



## Review

# Shift from morphological to recent advanced molecular approaches for the identification of nematodes

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## ABSTRACT

Nematodes are the most diverse but most minor studied microorganisms found in soil, water, animals, or plants. Either beneficial or pathogenic, they significantly affect human and animal health, plant production and ultimately affect the environmental equilibrium. Knowledge of their taxonomy and biology are the main issues to answer the different challenges associated with these microorganisms. The classical morphology-based nematode taxonomy and biodiversity studies have proved insufficient to identify closely related taxa and have challenged most biologists. Several molecular approaches have been used to supplement morphological methods and solve these problems with markable success. The molecular techniques range from enzyme analysis, protein-based information to DNA sequence analysis. For several decades, efforts have been made to integrate molecular approaches with digital 3D image-capturing technology to improve the identification accuracy of such a taxonomically challenging group and communicate morphological data. This review presents various molecular techniques and provides examples of recent advances in these methods to identify free-living and plant-parasitic nematodes.

## 1. Introduction

Nematodes are diverse, unsegmented roundworms comprising over a million species in terrestrial and aquatic ecosystems [1]. Among 26,000 described species, about 4100 are plant-parasitic, which cause enormous agricultural loss to all crop plants [2]. The free-living nematodes play an essential role in nutrient cycling and decomposition, and others have a significant role in medical and veterinary sciences [3]. Hence, correct identification is essential in understanding nematode diversity and designing effective control and management approaches. The earlier and commonly used morphological identification method is based on physical characteristics such as body length, morphology of snout and tail parts, sexual organs, and other physical characteristic features. This method proved insignificant due to the lack of apparent variation among closely related genera and species and the need for highly skilled taxonomists, whose number is declining continuously [4]. The

morphological method cannot distinguish most nematode species that are morphologically similar but genetically different [5,6]. These can be resolved by molecular approaches, opening the door to the DNA-based taxonomy [7]. Natural variation resulting from phenotypic plasticity [8] and cryptic and sibling species obscures a correct identification on a morphology basis [9,10].

Many biological study fields have ignored nematodes due to their tremendous species variety, shortage of trained nematode taxonomists, and difficulty of using morphological features to identify taxa. Most nematode species have not been taxonomically named and characterized; hence estimates of total nematode numbers vary. For millennia, morphology has been the only way used to identify nematodes. Over time, it became evident that morphology alone did not represent observed pathogenic distinctions among morphologically confined species. As a result, scientists have discovered methods for promptly detecting species pathology. Modern approaches are being used to aid

**Abbreviations:** 2DE, Two-dimensional electrophoresis; AFLP, Amplified fragment length polymorphism; CAPS, cleaved amplified polymorphic sequences; COI, Cytochrome C oxidase subunit I gene; DEPs, Differentially expressed proteins; DGGE, Denaturing gradient gel electrophoresis; EPN, Entomo pathogenic nematodes; HTS, High-throughput sequencing; ITS, Internal transcribed sequences; LSU, Large subunit; MOTU, Molecular operational taxonomic unit; SCAR, Sequenced characterized amplified region; SNPs, Single nucleotide polymorphism; SSP, Single polypeptides; SSR, Simple sequence repeats; SSU, Single subunit; T-RFLP, Terminal restrict fragment length polymorphism.

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species identification using the classical morphological method. These approaches help identify and quantify nematodes at the species level. Modern molecular techniques allow quick taxonomic identification and group genetic proximity determination [11–14]. Proteolysis, immunology, and the newly created DNA sequencing analysis are examples. Several agriculturally important nematodes have been identified and quantified using restriction fragment length polymorphism (RFLP) [15], random amplified polymorphic DNA (RAPD) [16], isoenzyme pattern analysis [17], polymerase chain reaction (PCR) [18], quantitative polymerase chain reaction (qPCR) [19], amplified fragment length polymorphisms (AFLP) [20], and sequencing of diagnostic rDNA regions [21–23].

Three molecular methods have been used to study free-living nematode biodiversity: Denaturing Gradient Gel Electrophoresis (DGGE), marker gene DNA sequences, and pyrosequencing. Nuclear rRNA sequence repeats have been used to identify parasitic isolates from various hosts and decipher species complexes with a lack of published taxonomy [24]. Several alternative DNA sequence markers are now widely used to identify nematodes. These markers include internal transcribed spacers [25,26] and hypervariable expansion regions of the nuclear 18S and 28S rRNA genes [27–30]. The morphological method is not always inadequate for taxonomic identification. Instead,

taxonomists' ignorance may be a deciding factor in some cases. Sudhaus and Kiontke [31] used classical and molecular taxonomy methods. Based on "cross-breeding experiments, biogeography, and DNA sequences," they proposed the nomenclature *Caenorhabditis breunneri*. Vogt [32] used DNA sequence data to identify North Sea benthic nematodes. They used an integrative approach combining morphological and molecular methods to characterize nematodes from different North Sea sampling sites.

