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Integrative Taxonomy of Marine Helminths from Carangoides spp.: Evidence from Morphology, ITS2, 28S rDNA and COI Gene Sequences

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Abstract

Marine carangid fishes of the genus Carangoides harbour diverse helminth assemblages including digeneans, monogeneans, and nematodes. Traditional morphology-based taxonomy has historically guided species descriptions, yet phenotypic plasticity and cryptic speciation frequently obscure species boundaries. Integrative taxonomy combining morphological evidence with molecular markers such as ITS2, 28S rDNA, and mitochondrial COI has significantly advanced marine helminth systematics. This study synthesizes evidence from various open-access studies demonstrating the complementary roles of ribosomal and mitochondrial markers in species delimitation, phylogenetic placement, and life-stage linkage. The literature consistently supports a multilocus framework, particularly for parasites infecting carangid hosts in Indo-Pacific systems. Integrative approaches reveal hidden diversity, clarify host specificity patterns, and provide robust phylogenetic context. For helminths infecting Carangoides spp., morphology combined with ITS2, 28S rDNA, and COI represents the current gold standard for accurate taxonomic resolution.

Keywords: Integrative taxonomy; Marine helminths; Carangoides; ITS2; 28S rDNA; COI; Monogenea; Digenea; DNA barcoding; Host–parasite relationships

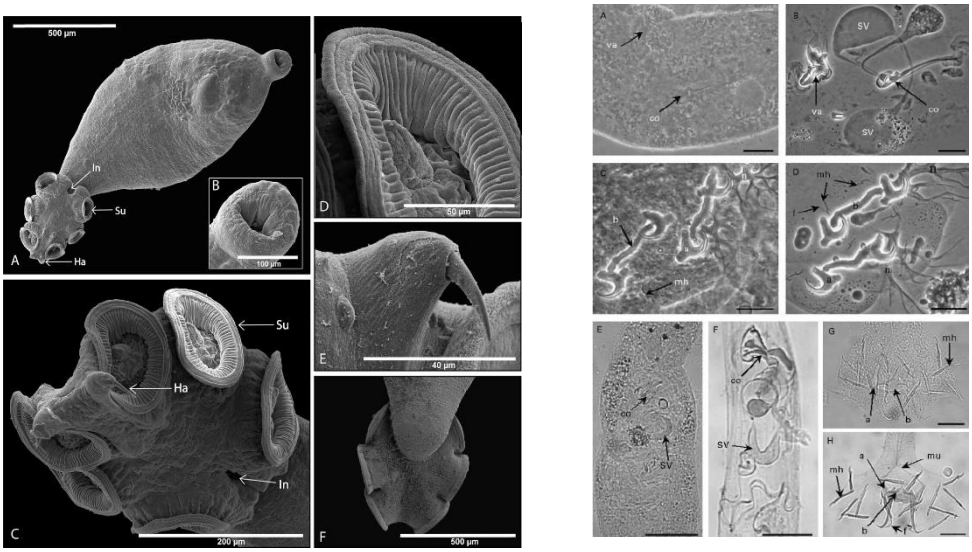
1. Introduction

Marine teleosts support extensive parasite biodiversity, yet taxonomic resolution remains incomplete for many helminth groups. Carangid fishes, including species of Carangoides, are ecologically significant predators widely distributed across tropical and subtropical oceans. These hosts harbour diverse

helminths, particularly monogeneans and digeneans, whose taxonomy has historically depended on morphological traits such as haptoral structures, reproductive anatomy, and body proportions (Justine et al., 2013; Mendoza-Franco et al., 2018). However, morphological convergence, ontogenetic variation, and environmentally induced plasticity frequently complicate species delimitation (Vanhove et al., 2013). Studies on marine trematodes and monogeneans increasingly report cryptic species complexes that cannot be reliably distinguished through morphology alone (de León et al., 2021; Schols et al., 2020).

