

Host-Associated Genetic Diversity and Phylogenetic Patterns of Raphidascarididae Nematodes in Iraqi Marine Fish Based on ITS-1 Sequences

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Abstract— A total of 24 nematode specimens belonging to the family *Raphidascarididae* were collected from three ecologically and economically important marine fish species inhabiting Iraqi marine waters: *Saurida macrolepis* Tanaka, 1917, *Nemipterus japonicus* (Bloch, 1791), and *Platycephalus indicus* (Linnaeus, 1758). The present study aimed to assess the degree of genetic variability and to explore the possible evolutionary relationships among the sampled nematode populations. Phylogenetic analysis based on the **Neighbor-Joining** method revealed that the *Raphidascarididae* specimens were divided into four main clades, supported by bootstrap values ranging from 48% to 87%, indicating a largely independent evolutionary history with varying degrees of genetic relatedness among lineages. Nematodes isolated from *S. macrolepis* exhibited the highest level of genetic divergence, suggesting pronounced differentiation that may reflect lineage-specific adaptation to the host or differences in transmission pathways. In contrast, nematodes recovered from *N. japonicus* formed a tightly clustered genetic group, supporting the presence of a host-specific parasite population. Similarly, specimens from *P. indicus* showed clear ecological and genetic structuring, indicating substantial intraspecific variability within this host-associated parasite population. The occurrence of shared gene sequences among nematodes collected from different host species suggests the possibility of host-switching events, most plausibly facilitated by co-feeding behavior among sympatric fish hosts. Overall, the findings demonstrate that parasite ecology including feeding behavior, spatial distribution, and trophic interactions plays a critical role in shaping the genetic differentiation of *Raphidascarididae* nematodes. The study further supports the concept of long-term coevolution between these parasites and their marine fish hosts, providing valuable insights into the evolutionary history of marine nematodes and establishing a

foundation for future investigations across diverse ecological settings.

Keywords — *Raphidascarididae*, ITS-1, host-associated genetic diversity, marine fish, phylogenetic patterns.

INTRODUCTION

Despite the well-recognized ecological and economic impacts of marine parasites, molecular studies on fish parasites in the Arabian Gulf remain extremely limited. In particular, there is a notable lack of research examining how host ecological specialization influences genetic diversity and population structure in parasitic organisms. Most studies conducted in the region have focused primarily on traditional parasitological surveys, leaving host-associated genetic differentiation and evolutionary patterns largely unexplored.

The present study contributes to addressing this knowledge gap by providing a molecular investigation of *Raphidascarididae* nematodes infecting economically important marine fish species, including *Saurida macrolepis*, *Nemipterus japonicus*, and *Platycephalus indicus*, from Iraqi marine waters of the Arabian Gulf. Members of the family *Raphidascarididae* are among the most significant parasitic nematodes affecting marine and aquaculture systems, predominantly inhabiting the digestive tract of teleost fishes, although infections may extend to other organs. Such infestations can impair host growth, reproduction, and survival, thereby posing serious ecological and economic concerns (1,2). The prevalence and intensity of these infections are strongly influenced by environmental factors, particularly water temperature and salinity, and are generally higher in tropical and temperate marine environments (2).

Understanding the evolutionary relationships between parasites and their hosts is essential for interpreting patterns of genetic diversity, host specificity, and population differentiation, as well as for evaluating the broader ecological consequences of parasitism. Molecular markers, particularly

the internal transcribed spacer 1 (ITS-1) region of ribosomal DNA, have proven effective in revealing cryptic diversity, host-associated structuring, and evolutionary trajectories in marine parasitic nematodes (3).

