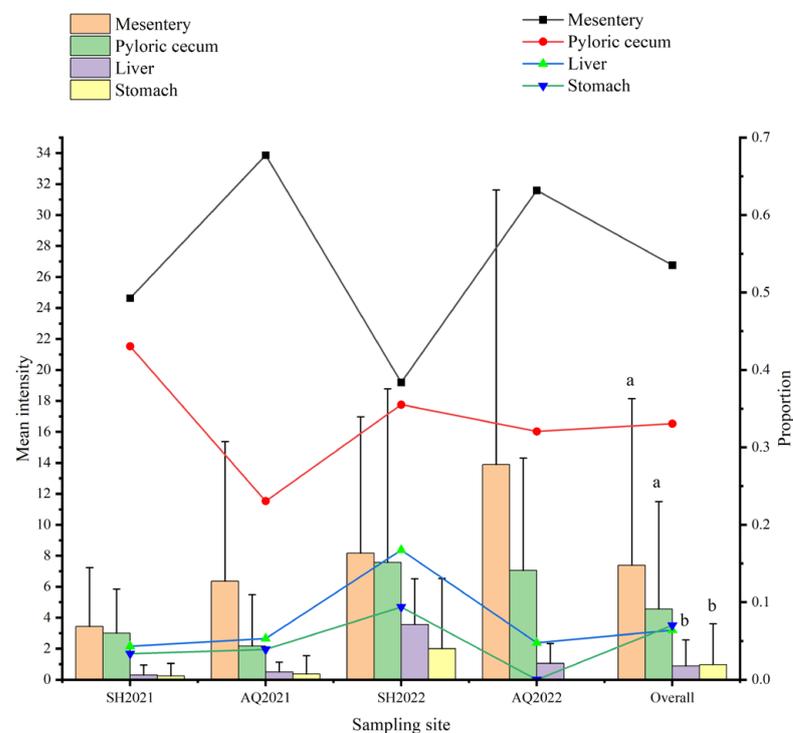


Figure 6. The distribution and mean infection intensity of parasitic nematodes in different tissues and organs of *C. nasus* at each sampling time and site. Bars represent standard deviation. The presence of distinct letters signifies statistically significant disparities ($p < 0.05$).

3.3. Composition of Nematode Species

A total of 242 ITS sequences of the 250 nematodes isolated from *C. nasus* ($n = 26$) were successfully obtained, accounting for 96.8%. Based on morphological characters and ITS sequence alignment search at the GenBank database, eight nematode species belonging to two families, Anisakidae and Raphidascarididae, were identified, respectively. There were two anisakid nematodes closely related to the following taxa in GenBank: *Anisakis pegreffii* and *Anisakis simplex*, and six raphidascaridid nematodes: *Hysterothylacium aduncum*, *Hysterothylacium fabri*, *Hysterothylacium sinense*, *Hysterothylacium amoyense*, and *Hysterothylacium* sp., *Raphidascaris* sp. (Table 2). In the 242 identified nematodes, *Raphidascaris* sp. was the dominant species, contributing 40.9% of individuals, and it was followed by *H. aduncum* and *A. pegreffii*, with 28.1% and 21.5%, respectively. *H. fabri* was 7.4%, and *H. sinense*, *H. amoyense*, and *Hysterothylacium* sp. accounted for only 0.4%, respectively (Figure 7).

Table 2. Nematodes collected from *Coilia nasus* (n = 26) and identified by ITS DNA sequence.

