

Role of DNA-Based Markers in Nematode Taxonomy

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Abstract

Nematodes, or roundworms, represent one of the most diverse phyla in the animal kingdom, with species occupying a broad range of ecological niches. Accurate identification and classification of nematodes are critical for various biological, agricultural, and ecological studies. Traditional morphological methods often fall short due to the high morphological plasticity and cryptic nature of many nematode species. DNA-based markers have revolutionized nematode taxonomy, offering more precise, rapid, and reliable identification methods. This article examines the role of DNA-based markers in nematode taxonomy, highlighting key techniques, their applications, and future prospects.

Introduction

Nematodes are ubiquitous, inhabiting soil, freshwater, and marine environments. They play significant roles in nutrient cycling, soil health, and as parasites of plants, animals, and humans. Accurate taxonomy is fundamental to understanding nematode ecology, evolution, and their interactions with hosts and ecosystems. Traditional taxonomic methods rely on morphological features, which can be ambiguous and insufficient for distinguishing closely related species. DNA-based markers have emerged as powerful tools for nematode taxonomy, offering higher resolution and specificity. Understanding the subtle environmental influences on organisms is challenging, and morphological differences do not always result from nucleotide alterations. Species evolution stems from changes in genetic makeup, which can occur through mutation, recombination, and segregation. DNA polymorphism arises from the presence of different alleles of a specific gene within animals or their populations. Molecular markers detect these variations to identify genes associated with particular traits. These markers have identifiable DNA or protein sequences, allowing their inheritance to be tracked.

DNA-Based Markers in Nematode Taxonomy

1. Ribosomal DNA (rDNA) Markers

Ribosomal DNA sequences, particularly the small subunit (SSU) and large subunit (LSU) rRNA genes, have been extensively used in nematode taxonomy. The internal



transcribed spacer (ITS) regions, which are more variable than coding regions, provide species-specific markers useful for differentiating closely related species.

- ✚ **Small Subunit (SSU) rRNA Gene:** The SSU rRNA gene, also known as 18S rRNA, is highly conserved and useful for higher taxonomic levels such as genera and families. It has been instrumental in constructing phylogenetic trees and understanding evolutionary relationships among nematode groups. The SSU rRNA gene was used to classify a diverse range of nematodes in marine sediment samples. Researchers successfully identified many nematode species and constructed phylogenetic trees to understand their evolutionary relationships better.
- ✚ **Large Subunit (LSU) rRNA Gene:** The LSU rRNA gene, or 28S rRNA, offers more variability than the SSU rRNA gene, making it suitable for resolving relationships at the genus and species levels. It is often used in conjunction with SSU rRNA sequences for comprehensive taxonomic studies. The LSU rRNA gene helped distinguish between closely related species of plant-parasitic nematodes in the genus *Meloidogyne*. This method provided clearer resolution than SSU rRNA alone, aiding in the accurate identification of species involved in crop damage.
- ✚ **Internal Transcribed Spacer (ITS) Regions:** The ITS1 and ITS2 regions are flanked by highly conserved SSU and LSU rRNA genes, respectively. These regions exhibit high interspecific variability and are widely used for species identification and delimitation. ITS sequencing is particularly useful in identifying cryptic species and understanding intraspecific variation. ITS sequencing was employed to differentiate species within the genus *Heterodera*, which includes many economically important cyst nematodes. The ITS regions provided distinct genetic signatures for each species, facilitating accurate identification and management strategies in agriculture.

2. Mitochondrial DNA (mtDNA) Markers

Mitochondrial DNA markers, such as the cytochrome c oxidase subunit I (COI) gene, provide high-resolution tools for nematode taxonomy. The COI gene is known for its rapid rate of evolution, making it ideal for distinguishing closely related species.

- ✚ **Cytochrome c Oxidase Subunit I (COI) Gene:** The COI gene, also referred to as the "barcode" region, has been widely adopted for species identification across various animal taxa, including nematodes. It enables the differentiation of morphologically



similar species and the discovery of new species. The COI gene was used to identify and classify nematode species from soil samples. This "barcode" approach enabled the detection of cryptic species that were not distinguishable by morphological features alone, enhancing our understanding of soil biodiversity.

3. Single Nucleotide Polymorphisms (SNPs)

SNPs represent single base pair variations in the genome and provide a wealth of information for population genetics and phylogeography. High-throughput sequencing technologies have facilitated the discovery and utilization of SNPs in nematode taxonomy, offering precise and detailed insights into genetic diversity and evolutionary history. SNP markers were utilized to study the population genetics of the pinewood nematode (*Bursaphelenchus xylophilus*). By analyzing SNP patterns, researchers could trace the geographic spread and genetic diversity of this invasive species, informing quarantine and control measures.