Figure 1: Representative species of Carangoides (Carangidae)

Molecular systematics has therefore become central to modern helminth taxonomy. Nuclear ribosomal markers such as 28S rDNA provide stable phylogenetic frameworks at family and genus levels (Mendoza-Franco et al., 2018), while ITS2 offers greater variability suitable for distinguishing closely related taxa and linking larval and adult stages (de León et al., 2021). The mitochondrial COI gene, widely used in DNA barcoding, frequently reveals cryptic lineages within morphologically defined species (Vanhove et al., 2013; Justine et al., 2013). Recent integrative studies demonstrate that combining morphology with 28S, ITS2, and COI enhances taxonomic accuracy and reproducibility (Rojas et al., 2025; Narciso et al., 2024). This is particularly relevant for parasites of Carangoides spp., where overlapping morphological traits and wide geographic distributions increase the likelihood of hidden diversity (Tambireddy et al., 2016).



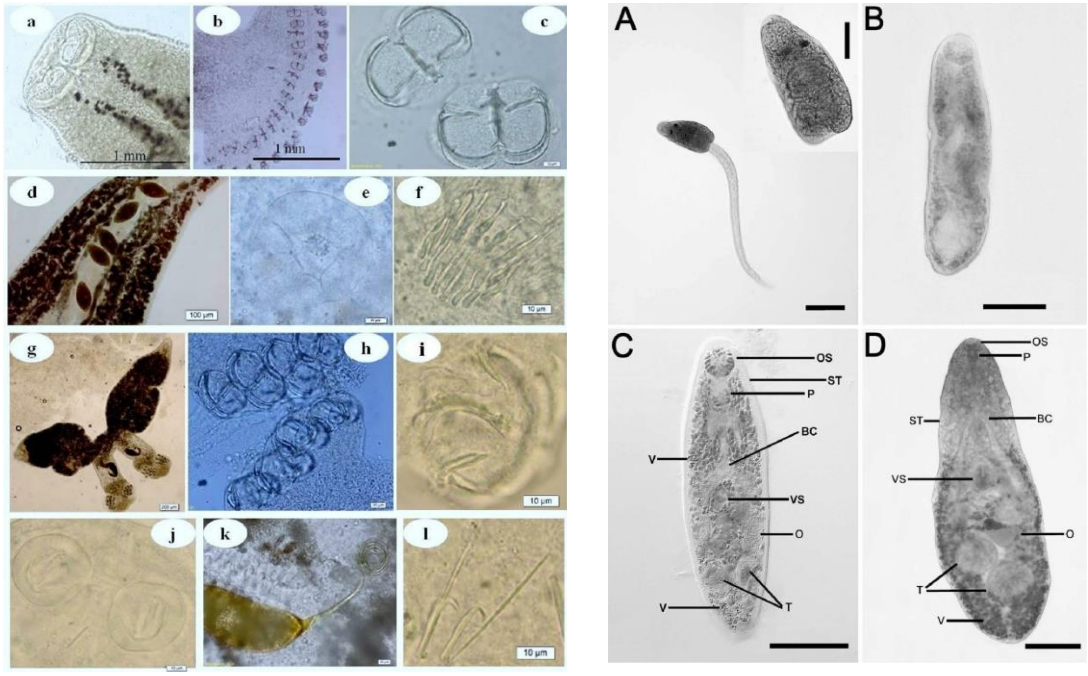


Figure 2: Diagnostic morphological characters used in helminth taxonomy.
(A) Monogenean haptor with clamps/anchors.
(B) internal reproductive structures of trematodes.
(C) copulatory organ morphology in monogeneans.
(D) stained whole mount of a digenean trematode internal reproductive structures of trematodes.

2. Methodology

A structured search of databases including PubMed Central, Parasite, PLOS ONE, Frontiers, and institutional repositories was conducted. Keywords used were marine helminths, integrative taxonomy, ITS2, 28S rDNA, COI, monogenean, digenean, carangid, and Carangoides. Inclusion criteria required availability, application of morphology combined with at least one molecular marker (ITS2, 28S rDNA, or COI), and relevance to marine fish parasites. Studies meeting these criteria were selected. Data extracted included parasite group, host taxa, geographic region, molecular markers used, and principal taxonomic findings.