Nematode Species	No. of Individuals Sequenced	Sequence Identity (%)	GenBank Acc. No.	Closely Related Nematode in GenBank	
				Species (Accession)	Sequence Similarity (%)
<i>Hysterothylacium aduncum</i>	68	99.4–100	PP029287	<i>Hysterothylacium aduncum</i> (MH211517)	98.3–100
<i>Hysterothylacium fabri</i>	18	99.4–100	PP029274	<i>Hysterothylacium fabri</i> (MH211492)	99.6–100
<i>Hysterothylacium amoyense</i>	1	-	PP029291	<i>Hysterothylacium amoyense</i> (MT269312)	99.89
<i>Hysterothylacium sinense</i>	1	-	PP029292	<i>Hysterothylacium sinense</i> (MH211574)	100
<i>Hysterothylacium</i> sp.	1	-	PP034301	<i>Hysterothylacium</i> sp. (MF061683)	100
<i>Anisakis simplex</i>	2	100	PP029277	<i>Anisakis simplex</i> (MT355320)	100
<i>Anisakis pegreffii</i>	52	99.9–100	PP029289	<i>Anisakis pegreffii</i> (MH211473)	99.6–100
<i>Raphidascaris</i> sp.	99	99.0–100	PP034302	<i>Raphidascaris</i> sp. (MW370774)	99.5–100

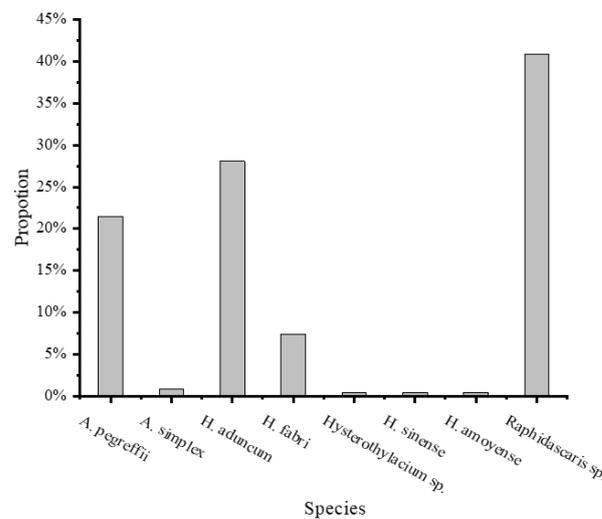


Figure 7. The species composition of nematodes isolated from *C. nasus* (n = 26).

In the 26 *C. nasus* from which nematodes were isolated and identified by ITS, *A. pegreffii* had the highest infection prevalence at 69.23%. The infection prevalence of *H. aduncum* and *Raphidascaris* sp. was both 61.54%. However, it is worth noting that the infection rate of *H. aduncum* at the Shanghai section (*C. nasus* n = 16) was 100%, and it was not detected at the Anqing section (*C. nasus* n = 10) (Figure 8).

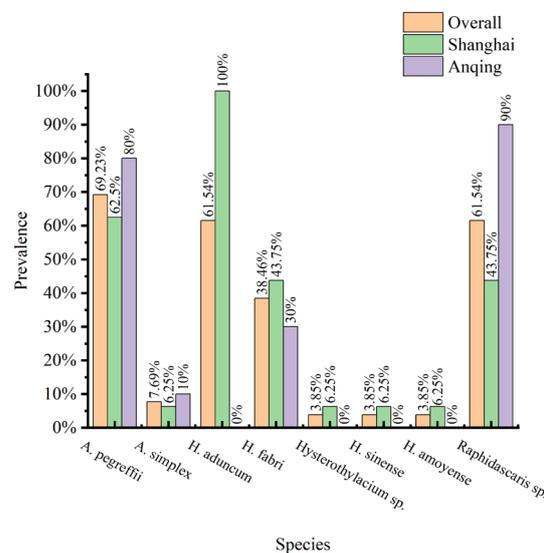


Figure 8. The species composition of nematodes isolated from *C. nasus* at each sampling site in 2022.

Figure 9 shows a phylogenetic tree constructed from eight ITS sequences of nematode specimens in this study. The nematodes from the family Raphidascarididae formed a separate clade.