Moreover, combining morphological and molecular methods has proven helpful for identifying species [29] and disentangling species complexes within Nematoda [9,33]. Data from morphological, molecular, typological taxonomic, and interbreeding studies were combined to generate a more integrative approach [34]. This review paper presents a detailed update of several techniques, from DNA to protein-based approaches, to better identify nematodes. The techniques used for identification of nematodes with help of a range of biomolecules and associated databases are shown in Fig. 1.

## 2. Protein-based methods

Many biochemical and molecular techniques have been used to identify nematodes. Genomic information has been used to identify



**Fig. 1.** Outline of molecular methods employed for identification of nematodes. After morphological methods, the identification of nematodes was started with biochemical analysis like PAGE and serological techniques. With advancement in taxonomy, PCR has been employed with some modification like RAPD, RFLP, and AFLP. Then with improvement in PCR techniques, SSR, multiplex-PCR, and quantitative-PCR have made taxonomical work little easy. Presently, high-through put methods are used like pyrosequencing, ITS, and a very powerful method is next-generation sequencing. Now, scientists are trying to make available genomic and other molecular markers for public through databases which improve and speedup taxonomy of nematodes.

nematodes [35]. Techniques used to identify nematodes at the species or subspecies level include isozyme analysis [36], polyacrylamide gel electrophoresis [37], antibody-based serological methods [38], and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) [39].

### 2.1. Biochemical method/allozymes

Allozyme pattern analysis was the first biochemical technique used to identify nematode species. Esterase, superoxide dismutase, and glutamate-oxaloacetate transaminase (GOT) were frequently employed enzymatically [17,36]. Biochemical analysis is a combination of morphological and molecular analysis. Since the 1960s, biochemical techniques based on proteins have been employed to classify species and determine their evolutionary relationships [40]. Multilocus enzyme electrophoresis (MEE), commonly referred to as isoenzyme typing, is one successful method. MEE was commonly employed in taxonomy and the naming of new species. It was employed in plant science, bacteriology [41], and parasitology [42]. This technique was utilized to determine the identity of many *Meloidogyne* species [17]. This approach was also utilized to investigate the phylogeny of the Anisakidae [43]. This approach is based on the relative mobility of the organisms' internal enzymes. Enzymes are separated by non-denaturing horizontal gel electrophoresis according to their molecular weight, electrical charge, and protein structure (isoelectric focusing). Their amino acid makeup determines the mobility and electrical charge of proteins. A comparable electrophoretic pattern does not necessarily imply identical amino acid sequences, which may mask genetic similarity. Occasionally, MEE is combined with morphological or molecular techniques [44,45]. The MEE approach requires that each frozen or fresh sample be processed individually and compared to recognized reference specimens [42]. However, this approach is time-consuming and requires established reference samples [36].

### 2.2. Two-dimensional gel electrophoresis (2-DGE)

The 2-DGE approach has been applied to nematode taxonomic investigations. It enables the charge-based resolution of complicated protein mixtures by employing isoelectric focusing in one dimension followed by a mass resolution in a dimension perpendicular to the first. Then, the resolution patterns of isolates are compared to identify similarities and differences that can be scored as presence/absence for phenetic and cladistic analyses of the resulting data matrix. The 2-DGE technique was utilized to demonstrate the proteome variation among 18 root-knot nematodes belonging to four species [46]. Their research indicated that while most of these differences were species-specific, others revealed evolutionary links between the various species. The primary advantage of 2-DGE is that it enables inferences about the taxa under consideration. Additionally, species-specific polypeptides can be isolated and examined using mass spectrometry, providing information about the encoding genes.