In this study, ITS-1 sequence data were employed to investigate the phylogenetic relationships and genetic variation of *Raphidascarididae* nematodes collected from three marine fish hosts. Phylogenetic reconstructions using Neighbor-Joining and Maximum Likelihood methods were conducted based on 24 nematode specimens to assess genetic differentiation and infer evolutionary patterns. By integrating molecular evidence with host ecological context, this research aims to identify potential host-driven divergence and cryptic diversification within *Raphidascarididae*. Similar molecular approaches applied to *Hysterothylacium* species infecting Iraqi marine fishes have demonstrated the importance of combining genetic data with host ecology to elucidate parasite population structure and evolutionary patterns (4–6). Furthermore, integrative morphological and molecular studies have proven effective in resolving taxonomic complexity and documenting parasite biodiversity in Iraqi marine waters (7). Collectively, these findings provide essential baseline data that support marine biodiversity documentation, contribute to parasite monitoring and risk assessment, and inform sustainable management strategies for fisheries and aquaculture in the Arabian Gulf.

MATERIALS AND METHODS

Three marine fish species the greater lizardfish *Saurida tumbil* (Tanaka, 1917), the Japanese threadfin bream *Nemipterus japonicus* (Bloch, 1791), and the bartail flathead *Platycephalus indicus* (Linnaeus, 1758) were collected from the Arabian Gulf in Iraqi waters during 2024. Fish specimens were obtained from local fish markets in Al-Faw, southern Iraq, and were identified based on standard taxonomic keys and validated using global ichthyological databases, including FishBase (8).

A total of 60 *Raphidascarididae* nematodes (20 individuals per host species) were recovered from the examined fish and preserved in 70% ethanol. All sampling procedures complied with ethical standards and local regulations governing marine biodiversity conservation.

Genomic DNA was extracted from individual nematodes using the Geneaid DNA Isolation Kit, following the manufacturer's protocol. The internal transcribed spacer 1 (ITS-1) region of ribosomal DNA was amplified using universal nematode primers. PCR products were visualized by agarose gel electrophoresis, purified, and sequenced bidirectionally using the Sanger method on an ABI 3500 Genetic Analyzer.

Sequence data were edited and aligned with reference sequences retrieved from GenBank using the ClustalW algorithm implemented in MEGA X. Phylogenetic relationships were inferred using the Neighbor-Joining method based on 24 representative sequences, with 1,000 bootstrap replicates to assess nodal support.

RESULT AND DISCUSSION

Genetic Diversity and Phylogenetic Patterns of *Raphidascarididae* Nematodes

A total of 24 representative ITS-1 sequences of *Raphidascarididae* nematodes obtained from three marine fish hosts *Saurida tumbil*, *Nemipterus japonicus*, and *Platycephalus indicus* were analyzed. Representative sequences were selected based on sequence quality and haplotype uniqueness to reduce redundancy while preserving the observed genetic diversity.

Analysis of the ITS-1 region revealed substantial genetic variation among the examined nematode samples. Neighbor-Joining (NJ) phylogenetic reconstruction resolved the sequences into four major clades with bootstrap support values ranging from 48% to 87% (Figure 2). Considerable variation in branch lengths was observed across the tree, reflecting different degrees of genetic divergence among lineages.

Clade I grouped *Hysterothylacium* sp. and *H. amoyense* sequences derived mainly from *S. tumbil* and *P. indicus*, with moderate bootstrap support (48–49%). Clade II included *Hysterothylacium* sp. and *H. amoyense* isolates from *S. tumbil* and *N. japonicus*, supported by similar bootstrap values (48–49%). Clade III comprised *H. amoyense* and *Hysterothylacium* sp. sequences recovered from *P. indicus* and *N. japonicus*, with bootstrap support ranging from 48% to 57%. In contrast, Clade IV showed the highest bootstrap support (up to 87%) and included *H. amoyense* and *Hysterothylacium* sp. sequences from additional paratenic or intermediate hosts reported in GenBank, such as *Lophius piscatorius* and *Johnius glaucus*.

ITS-1 sequences obtained from *S. tumbil* were distributed across multiple clades (e.g., A6_C3 ITS1, A7_C4 ITS1), indicating pronounced genetic heterogeneity. In contrast, sequences from *N. japonicus* (e.g., C13 ITS1, C19 ITS1, C21 ITS1) formed a compact and well-defined cluster characterized by short branch lengths. Sequences obtained from *P. indicus* (e.g., C31 ITS1, C28 ITS1, C49 ITS1) exhibited comparatively higher genetic divergence, with several isolates occupying distinct branches separated by longer genetic distances.