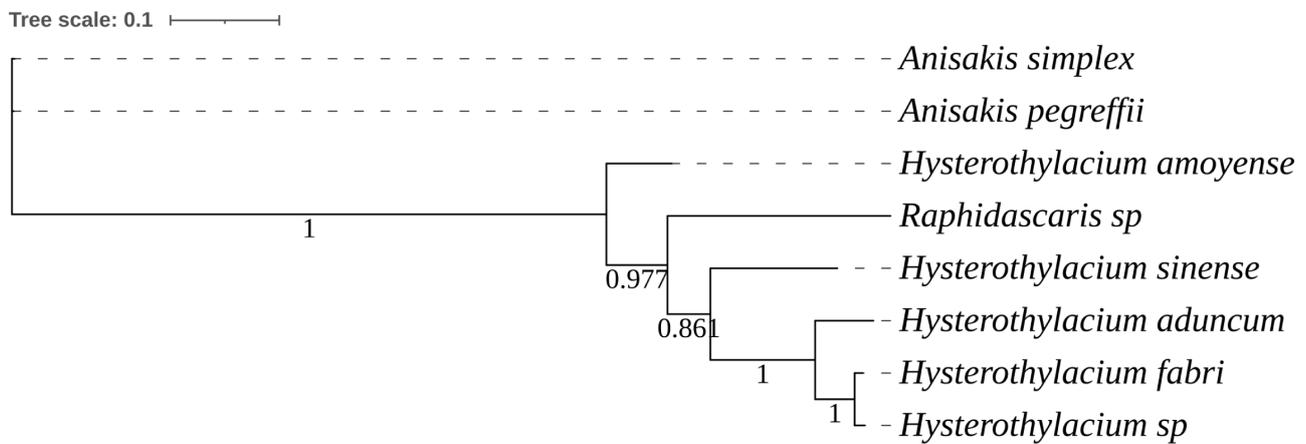


Figure 9. Bayesian inference phylogenetic tree based on the obtained sequences of ITS1-5.8s-ITS2.

3.4. Organ Tropism

Raphidascaris sp., *H. fabri*, and *H. aduncum* did not show strict organ/tissue preference, and were found in the pyloric cecum, stomach, mesentery, and liver of *C. nasus*. The dominant species *Raphidascaris* sp. consisted of 74.36%, 39.81%, and 36.21% of individuals in the stomach, mesentery, and cecum, respectively. *A. pegreffii* occurred in the pyloric cecum, mesentery, and liver, but not in the stomach, and contributed 48.65% of individual numbers in the liver. In the *C. nasus* examined here, a few *A. simplex* and *H. sinense* were detected in the mesentery and liver, respectively (Figure 10).

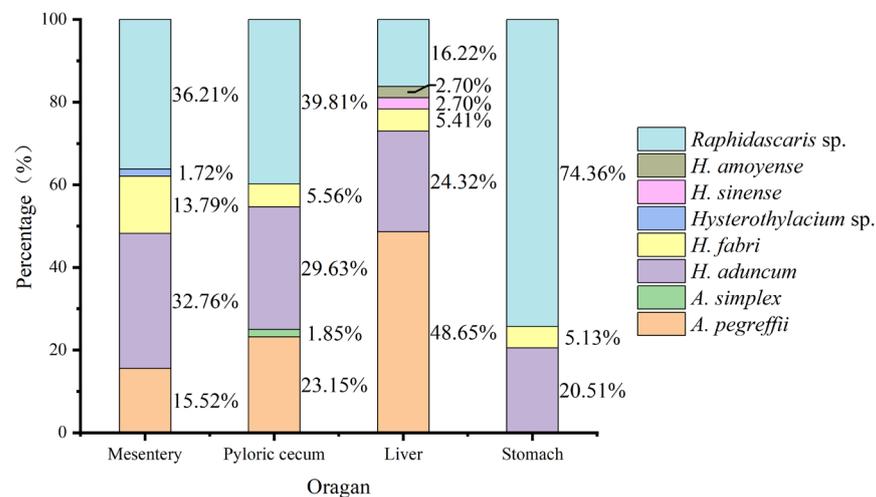


Figure 10. The proportion of parasitic nematode species identified in each organ and tissue of *C. nasus* in 2022.