### 2.3. Serological analyses

Many researchers have studied the use of polyclonal and monoclonal antibodies (mAbs) with mixed results [47,48]. In the Ouchterlony double diffusion method, Lee [49] showed that *M. incognita* antiserum did not create an arc-shaped precipitation band when coupled with antigens from *M. hapla*, indicating a lack of cross-reactivity. Additional research demonstrated the lack of specificity of *Meloidogyne* antisera [50]. Similarly, *Globodera* and *Heterodera* cyst nematodes showed similar results [48]. The nematology society's optimism for diagnostic mAbs grew with hybridoma technology [51]. After immunization with nematode antigens, mature B-cells are isolated and fused with mouse lymphoid tumor cells to generate hybridomas that may be kept in vitro for ongoing antibody production. Monoclonal antibodies are more

selective depending on the antigen produced. mAbs were produced against *M. incognita* [52], *H. glycines* [53], *G. pallida* [48], and *G. rostochiensis* [48]. Schots [48] discovered that some mAbs could differentiate *G. pallida* from *G. rostochiensis*. The hybridoma technology is crowded with nematode samples. Another issue is the low success rate of B-tumor cell fusions. For comparative research, single B-cell receptor sequencing (scBCR-seq) can reconstruct antigen-binding site sequences [54].

## 3. PCR based methods

Numerous investigations conducted decades later showed that morphological identification was insufficient for nematode taxonomy [55]. Certain authors proposed reclassifying nematodes based on their 18S ribosomal RNA sequence homology as determined by PCR [56]. In 1993, a parasitic nematode species-specific PCR was proposed [57]. Additionally, satellite DNA can be used to detect *Meloidogyne hapla* (a plant-parasitic nematode) [58]. In vitro amplification of DNA sequence molecules that have been precisely constructed [59]. This approach has been used to identify Anisakidae nematodes isolated from human, fish, and frozen samples [42] and soil nematodes obtained in northwestern Iran [60]. *Meloidogyne enterolobii* was isolated from South African potatoes using PCR targeting mitochondrial and ribosomal DNA [61]. According to Gasser [62], identifying and selecting a relevant DNA region (genetic marker) is critical. The small (18S rRNA) and large subunit (28S rRNA) of ribosomal RNA [63], the mitochondrial cytochrome b locus (mt DNA cytb) [64], mitochondrial cytochrome oxidase 2 (COX2) [45], and mitochondrial cytochrome oxidase 1 (COX1) [65] were used to identify nematodes. ITS1 of rDNA [66], ITS2 of rDNA, and other ribosomal DNA sequences are also utilized to identify Anisakidae and Strongilidae [67,68]. PCR can be combined with other methods to increase the number of genetic markers. The polymorphism of single-stranded DNA molecules in parasites has been discovered using PCR-single strand conformational polymorphism (PCR-SSCP) [69]. PCR-SSCP was used to identify the Anisakidae [70]. Its fundamental principle is that the mobility of ssDNA molecules over a non-denaturing polyacrylamide gel is connected to the shape of the DNA. Single-stranded DNA molecules can adopt secondary or tertiary structures depending on their length and primary sequence. A single nucleotide variation of 300–530 bp can be calculated using PCR-SSCP [70]. A detailed comparison between various PCR-methods and other molecular techniques is given in Table 1.

### 3.1. PCR restriction fragment length polymorphism (PCR-RFLP)

One or more endonucleases can be used to generate fingerprints from restriction digestion of genomic DNA (gDNA). PCR-amplicons (PCR-RFLPs) can also create fingerprints [71]. The PCR-restriction fragment length polymorphism (PCR-RFLP) helps identify closely related species. PCR-RFLP uses endonuclease enzymes to degrade an amplified product. The PCR product's nucleotide sequence differs between species due to the placement of restriction sites, resulting in fragments of various sizes. Enzymatic digestion profiles of various species can be compared to identified sample profiles after gel electrophoresis. Several authors choose PCR-RFLP over basic PCR for *Anisakis* species identification [72]. gDNA-RFLPs do not require prior sequence knowledge, unlike PCR-RFLPs. However, incomplete restriction digestion can lead to non-reproducible fingerprints. Band pattern differences or similarities distinguish species [73,74]. There are more than two species of *Pratylenchus coffeae*, according to PCR-RFLP results on sweet potato [75]. PCR-RFLP is used to identify species and intraspecific variation. This approach can be coupled with radioactive or nonradioactive DNA hybridization [76]. Other approaches based on random amplification of DNA sequences include RAPD [77], RFLP [78,79], amplified fragment length polymorphism (AFLP) [80,81] and sequence characterized amplified DNA regions (SCAR) [82,83]. These random DNA target-based