All ITS-1 sequences generated in this study were deposited in the NCBI GenBank database (Table 1).

Table 1. NCBI GenBank accession numbers of ITS-1 sequences of *Raphidascarididae* nematodes isolated from three marine fish hosts (*Saurida macrolepis*, *Nemipterus japonicus*, and *Platycephalus indicus*) collected from Iraqi marine waters of the Arabian Gulf.

Host species	Accession number	Isolate ID
<i>Saurida macrolepis</i>	PQ811749	C47 ITS1
	PQ812518	C11 ITS1
	PQ812545	C23 ITS1
	PQ811740	C10 ITS1
	PQ811738	C8 ITS1
	PQ811737	C7 ITS1
	PQ811131	C6 ITS1
	PQ811120	C5 ITS1
	PQ810057	C1 ITS1
	<i>Nemipterus japonicus</i>	PQ812523

	PQ812524	C14 ITS1
	PQ812525	C15 ITS1
	PQ812527	C16 ITS1
	PQ812528	C17 ITS1
	PQ812529	C18 ITS1
	PQ812530	C19 ITS1
	PQ812533	C20 ITS1
	PQ812535	C21 ITS1
	PQ812536	C22 ITS1
	PQ812537	C48 ITS1
<i>Platycephalus indicus</i>	PQ812553	C27 ITS1
	PQ812554	C28 ITS1
	PQ812558	C29 ITS1
	PQ812568	C30 ITS1
	PQ812586	C48 ITS1
	PQ812588	C49 ITS1
	PQ819507	C32 ITS1

Host-Associated Structuring and Trophic-Related Patterns

The NJ phylogenetic tree based on ITS-1 sequences (Fig. 3) revealed clear host-associated genetic structuring among *Raphidascarididae* nematode isolates. Several clusters were observed in which isolates grouped predominantly according to host species, although partial intermixing among hosts was evident.

Isolates recovered from *Nemipterus japonicus* (e.g., C13 ITS1, C17 ITS1, C18 ITS1, C19 ITS1, C20 ITS1, C21 ITS1, and C22 ITS1) formed a relatively compact cluster with short branch lengths, indicating high genetic similarity among parasites associated with this host. In contrast, isolates from *Saurida tumbil* (e.g., C2 ITS1, C3 ITS1, C4 ITS1, C5 ITS1, C7 ITS1, and C8 ITS1) were distributed across multiple branches, reflecting greater genetic heterogeneity.

Nematodes isolated from *Platycephalus indicus* (e.g., C27 ITS1, C28 ITS1, C29 ITS1, C30 ITS1, C31 ITS1, and C32 ITS1) showed increased genetic dispersion, with several isolates forming distinct branches separated by longer genetic distances. Shared ITS-1 haplotypes were detected between isolates from *S. tumbil* and *N. japonicus* (e.g., C10 ITS1 and C14 ITS1), whereas no identical haplotypes were observed between *P. indicus* and the other two host species.

Branch length variation across the phylogenetic tree further illustrated differing degrees of genetic divergence among parasite lineages, as indicated by the scale bar (0.05 substitutions per site). These patterns highlight distinct genetic structuring of *Raphidascarididae* nematodes in relation to their fish hosts and associated trophic pathways.