3.5. Community Structure of Nematodes

The abundance of nematodes in *C. nasus* was 8, and the average richness was 2.73 ± 1.22 . The Margalef richness index was calculated as 1.28, while the Brillouin diversity index was 1.30. The Shannon–Wiener diversity index was 1.35 and the Pielou homogeneity index showed a value of 0.65. The population characteristic values of each nematode in *C. nasus* were employed to assess the distribution types (Table 3). The results showed that *Raphidascaris* sp., *A. pegreffii*, and *H. aduncum* had an aggregated distribution pattern, whereas *H. sinense*, *H. amoyense*, *Hysterothylacium* sp., and *A. simplex* displayed random distribution.

Table 3. The population characteristics of nematodes in *C. nasus*.

Nematode Species	Mean Value	Variance	Distribution Index	d	Aggregate Index	Infection Index
<i>Anisakis pegreffii</i>	2.000	4.560	2.280	3.677	0.025	1.385
<i>Anisakis simplex</i>	0.077	0.074	0.960	−0.072	−0.040	0.006
<i>Hysterothylacium aduncum</i>	2.615	7.206	2.755	4.737	0.026	1.609
<i>Hysterothylacium fabri</i>	0.692	1.022	1.476	1.589	0.028	0.266
<i>Hysterothylacium</i> sp.	0.038	0.038	1.000	0.071	-	0.001
<i>Hysterothylacium sinense</i>	0.038	0.038	1.000	0.071	-	0.001
<i>Hysterothylacium amoyense</i>	0.038	0.038	1.000	0.071	-	0.001
<i>Raphidascaris</i> sp.	3.808	32.882	8.636	13.779	0.078	2.343

Furthermore, community characteristics of nematodes in *C. nasus* from the Shanghai and Anqing sections of the Yangtze River were assessed, respectively (Tables 4 and 5). *Raphidascaris* sp. was found to have an aggregated distribution, while *A. pegreffii* had an aggregated distribution at the Shanghai section but not in the Anqing section.

Table 4. The population characteristics of nematodes in *C. nasus* at Shanghai section of the Yangtze River.

Nematode Species	Mean Value	Variance	Distribution Index	d	Aggregate Index	Infection Index
<i>Anisakis pegreffii</i>	1.625	4.783	2.944	4.012	0.078	1.016
<i>Anisakis simplex</i>	0.063	0.063	1.000	0.092	-	0.004
<i>Hysterothylacium aduncum</i>	4.250	4.600	1.082	0.313	0.001	4.250
<i>Hysterothylacium fabri</i>	0.750	1.000	1.333	0.939	0.030	0.328
<i>Hysterothylacium</i> sp.	0.063	0.063	1.000	0.092	-	0.004
<i>Hysterothylacium sinense</i>	0.063	0.063	1.000	0.092	-	0.004
<i>Hysterothylacium amoyense</i>	0.063	0.063	1.000	0.092	-	0.004
<i>Raphidascaris</i> sp.	3.938	44.863	11.394	13.103	0.168	1.723

Table 5. The population characteristics of nematodes in *C. nasus* at Anqing section of the Yangtze River.

