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REVIEW ARTICLE

Morphological and Molecular Characterization of Parasitic Helminths in Fishes: An Integrative Approach

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ABSTRACT

Parasitic helminths, including trematodes, cestodes and nematodes are ubiquitous pathogens in aquatic ecosystems, posing substantial threats to fish health, aquaculture and biodiversity. The diverse group of parasitic helminths exhibit a variety of morphological and molecular adaptations that enable them to infect and thrive within their fish hosts. Accurate identification and characterization of these parasites are critical for understanding their epidemiology, host-parasite interactions and impact on fish health. This review presents an integrative approach combining traditional morphological analysis with advanced molecular techniques to characterize helminth parasites in fish. Morphological identification, while foundational, often faces challenges due to phenotypic plasticity, ontogenetic variation and taxonomic ambiguities within helminth species. In contrast, molecular tools such as polymerase chain reaction (PCR), DNA sequencing and molecular barcoding provide high-resolution species identification, revealing cryptic diversity and facilitating the study of complex life cycles. Molecular characterization plays a crucial role to validate species identification, assess genetic diversity and construct phylogenetic relationships based on either whole genome or partial gene sequence analysis of highly conserved regions like 18SrRNA, mt DNA (cytochrome oxidase subunit I, CoxI or coi gene), ITS regions of 5.8S rRNA gene, 28S rRNA gene etc. This paper aims to highlight the importance of integrating morphological and molecular methods for accurate diagnosis and the need for monitoring programs to mitigate helminthic infections, thus supporting sustainable fisheries and aquaculture practices.

Keywords: *Parasitic helminths, DNA sequencing, Molecular barcoding, Phenotypic plasticity, Cryptic diversity.*

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INTRODUCTION

Helminthes are multicellular organisms that are triploblastic, dorsoventrally flattened or round, acoelomate or pseudocoelomate and primarily exhibit parasitic behaviour. Parasitic helminths, including nematodes, trematodes and cestodes, represent a diverse group of organisms that can have profound impacts on the health and survival of fish populations [1-3]. These parasites are ubiquitous which infect both wild and farmed fish and are responsible for significant economic losses in aquaculture. In addition to the direct effects on fish health, some parasitic helminths also serve as vectors for other infectious agents, further complicating the ecology and management of parasitic diseases in aquatic environments [4-6]. The identification, characterization and management of parasitic helminths are therefore essential for sustainable fishery management, biodiversity conservation and public health.

Traditionally, the identification and characterization of parasitic helminths have typically been performed through conventional methods including morphological, numerical, histological and biochemical approaches [7,8]. The identification of parasitic helminths is primarily based on their morphological characteristics, including size, shape, internal structure and reproductive organs. However, the reliance on morphological taxonomy alone can present significant challenges. Many parasitic helminths exhibit cryptic diversity, where closely related species look morphologically similar, or have overlapping features that complicate accurate identification. Furthermore, life stages of the same species can exhibit significant morphological variation, making species determination even more difficult. Additionally, environmental factors such as water temperature, pH, oxygen levels or salinity can induce morphological plasticity, further hindering taxonomic consistency [9].

To overcome these limitations, molecular techniques have become an indispensable tool for the study of parasitic helminths. The molecular taxonomy has been pivotal in resolving complex taxonomic issues, discovering new species and understanding the genetic diversity within and between species. Molecular characterization methods, including DNA barcoding, Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS) have revolutionized the ability to accurately identify species, determine genetic diversity and uncover cryptic species. By sequencing specific genetic markers (like 18SrRNA, mt DNA (cytochrome oxidase subunit I, CoxI or coi gene), ITS regions of 5.8S rRNA gene, 28S rRNA gene etc., parasitic helminths can be assigned to their respective species, providing essential information for the understanding of host-parasite dynamics, evolutionary relationships and population structure [10-12].

Despite the powerful capabilities of molecular tools, these methods are not without their own set of challenges. The reliability of molecular data heavily depends on the availability of reference databases, the quality of DNA extracted and the careful design of primers. Furthermore, molecular identification can sometimes reveal surprising genetic divergence even within species, complicating the understanding of species boundaries and intraspecific variation. As such, molecular tools are often best used in conjunction with traditional morphological techniques, creating an integrative taxonomic approach that combines both morphological and molecular data for a more comprehensive and accurate characterization of parasitic helminths [13,14].

An integrative approach in parasitology involves a unified framework that provides a fuller understanding of parasitic diversity, life history and evolutionary processes. The use of multiple lines of evidence enhances the robustness of species identification and helps resolve ambiguities that may arise when using either morphological or molecular data alone. The aim of this review paper is to explore the morphological and molecular characterization of parasitic helminths in fish using an integrative approach. By examining these methodologies and their applications in parasitology, this review aims to provide a comprehensive overview of the current state of research on parasitic helminths in fish and highlight the benefits of adopting an integrative approach to parasite characterization. Ultimately, this integrated perspective will foster a more holistic understanding of parasitic biodiversity, promote improved management practices in aquaculture and contribute to advancing knowledge in the field of aquatic parasitology.

