Nematode Identification Techniques

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Nematodes are among the most diverse but least studied organisms. Classic morphology-based identification is often insufficient for nematode identification, mainly due to the lack of sufficient variations among closely related species. Various molecular methods are used to supplement and/or circumvent these problems. These methods range from DNA fingerprinting to sequence analyses of DNA- and/or protein-based information. Computational image analyses have also contributed towards improved nematode identification and classification. Each of these methods have unique benefits, and potential issues, usually depending on the goal and circumstance of identification. However, together, these methods have aided nematode identification and increase our understanding of nematode diversity and phylogeny.

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1. Introduction

Comprising over a million species[1], nematodes are likely the most diverse and numerous metazoans in soil and aquatic sediments. Despite this, nematodes are among the least studied organisms with less than 0.01% of their species diversity described to date[2]. Among some 26,000 described species, about 4100 are plant parasitic, which cause drastic economic losses to all crops[3]. Nematodes are also of significant medical and veterinary importance [4], and free-living nematodes are crucial to nutrient recycling in the environment. Therefore, accurate identification is of paramount significance to understand nematode diversity and design efficient control and management strategies. Traditionally, identification is based on characteristics such as body length, morphology of sexual organs, mouth and tail parts, and other physical characters. This morphology-based classification can prove inadequate due to lack of clear variation among closely related taxa and the need for highly skilled taxonomists, whose number is on the decline[5]. Morphology-based identification is also a demanding endeavor, especially when large numbers of samples are involved. Various sub-organismal (protein- and DNA-based) methods have been employed to supplement or circumvent the limitations associated with morphology-based classification of nematodes. The highly influential work of Blaxter et al.[6] employed sequencing of nematode ribosomal DNA (rDNA) and led to improved understanding of nematode evolutionary relationships and identification. We will not spend time discussing the evolution of nematodes and phylogenetic relationships, but it is important to understand the significance of correct nematode identification and, more to the point, how we define a nematode species. As pointed out by Adams[7] there is a trade-off between an operational species definition and that with a strong philosophical integrity. While there is a justified need to place species within the correct evolutionary lineage, more often, nematode identification techniques are driven by an operational definition of species to assess potential threats to animal and plant health.

2. Identification of a Nematode Sample

The purpose of taxonomy is to understand biodiversity, categorize organisms, and aid the communication of biological information. Scientific naming is a prerequisite for communication in taxonomy, and valid naming is only possible with type specimens and corresponding morphological information. However, this is not always possible, particularly when dealing with environmental samples (eDNA). Furthermore, it is now generally accepted that there are insufficient morphological features to describe biological diversity, and the use of molecular information to supplement and/or circumvent this limitation is commonplace. Nonetheless, a taxon is more meaningful if its members possess unique biological features, rather than the taxon only representing a group of individuals sharing similar morphological or molecular features.

Morphology-based classification forms the foundation of taxonomy. It has benefited from recent advances in image analysis. AI helps circumvent limitations associated with the scarcity of highly qualified taxonomists and enables objective decision making, coupled with fast and accurate identification. Spectroscopic features and lifetime value measurements of autofluorescence also provide additional traits that can be exploited for identification purposes.

The relative ease of molecular methods (Table 1) has led to the recognition of many new taxa; some, based on sequence information alone. These taxa would have been impossible to describe morphologically not only for lack of taxonomists and sufficient morphological differences, but also because members of these taxa are difficult to culture. Taxa identified using different molecular approaches, however, are not always congruent; for example, when sequence information from different regions of the DNA is used in different studies, or when sequence data generated from the same DNA region are analyzed differently between studies. Likewise, taxa based on morphological features do not always correspond with those based on molecular information and vice versa. Consequently, no single method by itself provides all the answers all the time; and the choice of method(s) depends on the question asked, the nature of the samples and the availability of resources.

Table 1. Comparison of different nematode identification methods.

Method	Expertise	Cost	Resolution
Morphological and Image-Based			
Classical Morphometrics	High	Low	Medium
Machine Learning	High	Low	Medium
Autoflorescence	High	Low	Medium
DNA-Based			
Fingerprint	Medium	Medium	Medium
Microarray / Probe-Based	Medium	Low	Medium

Method	Expertise	Cost	Resolution
Sequencing	Medium	High	High
Protein-Based			
Isozyme Analyses	Medium	Medium	Medium
2-D Gel Analyses	Medium	Low	Medium
Mass Spectrometry	Medium	Medium	Medium
Serological Analyses	High	High	Medium

If the question at hand is identification of a nematode sample, the most direct approach would be to examine the sample microscopically and assign the nematode to the lowest taxonomic rank possible. The source of the sample may also provide a clue in this regard. However, this may require some level of taxonomic expertise. Based on this information, a molecular technique may then be employed to identity the nematode to species or even subspecies level. If the question has to do with quarantine, molecular methods that are specific to the quarantined nematode species may be employed to ascertain whether the nematode at hand is quarantined. If the objective is assessment of diversity in a field population(s), any of the fingerprinting techniques and/or sequence analyses based on one or a few genes may do. High-throughput sequencing using second or third generation technologies and the appropriate bioinformatic techniques are useful to study the diversity of nematodes in an environmental sample (eDNA).

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