3. Results

Across the reviewed studies, integrative approaches consistently combined detailed morphological documentation with molecular phylogenetic analysis. 28S rDNA was the most frequently employed marker for resolving higher-level relationships, ITS2 provided reliable species-level discrimination, and COI revealed cryptic diversity within morphologically defined taxa. Studies involving carangid hosts confirmed placement of monogeneans within Protomicrocotylidae and clarified species boundaries using multilocus datasets (Tambireddy et al., 2016). Several trematode studies demonstrated successful linkage of larval and adult life stages using ITS2 and COI sequences (de León et al., 2021; Schols et al., 2020). Collectively, the literature shows that multilocus frameworks outperform single-marker or morphology-only approaches in resolving marine helminth diversity.

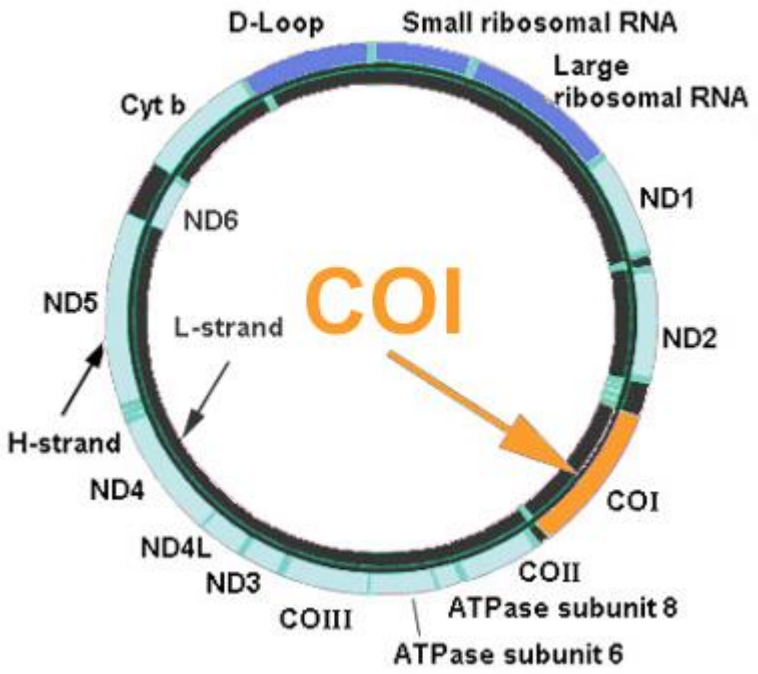


Figure 3: COI (cytochrome c oxidase subunit I), a mitochondrial marker widely applied in DNA barcoding.

Table 1. Summary of the studies

Study Type	Parasite Group	Host Taxa	Markers Used	Key Findings
Species description (marine trematodes)	Digenea	Indo-Pacific teleosts	28S, ITS2, COI	New species validated by multilocus phylogeny
Monogenean from carangid fish	Monogenea	Carangoides fulvoguttatus	28S, COI	Confirmed species placement within Protomicrocotylidae
Integrative review	Mixed helminths	Marine fishes	ITS, 28S, COI	Recommended multilocus taxonomy
Life-cycle linkage study	Digenea	Marine fish + intermediate hosts	ITS2, COI	Linked larval and adult stages
DNA barcoding evaluation	Trematoda	Marine fishes	COI	Identified cryptic diversity
Regional Indo-Pacific survey	Digenea	Reef fishes	28S, COI	Revealed undescribed diversity
Morphology + phylogeny	Monogenea	Carangids	28S	Resolved genus-level placement

Integrative copepod study (method template)	Parasitic copepods	Marine fishes	COI, 28S	Demonstrated workflow transferable to helminths
Nematode phylogeny	Nematoda	Marine/freshwater fishes	COI, nuclear markers	Confirmed morphological species boundaries
Methodological review	Flatworms	General	COI, 28S	Highlighted primer limitations