MORPHOLOGICAL CHARACTERIZATION OF PARASITIC HELMINTHS

Morphological taxonomy is based on the external and internal physical features of the parasite, which are observed under a microscope or through histological examination. Despite the advent of molecular techniques, morphology remains an essential tool in parasitology due to its simplicity, historical importance and cost-effectiveness. However, this approach does face several challenges, especially when identifying cryptic or closely related species.

1. Techniques of Morphological Taxonomy

- **Light Microscopy:** This is the primary tool for examining parasitic helminths. The morphology of the parasite can be assessed through various features, such as body shape, segmentation and organ structure (e.g., suckers, testes, ovaries, etc.). Light microscopy is useful for observing external features such as the shape of the body, hooks and other structures like the scolex of cestodes or the body flaps of trematodes [15-16].
- **Scanning Electron Microscopy (SEM):** SEM provides high-resolution images of the surface structures of parasitic helminths, allowing for detailed examination of features like the cuticle, hooks and spines, which can be essential for distinguishing species. This technique is especially useful in observing fine details that are not visible with light microscopy [17].
- **Histological Techniques:** Tissue sections of infected fish can be examined to study the internal features of parasitic helminths, such as reproductive organs, digestive systems, and excretory structures. Histology is particularly useful for identifying parasites in their larval stages, which may not be distinguishable in their free-living forms [18].
- **Morphometric Analysis:** This involves the measurement and quantification of morphological features, such as body length, width, and organ dimensions, which can be compared across different species. Advanced software tools can be used to analyze these measurements statistically, providing a more objective way of distinguishing species [19,20].

2. **Advantages of Morphological Techniques**
   - Practical and Cost-Effective: Morphological identification can often be performed with basic laboratory equipment, making it relatively affordable and accessible.
   - Broad Applicability: This technique can be used to identify parasitic helminths at various life stages, including larvae, which is useful for understanding the entire life cycle of a parasite.

3. **Disadvantages of Morphological Techniques**
   - Limited Precision: Many parasitic species share similar morphological traits, which makes it difficult to differentiate closely related species.
   - Subjectivity: The identification process can be subjective, depending on the expertise of the researcher. Variability in morphology within species also complicates the process.
   - Time-Consuming: Detailed morphological analysis requires significant time and effort to prepare, observe, and interpret specimens.

MOLECULAR CHARACTERIZATION OF PARASITIC HELMINTHS

Molecular techniques have transformed the field of parasitology by enabling more precise, sensitive and comprehensive identification of parasitic species. In particular, for parasitic helminths in fish, molecular methods provide a powerful means of overcoming the limitations inherent in traditional morphological identification [21]. The following is a detailed exploration of the key molecular techniques employed in the study of parasitic helminths in fish.

1. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is one of the most widely used molecular techniques in parasitology. PCR amplifies specific regions of DNA, allowing to target and replicate parasitic genetic material for identification and analysis [21]. PCR-based methods are particularly useful for identifying parasitic helminths in fish, even when traditional morphological features are insufficient for accurate species identification.

PCR Technique Overview

- PCR involves a three-step process: denaturation (separating DNA strands), annealing (binding primers to the target DNA) and extension (synthesizing new DNA strands).
- This process is repeated in cycles, amplifying the target DNA exponentially.
- Specific primers are designed to match known sequences in the parasite's genome, allowing the to target particular genes that are species-specific or highly conserved across related species.

PCR-Based Approaches for Parasitic Helminths

- **General PCR (Universal PCR):** Universal primers are used that target highly conserved regions of the DNA (e.g., 18S rRNA, ITS-1, or COI) to amplify sequences that are present across many different parasitic species. These primers allow broad identification and analysis of various parasites.
- **Species-Specific PCR:** In cases where the species of the parasite is known, primers targeting unique genetic markers (e.g., ribosomal RNA genes, mitochondrial genes) specific to that species can be designed to ensure accurate identification.
- **Nested PCR:** This method involves two rounds of PCR amplification. The first round amplifies a larger DNA fragment, and the second round amplifies a smaller region within that fragment. Nested PCR increases the sensitivity and specificity of the technique, especially when working with small amounts of DNA.

2. DNA Barcoding

DNA barcoding is a molecular technique that uses a standardized region of DNA, typically from the mitochondrial genome, to identify and classify species. The most commonly used barcoding marker for parasitic helminths is the Cytochrome c oxidase I (COI) gene, although other regions like 18S rRNA and ITS-1 have also been used [23,24].

DNA Barcoding Overview

- DNA barcoding uses a short, consistent segment of DNA as a molecular “barcode” to distinguish between species.
- The COI gene is widely used in animal species, including parasitic helminths, because it evolves relatively quickly, making it effective for distinguishing closely related species.

DNA Barcoding Process

- DNA Extraction: Parasite DNA is extracted from fish tissues or directly from the parasite.
- PCR Amplification: The barcode region (such as the COI gene) is amplified using PCR.