**Table 1**  
Application and limitations of molecular methods used for nematode identifications

Method	Applications/ Uses	Examples	Limitations	
Protein based methods	<b>Biochemical method/ allozymes</b>	<ul style="list-style-type: none"> <li>➤ First biochemical method used in nematode taxonomy and provided comparable data between geographical isolates</li> <li>➤ Helps in detection of genetic diversity within and among species of cyst nematodes</li> </ul>	<ul style="list-style-type: none"> <li>➤ Nematode genera identified by this approach are <i>Pratylenchus</i>, <i>Meloidogyne</i>, <i>Ditylenchus</i>, <i>Heterodera</i>, and <i>Aphelenchus</i> [36,209,210,and]</li> </ul>	<ul style="list-style-type: none"> <li>• Cannot be applied for single nematode studies and is life stage specific</li> <li>• Requires known samples for reference purposes [36]</li> </ul>
	<b>Two-dimensional gel analyses</b>	<ul style="list-style-type: none"> <li>➤ Used for the taxonomic studies of nematodes</li> <li>➤ It permits in evolutionary inferences to be made about the taxa considered</li> </ul>	<ul style="list-style-type: none"> <li>➤ Proteomic variation among 18 root knot nematodes of four species [46]</li> </ul>	<ul style="list-style-type: none"> <li>• The number of polypeptides resolved, and polymorphism observed depends on sample number analysed and procedure used [46]</li> </ul>
	<b>Restriction fragment length polymorphism (RFLP)</b>	<ul style="list-style-type: none"> <li>➤ Differentiates closely related species based on the presence/ absence of restriction fragments and helps in the physical mapping</li> <li>➤ Simple and rapid method and does not involve a restriction step like AFLP</li> </ul>	<ul style="list-style-type: none"> <li>➤ Detection of species or populations within species [78,211,212,and]</li> <li>➤ Species specific identification in <i>Anaskids</i> [213]</li> </ul>	<ul style="list-style-type: none"> <li>• Large amounts of PCR products are required to use for different restriction enzymes</li> </ul>
	<b>Random amplified polymorphic DNA (RAPD)</b>	<ul style="list-style-type: none"> <li>➤ Does not require prior knowledge of sequence information about the template DNA</li> <li>➤ Helps in Phylogenetic analysis</li> </ul>	<ul style="list-style-type: none"> <li>➤ Detection of <i>G. rostochiensis</i>, <i>G. pallida</i>, <i>M. incognita</i>, <i>M. javanica</i>, and <i>M. arenaria</i> [82,212]</li> <li>➤ Identification of single juveniles of seven important species of <i>Meloidogyne</i> [98]</li> </ul>	<ul style="list-style-type: none"> <li>• Not suitable for all organisms and has poor reproducibility</li> <li>• Sensitive to variations in DNA and primer concentrations</li> </ul>
PCR-based methods	<b>Denaturing gradient gel electrophoresis (PCR-DGGE)</b>	<ul style="list-style-type: none"> <li>➤ Easy and straightforward method for nematode community analysis</li> <li>➤ Used to differentiate multiple species in a sample</li> </ul>	<ul style="list-style-type: none"> <li>➤ Community analysis in different environments [93,215,216,and] and identification of nematodes in pepper growing soil in Vietnam [95]</li> </ul>	<ul style="list-style-type: none"> <li>• Lacks detection sensitivity when the nematode community being assessed consists of many species</li> <li>• Not applicable for quantification of species richness in marine communities [217]</li> </ul>
	<b>Amplified fragment length polymorphism (AFLP)</b>	<ul style="list-style-type: none"> <li>➤ Used to evaluate variation among individuals of the same species</li> <li>➤ Selectively amplifying fewer restriction products and produces less-complex fingerprints</li> </ul>	<ul style="list-style-type: none"> <li>➤ Detection of species in <i>Heterodera avenae</i> group [218]. Molecular characterization of <i>Pratylenchus</i> species [219]</li> <li>➤ Study of intraspecific variation in <i>Radopholus similis</i> [25] and genetic diversity and virulence of <i>M. Incognita</i> populations [220]</li> </ul>	<ul style="list-style-type: none"> <li>• Lengthy protocol</li> <li>• Development cost is high</li> </ul>