Nematode Species	Mean Value	Variance	Distribution Index	d	Aggregate Index	Infection Index
<i>Anisakis pegreffii</i>	2.600	4.044	1.556	1.168	0.022	2.080
<i>Anisakis simplex</i>	0.100	0.100	1.000	0.120	-	0.010
<i>Hysterothylacium fabri</i>	0.600	1.156	1.926	1.765	0.185	0.180
<i>Raphidascaris</i> sp.	3.600	16.489	4.580	4.957	0.102	3.240

4. Discussion

The longjaw tapertail anchovy *C. nasus* is a commercially valuable fish in China, and was also listed as an endangered (EN) species in a 2018 Red List of Threatened Species report from the International Union for Conservation of Nature (IUCN) (www.iucnredlist.org (accessed on 15 October 2023)) [32,33]. In this study, anadromous *C. nasus* collected from the channel of the Yangtze River at Shanghai and Anqing sections in 2021 and 2022 showed a very high infection rate of nematodes, with 100% prevalence and a mean intensity of 13.81 ± 16.45 (Table 1, Figure 2). In a *C. nasus* individual, even over 80 nematodes were detected. Eight ascaridoid nematodes belonging to the family Anisakidae and Raphidascarididae were detected and identified in the internal organs, body cavity, mesentery, pyloric cecum, stomach, and liver. The nematode infection posed significant challenges to the health of *C. nasus*, and the high infection rate of *Anisakis* spp. was a potential zoonosis risk to the consumer. Additionally, due to the limitation of visual examination employed in this study, some nematode larvae, such as *Contracaecum* spp. and *Spiroxys* spp., with a low infection rate in *C. nasus* may be overlooked [34–36].

In the present study, nematode infection in *C. nasus* was 100%, which is much higher than previous studies [24,25,28,36]. And, it is worth noting that the mean intensity of nematodes in *C. nasus* between 2021 and 2022 was significantly increased from 8.18 ± 9.12

to 21.63 ± 20.80 . The mean intensity in 2022 was the highest recorded, at almost twice the mean intensity of 10.16 ± 9.67 reported by Dai et al. [25] in 2018. This could be attributed to the fact that *C. nasus* consumed a substantial number of copepods and krill that had been infected by many nematodes, indirectly indicating the severity of offshore organism infections. Meanwhile, according to the results in the present study, the infection rate of nematodes has no significant correlation with the body length. However, if the host diet does not change, the infection rate and intensity of parasites are usually positively correlated with body length [37]. This discrepancy in *C. nasus* may be attributed to the larger individuals being parasitized with more nematodes, leading to potential damage or mortality during migration processes and consequently resulting in lower nematode abundance in the large *C. nasus* examined here.

The intensity of nematodes in *C. nasus* showed no significant variation during the migration at the Shanghai and Anqing sections in the Yangtze River (Figure 2). This could be ascribed to nematodes being in the abdominal cavity and not being susceptible to osmotic pressure generated by migration and changes in the external environment [24]. The nematodes were frequently detected from the mesentery, pyloric cecum, stomach, and liver of *C. nasus*. Specifically, the highest percentage of infection was found in the mesentery, followed by the pyloric cecum, while the lowest percentage was observed in the stomach or liver. In this study, there were a total of 53.52% nematodes parasitized in the mesentery and 33.05% in the pyloric cecum. The results were consistent with the report of Dai et al. [25]. In addition, nematodes in *C. nasus* exhibited organ preference, with the highest prevalence observed in the mesentery, constituting over 50% of total nematode occurrences at each site.

Nematode infection caused a certain degree of damage to the liver of *C. nasus* (Figure 1), and this phenomenon was also reported by Liu et al. [10]. Meanwhile, due to density or developmental requirements, existing nematodes in the host migrated among different organs [38,39]. Balbuena et al. [38] reported that the larvae of *H. aduncum* could be transferred from the rectum to brain in herring under laboratory rearing conditions. The migration of nematodes would lead to the puncture of the stomach and other organs, which is associated with inflammation and organ damage. Previous studies have demonstrated that ascaridoid infection elicits inflammatory responses and adaptive immunity in *C. nasus* [28,40,41]. A high nematode infection rate may result in mortality during migration and failure to reach spawning grounds, consequently contributing to a decline in the *C. nasus* population.