- Sequencing: The amplified DNA is sequenced, generating a unique genetic sequence for the target region.
- Sequence Comparison: The obtained sequences are compared to a reference database, such as the Barcode of Life Data Systems (BOLD), to identify the species.

3. Next-Generation Sequencing (NGS)

Next-Generation Sequencing (NGS) is a powerful molecular tool that allows the sequencing of entire genomes or metagenomes in a single run. NGS has transformed many areas of biology, including parasitology, by providing detailed genetic information that can be used to study biodiversity, genetic diversity and evolutionary relationships in parasitic helminths [25].

NGS Overview

- NGS involves high-throughput sequencing of DNA, generating millions of short DNA sequences that are then assembled and analyzed.
- NGS platforms, such as Illumina, PacBio and Oxford Nanopore, enable massive parallel sequencing, significantly reducing the cost and time required to obtain comprehensive genetic data.
- The technology can be used for a range of applications, from full genome sequencing of parasitic helminths to sequencing the collective genetic material of entire parasitic communities in a host (metagenomics).

NGS Applications for Parasitic Helminths

- **Whole Genome Sequencing**: NGS can be used to sequence the entire genome of a parasitic helminth, providing insights into its genetic structure, gene content, and evolutionary history.
- **Metagenomics:** NGS allows researchers to study the genetic material from mixed samples (e.g., a fish gut or tissue) to detect and identify multiple parasitic species simultaneously.
- **Transcriptomics:** NGS can also be used to study the RNA transcripts (expressed genes) of parasitic helminths, providing insights into their biological functions, life cycle stages, and host-parasite interactions.

4. Advantages of Molecular Techniques

- **High Sensitivity and Specificity:** Molecular techniques can identify parasitic helminths at the species level with great precision, even in mixed infections.
- **Ability to Detect Cryptic Species:** Molecular methods can detect genetically distinct species that appear morphologically identical but differ at the molecular level.
- **Quantitative Analysis:** PCR-based methods can quantify parasite load in fish tissues, helping to assess the intensity of infections.

5. Disadvantages of Molecular Techniques

- **Cost:** Molecular techniques can be expensive due to the need for specialized equipment and reagents.
- **Technical Expertise:** Molecular methods require skilled personnel to conduct the analysis and interpret the results accurately.
- **Incomplete Databases:** The effectiveness of molecular techniques depends on the availability of comprehensive reference databases. For many parasitic species, genetic reference data may be limited or lacking.

INTEGRATIVE APPROACH IN TAXONOMY OF PARASITIC HELMINTHS

The integrative approach in taxonomy has emerged as an essential strategy in modern parasitology, particularly for studying parasitic helminths in fish. This approach leverages the strengths of each technique to provide robust taxonomic classifications that are both precise and reliable [26,27].

1. Benefits of the Integrative Approach

- **Improved Accuracy:** Integrating both morphological and molecular data leads to more accurate identification of species. Morphology can be used to distinguish species with unique traits, while molecular data can confirm these identifications or clarify cases where morphological features are not sufficient.
- **Resolution of Taxonomic Ambiguities:** In cases where morphological traits are ambiguous, molecular data can provide additional information to resolve taxonomic uncertainties. For instance, genetic barcoding can confirm the identity of species that may be morphologically similar.

- **Detection of Cryptic Diversity:** Many parasitic species exhibit cryptic diversity, where genetically distinct populations appear morphologically identical. An integrative approach helps detect these cryptic species by combining both genetic and morphological data.
- **Life Cycle Studies:** Morphological techniques are invaluable for studying the life cycle of parasitic helminths, while molecular tools can trace the genetic relationships of life stages. Together, these methods offer a more holistic understanding of parasitic biology.

2. **Applications of Integrative Approaches**
   - **Host-Parasite Interaction Studies:** An integrative approach can be used to study how parasitic helminths interact with their fish hosts at both the physiological and genetic levels.
   - **Aquaculture Disease Management:** Accurate identification of parasitic species is essential for managing fish health in aquaculture settings. Molecular tools can detect early infections, while morphological analysis can confirm species identity and assist in treatment decisions.
   - **Conservation Biology:** Understanding the diversity and distribution of parasitic helminths in natural fish populations helps in assessing the health of aquatic ecosystems and supports conservation efforts.

CONCLUSIONS

In conclusion, both morphological and molecular techniques are crucial for the characterization of parasitic helminths in fishes. Morphological methods offer simplicity, cost-effectiveness and historical significance, but they are limited in their ability to resolve species-level identification, especially in cases of cryptic diversity. Molecular techniques, on the other hand, provide greater precision and can detect genetic variation within and between species, facilitating the identification of cryptic species and offering deeper insights into the evolution and ecology of parasites. By combining these two approaches in an integrative manner, a more accurate and comprehensive understanding of parasitic helminths can be achieved. This integrative approach has significant implications for the study of parasite biodiversity, ecology and management and it is essential for advancing the knowledge of parasitic diseases in aquatic ecosystems. The future of parasitology lies in the continued development and application of integrative techniques that combine the best aspects of both morphological and molecular methods to enhance the understanding of parasitic helminths and their impact on fish populations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for work reported in this paper

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