Table 2. Marker Performance Comparison

Marker	Resolution Level	Strengths	Limitations
28S rDNA	Family/Genus	Stable alignment, broad phylogenetic signal	Limited species-level divergence
ITS2	Species	High variability, useful for life-stage linkage	Alignment difficulty in distant taxa
COI	Species/Cryptic taxa	High discriminatory power	Primer mismatch, variable amplification success

4. Discussion

The synthesis of the reviewed studies clearly demonstrates that integrative taxonomy has become the prevailing methodological standard in marine helminth systematics. Morphological evidence remains foundational for species diagnosis and formal description, yet repeated findings of cryptic diversity underscore its limitations when used alone (Vanhove et al., 2013). Ribosomal 28S rDNA provides stable alignment across divergent taxa and reliably resolves intergeneric relationships (Mendoza-Franco et al., 2018). In carangid parasites, 28S data have confirmed systematic placement within Protomicrocotylidae and related families (Tambireddy et al., 2016). However, its relatively conserved nature sometimes limits discrimination among recently diverged species.

ITS2 addresses this limitation by offering higher variability suitable for species-level delimitation. Studies linking metacercariae to adult trematodes through ITS2 sequence identity illustrate its importance in clarifying complex life cycles (de León et al., 2021). In marine systems where trophic transmission dominates, such linkage is essential for accurate ecological interpretation.

COI consistently exhibits the highest interspecific divergence among markers reviewed. Its utility in revealing cryptic lineages is well documented (Justine et al., 2013). Nevertheless, primer universality issues in flatworms caution against reliance on COI alone (Vanhove et al., 2013). Integrative studies

therefore recommend combining COI with nuclear markers to ensure robust phylogenetic inference (Rojas et al., 2025).

For parasites infecting Carangoides spp., the need for integrative approaches is particularly compelling. Carangids are wide-ranging, migratory predators occupying reef, pelagic, and coastal habitats. Such ecological breadth facilitates parasite acquisition across multiple intermediate hosts and geographic regions, increasing opportunities for diversification. Molecular data have already revealed unexpected genetic divergence within morphologically similar monogeneans infecting carangids (Tambireddy et al., 2016). Beyond species delimitation, integrative taxonomy enhances reproducibility and transparency. Depositing voucher specimens and linking sequence accession numbers to morphological descriptions enables re-examination and comparative analyses (Narciso et al., 2024). This practice strengthens systematic stability and reduces synonymy.

The reviewed literature also highlights technical challenges. Formalin fixation impedes DNA extraction, and limited reference sequences constrain comparative phylogenetics. Expanding regional surveys, particularly in the Indo-Pacific biodiversity hotspot, is therefore a priority.

Overall, the convergence of morphological and molecular data provides a coherent framework for understanding helminth evolution, host specificity, and biogeographic history in marine systems.

5. Conclusion

Integrative taxonomy combining morphology, ITS2, 28S rDNA, and COI represents the most reliable approach for resolving marine helminth diversity. Evidence from reviewed studies consistently shows that morphology alone underestimates species richness and fails to detect cryptic lineages (Vanhove et al., 2013; de León et al., 2021). Ribosomal markers provide stable phylogenetic scaffolding, while COI enhances species-level discrimination and reveals hidden diversity (Justine et al., 2013; Rojas et al., 2025).

For helminths infecting Carangoides spp., this multilocus framework is especially critical due to host mobility, ecological complexity, and morphological similarity among parasite taxa. Integrative studies improve life-cycle resolution, clarify host specificity patterns, and strengthen systematic reproducibility.

Future research should prioritize comprehensive geographic sampling, multilocus phylogenetic reconstruction, and deposition of well-curated voucher specimens. Such efforts will refine our understanding of marine parasite biodiversity and contribute to broader ecological and evolutionary insights in tropical marine ecosystems.

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