The species composition and abundance of parasites infecting fish are affected by a variety of factors, such as the physicochemical properties of the water, feeding, and the seasons [42]. The anadromous *C. nasus* exhibits a complex life cycle characterized by feeding offshore and breeding in freshwater (the Yangtze River and its tributaries), followed by a return to diverse habitats encompassing seawater and brackish water after spawning [43]. In this study, eight ascaridoid nematodes were detected in the population of *C. nasus* migrating from offshore to the spawning grounds. All of these identified nematodes are known as parasites in marine environments [44]. However, nematode *Contracaecum* spp. and *Eustrongylides* spp., which were previously reported by Song [36], were not detected in this survey. The difference may be attributed to either the change in nematode community in marine environments or the limitations of the parasite investigation in this study. Moreover, some nematodes had a stronger lethal ability, such as *Eustrongylides* spp. [45], and result in the death of the infected *C. nasus*. Meanwhile, no freshwater nematodes were detected in the *C. nasus* populations as per the previous report [24]. *C. nasus* refrained from feeding during migration and avoided the infection of freshwater nematodes [46]. Ascaridoid nematodes mainly infect marine fish, and have been detected in fish, krill, and other animals in the East China Sea and the Yellow Sea, exhibiting a broad range of hosts [47,48]. *C. nasus* serves as a common second intermediate host of ascaridoid nematodes [49]. Krill and copepods, as first intermediate hosts of ascaridoid nematodes, are the main food sources of *C. nasus*. *C. nasus* was infected by nematodes mainly through the intaking of krill harboring the larvae of nematodes. Therefore, the variation in nematode species and dominant species observed in this study may be attributed to the changes in

natural baits and parasites in coastal waters. In recent reports, a decline in ascaridoids in marine fish has been observed in Japan and Australia [50,51], and this has been ascribed to lower populations of zooplankton and crustaceans [52]. Here, the inconsistent observations were not clear, and further studies are needed to assess the nematode infection in marine fish from China's offshore water.

Tissue or organ tropism in many parasites is essential for their development and transmission. In nematode genus *Contracaecum*, *Contracaecum radiatum* was often found in the gastric wall of the fish host [53]; however, *Contracaecum osculatum* preferred the liver and body cavity [54]. In arctic ice fish, Ning [55] also discovered that a significant proportion of nematodes parasitized in the liver due to the larger volume and more abundant nutritional resources compared to other tissues and organs. In *C. nasus* detected here, *A. pegreffii*, *Raphidascaris* sp., and *H. aduncum* were the dominant parasitic nematodes; however, no obvious organ/tissue preference was observed (Figure 10), and they were found in the pyloric cecum, stomach, mesentery, and liver of *C. nasus*. *A. pegreffii* accounted for the highest proportion (48.65%) in the liver, and *Raphidascaris* sp. had the highest proportions in the other tissues: 36.21% in the pyloric cecum, 39.81% in the mesentery, and 74.36% in the stomach. Interestingly, *A. simplex* and *H. sinense* were only detected in the mesentery and liver, respectively. However, in this study, only limited nematodes from 26 *C. nasus* individuals were identified using both morphological and molecular data. Therefore, the tissue or organ distribution of nematodes with a low infection rate may be overlooked.

The application of parasites as biological indicators for the fish host population and environment assessment has recently attracted much attention [56,57]. Xu et al. [22] found that *A. simplex* only infected the anadromous *C. nasus* in the Yangtze River, and was not detected in the freshwater residents *C. nasus*. *A. simplex* was considered as a "biological indicator" of the anadromous *C. nasus* in the Yangtze River. Li et al. [23] also reported that parasites infecting *C. nasus* could be used to indicate the migration between the coast, estuary, and Yangtze River. However, according to the recent parasite investigation on anadromous *C. nasus* in the Yangtze River, *A. simplex* had a low infection rate, while *A. pegreffii*, *Raphidascaris* sp., and *H. aduncum* were the common and dominant parasites [25,26].

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