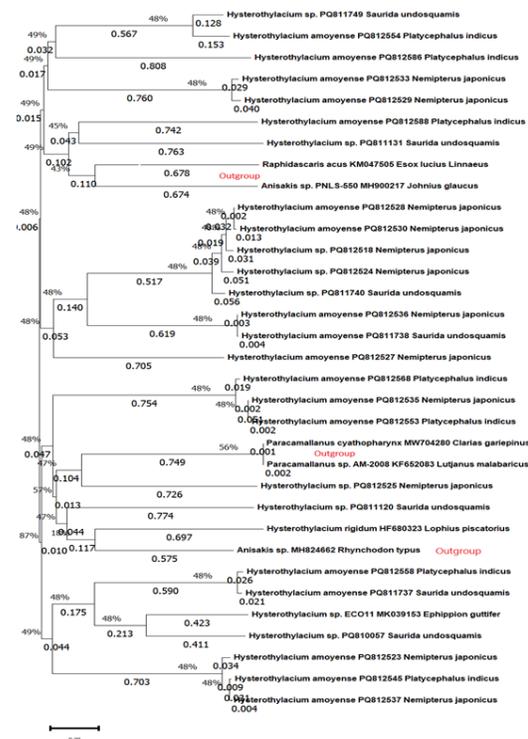


Figure 2. The phylogenetic relationships of the taxa using the Neighbor-Joining method, focus on *Raphidascarididae* nematodes analyzed in the present study.

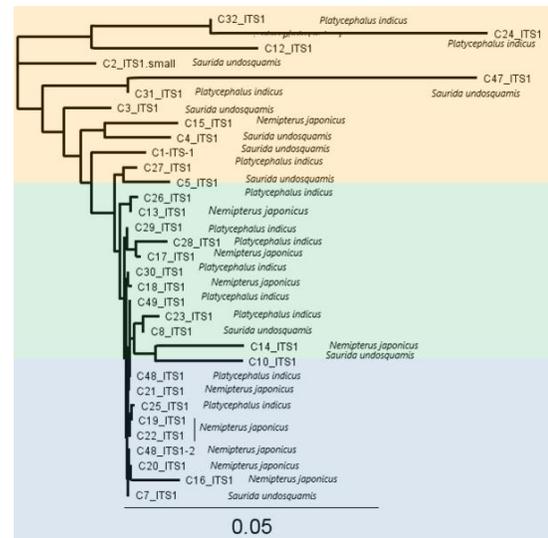


Figure 3. Neighbor-Joining phylogenetic tree based on ITS-1 sequences of *Raphidascarididae* nematodes. Colored background shading indicates host association (blue: *Saurida tumbil*; green: *Nemipterus japonicus*; orange: *Platycephalus indicus*) and is provided for visual guidance only; branch lengths and topology are not affected.

Genetic Resolution and Phylogenetic Structuring Based on ITS-1

The present study confirms that the ribosomal ITS-1 region provides sufficient genetic resolution to detect phylogenetic structuring and host-associated genetic patterns within

Raphidascarididae nematodes from Iraqi marine waters. Although ITS-1 is a non-coding marker, its relatively high mutation rate and concerted evolution make it particularly informative for population-level differentiation and interspecific comparisons in parasitic nematodes (9,14,17).

The recovery of four phylogenetic clades with variable bootstrap support reflects substantial intra- and interspecific genetic differentiation within *Hysterothylacium* populations. Although bootstrap values were moderate in some clades, similar levels are commonly reported in ITS-based analyses of anisakid and *Raphidascarididae* nematodes and are considered sufficient for identifying phylogenetic structuring and host-associated patterns rather than deep evolutionary relationships (19,20,7). Accordingly, the observed clustering patterns provide meaningful insights into genetic differentiation linked to host association and ecological context.

Host-Associated Genetic Structuring and Ecological Drivers

Marked differences in genetic structuring were observed among nematodes associated with different host species, indicating that host ecology plays a central role in shaping parasite population structure. Nematodes isolated from *Saurida tumbil* were distributed across multiple clades, suggesting relatively high genetic mixing. This pattern may reflect the pelagic or semi-demersal feeding behavior of *S. tumbil* and its interaction with a broad range of prey items, which can facilitate parasite transmission and gene flow among ecologically connected hosts (12,13).

In contrast, nematodes recovered from *Nemipterus japonicus* formed a compact and well-defined genetic cluster, indicative of stronger host-associated population structuring. Such clustering may arise from ecological fidelity, host-mediated selection, or limited trophic overlap with other fish species, all of which can restrict parasite dispersal and promote genetic cohesion within host-specific parasite populations (21,15).