4. Microsatellites/Simple Sequence Repeats

Microsatellites, or simple sequence repeats (SSRs), consist of short, tandemly repeated DNA motifs. They are highly polymorphic and serve as excellent markers for population genetics, species differentiation, and studies of genetic structure and gene flow in nematode populations. Microsatellite markers were applied to investigate the genetic structure of the soybean cyst nematode (*Heterodera glycines*). These highly polymorphic markers revealed significant genetic diversity within and among nematode populations, helping to understand the spread and evolution of this agricultural pest.

Role of DNA-Based Markers in Nematode Taxonomy

The taxonomy of nematodes, particularly plant-parasitic has traditionally relied on morphological characteristics and host range. However, these methods often face limitations due to the cryptic nature of many nematode species and the plasticity of morphological traits. DNA-based markers have revolutionized nematode taxonomy, providing more precise, reliable, and comprehensive tools for species identification, phylogenetic studies, and population genetics.

1. Precise Species Identification



DNA-based markers, such as ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) sequences, enable precise species identification by targeting specific genetic regions unique to each species. Common markers include:

- **ITS (Internal Transcribed Spacer) regions:** These regions are part of the rDNA and vary significantly between species, making them useful for distinguishing closely related nematodes.
- **COI (Cytochrome Oxidase I/COX1) gene:** Part of the mitochondrial genome, the COI gene is often used in DNA barcoding for species identification due to its high mutation rate and species-specific sequences.

2. Phylogenetic Studies

DNA-based markers facilitate the construction of phylogenetic trees, elucidating evolutionary relationships among nematode species. By comparing genetic sequences, researchers can infer the evolutionary history and divergence of species within the genus *Meloidogyne* and other nematode groups. This helps in understanding the genetic diversity and evolutionary adaptations of nematodes.

3. Detection of Cryptic Species

Morphologically indistinguishable species, known as cryptic species, are common in nematodes. DNA markers can reveal these cryptic species by uncovering genetic differences that are not evident morphologically. This has significant implications for nematode management, as different species may have varying host ranges, pathogenicity, and resistance to control measures.

4. Population Genetics and Epidemiology

DNA-based markers are essential for studying the genetic structure of nematode populations. Techniques such as microsatellite analysis, single nucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs) allow researchers to:

- Assess genetic diversity within and between nematode populations.
- Track the spread and origin of invasive species.
- Understand gene flow and reproductive strategies in nematode populations.

5. Development of Diagnostic Tools

The identification of specific DNA sequences unique to particular nematode species has led to the development of molecular diagnostic tools. Techniques such as polymerase chain reaction (PCR) and quantitative PCR (qPCR) are widely used for:

- Rapid and accurate detection of nematodes in soil and plant samples.
- Monitoring nematode populations in agricultural fields.
- Early detection of nematode infestations, enabling timely management interventions.

6. Advancements in Genomic Studies

Next-generation sequencing (NGS) technologies have further advanced nematode taxonomy by allowing whole-genome sequencing and transcriptome analysis. These approaches provide comprehensive insights into the genetic makeup, functional genes, and regulatory mechanisms of nematodes. Genomic studies can identify genes associated with pathogenicity, resistance, and adaptation, informing breeding programs and the development of targeted control measures.

Challenges and Future Directions

Despite their advantages, DNA-based markers have limitations, including the need for high-quality DNA samples, potential contamination issues, and the requirement for specialized equipment and expertise. Addressing these challenges is crucial for broader application and accessibility of molecular methods in nematode taxonomy. Integrating DNA-based markers with traditional morphological methods is essential for a holistic approach to nematode taxonomy. Combining these datasets can enhance taxonomic resolution and provide a comprehensive understanding of nematode diversity and evolution. Ongoing advancements in sequencing technologies, such as next-generation sequencing (NGS) and long-read sequencing, promise to further revolutionize nematode taxonomy. These technologies enable the generation of large-scale genomic data, facilitating more detailed and accurate taxonomic studies. Creating and maintaining comprehensive databases of DNA sequences and associated taxonomic information is crucial for the effective use of molecular markers in nematode taxonomy. Such databases will support accurate species identification, data sharing, and collaborative research efforts.

Conclusion

DNA-based markers have transformed nematode taxonomy, offering precise, reliable, and rapid tools for species identification and phylogenetic analysis. While challenges remain, ongoing technological advancements and integrative approaches hold great promise for further enhancing our understanding of nematode diversity and evolution. The continued development and application of molecular markers will undoubtedly play a pivotal role in advancing nematode taxonomy and addressing pressing ecological and epidemiological questions.