Trophic Pathways, Habitat Specialization, and Genetic Divergence

Nematodes associated with *Platycephalus indicus* exhibited comparatively higher genetic divergence, which is likely linked to the benthic feeding strategy and habitat specialization of this host. Benthic feeders typically exploit more spatially restricted prey assemblages, potentially reducing opportunities for parasite exchange with pelagic or semi-pelagic hosts. Such ecological isolation can limit gene flow among parasite populations and promote lineage divergence over time (11,18).

The absence of shared ITS-1 haplotypes between *P. indicus* and the other examined hosts further supports the role of habitat specialization and trophic segregation in shaping parasite genetic structure. Comparable patterns have been documented in other marine nematode systems, where host feeding ecology and habitat use act as primary drivers of parasite diversification (19,17).

Host Switching, Gene Flow, and Co-feeding Interactions

The detection of shared ITS-1 haplotypes between nematodes isolated from *S. tumbil* and *N. japonicus* suggests potential host-switching events or ongoing gene flow among

parasite populations. Such processes are often facilitated by overlapping trophic pathways, shared prey resources, or co-feeding interactions within the same ecological niche [(5,18). This interpretation is made conservatively and is based on patterns of genetic overlap combined with ecological context rather than direct experimental evidence.

Host switching is increasingly recognized as a major mechanism driving parasite diversification in marine systems, particularly in semi-enclosed environments such as the Arabian Gulf, where ecological connectivity among hosts may enhance transmission opportunities (21,16).

Taxonomic Considerations and Future Perspectives

Species names applied in this study (e.g., *Hysterothylacium* sp. and *H. amoyense*) are used in a comparative framework based on available GenBank reference sequences and do not imply definitive taxonomic resolution. Given the genetic complexity observed within and among clades, future studies incorporating additional nuclear and mitochondrial markers, such as COI or complete rDNA arrays, will be essential to refine species boundaries, detect cryptic diversity, and clarify evolutionary relationships within *Raphidascarididae* (10,17,22).

Implications for Parasite Evolution in the Arabian Gulf

Overall, this study highlights the pivotal role of host ecology particularly feeding strategy, habitat preference, and trophic interactions in shaping genetic diversity and phylogenetic patterns of *Raphidascarididae* nematodes in the semi-enclosed marine system of the Arabian Gulf. By integrating molecular genetic data with host and ecological context, the present findings contribute to a broader understanding of parasite–host coevolution, population divergence, and adaptive diversification in marine parasites. Such insights are essential for advancing parasite evolutionary theory and for informing fisheries management and aquaculture health strategies in the region (15,2).

CONCLUSION

The analysis of ITS-1 sequences in the present study revealed clear genetic variation and distinct phylogenetic patterns among *Raphidascarididae* nematodes infecting marine fish hosts from Iraqi waters of the Arabian Gulf. The observed genetic structuring indicates that parasite populations are shaped by both host-associated and ecological factors. Nematodes associated with *Platycephalus indicus* exhibited pronounced genetic divergence, suggesting a combination of ecological specialization and reduced gene flow, likely driven by habitat preference and benthic feeding behavior of the host. In contrast, parasites recovered from *Saurida macrolepis* and *Nemipterus japonicus* showed lower levels of genetic clustering, indicating greater genetic connectivity and the potential for transmission among host species sharing overlapping ecological niches.

Phylogenetic analyses further demonstrated that evolutionary trajectories of *Raphidascarididae* nematodes are closely linked to host ecology and environmental conditions within the semi-enclosed marine system of the Arabian Gulf. These findings provide new insights into host–parasite coevolution and highlight the importance of integrating molecular data with ecological context to understand parasite

diversification. Future studies incorporating additional mitochondrial and nuclear genetic markers, along with broader host and geographic sampling, are recommended to further resolve species boundaries and evolutionary relationships within this group.

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