	<b>Microarrays</b>	<ul style="list-style-type: none"> <li>➤ Several genetic features can be examined in a single experiment</li> <li>➤ High throughput capacity and high specificity for identification</li> </ul>	<ul style="list-style-type: none"> <li>➤ Used for identification of gastrointestinal nematodes [113], gene expression variation in <i>C. elegans</i> under different conditions [111] and specific diagnosis of plant parasitic nematode <i>Meloidogyne chitwoodi</i> [221]</li> </ul>	<ul style="list-style-type: none"> <li>• High cost</li> <li>• Amplification of unknown sequences in mixed samples and better hybridization of mixed targets than the perfectly matched ones [99]</li> </ul>
	<b>Simple Sequence Repeats (SSR)</b>	<ul style="list-style-type: none"> <li>➤ High Reproducibility</li> <li>➤ Used to evaluate paternity analysis, linkage mapping, and genetic diversity</li> </ul>	<ul style="list-style-type: none"> <li>➤ Determination of exact geographical origin of plant-parasitic nematodes [107] and type of sexual reproduction of nematodes [108].</li> </ul>	<ul style="list-style-type: none"> <li>• Need for sequence information</li> </ul>
PCR based methods	<b>Real-time PCR</b>	<ul style="list-style-type: none"> <li>➤ Quantitative and qualitative detection of species</li> <li>➤ Rapid and specific, provides immediate results without agarose gel electrophoresis</li> </ul>	<ul style="list-style-type: none"> <li>➤ Detection of beet cyst nematode (<i>H. schachtii</i>), potato cyst nematode (<i>G. pallida</i>), and <i>Bursaphelenchus xylophilus</i> [125,128]</li> <li>➤ Quantification of <i>Meloidogyne</i> spp. from tomatoes, <i>P. thornei</i> and <i>P. neglectus</i>. Identification and quantification of <i>P. penetrans</i> from field soil [222]</li> <li>➤ Identification of <i>Globodera pallida</i> and <i>G. rostochiensis</i> [214], <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> [223], <i>Pratylenchus penetrans</i> and <i>P. scribneri</i> [224] and <i>P. loosi</i> and <i>P. coffeae</i> [225]</li> <li>➤ Differentiation of closely related species, <i>Anisakis simplex sensu stricto</i> and <i>A. pegreffii</i> [114]</li> <li>➤ Identification and detection of <i>Meloidogyne incognita</i>, <i>M. enterolobii</i>, and <i>M. javanica</i> from individual galls [63]</li> </ul>	<ul style="list-style-type: none"> <li>• Requires species-specific primers</li> <li>• The stability of DNA from dead specimens in a sample especially from cysts is another barrier [128]</li> </ul>
	<b>Multiplex PCR</b>	<ul style="list-style-type: none"> <li>➤ Detects more than one species at a time</li> <li>➤ Rapid and cost-effective and helps in linkage analysis</li> </ul>	<ul style="list-style-type: none"> <li>➤ Identification of <i>Globodera pallida</i> and <i>G. rostochiensis</i> [214], <i>Meloidogyne chitwoodi</i> and <i>M. fallax</i> [223], <i>Pratylenchus penetrans</i> and <i>P. scribneri</i> [224] and <i>P. loosi</i> and <i>P. coffeae</i> [225]</li> <li>➤ Differentiation of closely related species, <i>Anisakis simplex sensu stricto</i> and <i>A. pegreffii</i> [114]</li> <li>➤ Identification and detection of <i>Meloidogyne incognita</i>, <i>M. enterolobii</i>, and <i>M. javanica</i> from individual galls [63]</li> </ul>	<ul style="list-style-type: none"> <li>• Requires optimization of reagent concentrations, variation in annealing temperatures, primer interactions, and amplification of non-specific products</li> <li>• Requires species-specific primers</li> </ul>
	<b>Internal transcribed sequences (ITS)</b>	<ul style="list-style-type: none"> <li>➤ High Reproducibility</li> <li>➤ Used to study genetic diversity and population studies</li> <li>➤ Rapid and accurate</li> <li>➤ Preferred method as factors that affect patterns in other methods like a variation in primer and DNA concentration, DNA template quality, type of DNA polymerase, and gel</li> </ul>	<ul style="list-style-type: none"> <li>➤ Species identification in <i>Caenorhabditis</i> [26]</li> <li>➤ Commonly used in recent years in various groups including family <i>Hoplolaimidae</i> [145], order <i>Tylenchida</i> [23], suborder <i>Criconeimatina</i> [129], <i>Pratylenchus</i> [226], <i>Longidorus</i> [227], <i>Acrobeloides</i> [228], <i>Meloidogyne</i> [229] and <i>Steinernema</i> [230]</li> </ul>	<ul style="list-style-type: none"> <li>• High development cost</li> </ul>
	<b>DNA sequencing</b>			<ul style="list-style-type: none"> <li>• Difficulty in finding an ideal gene for taxonomic documentation as well as phylogenetic inference, that works in all nematode groups</li> </ul>

(continued on next page)

Table 1 (continued)

Method	Applications/ Uses	Examples	Limitations
Pyrosequencing	<p>electrophoresis can be controlled, and the sequencing step can be optimized</p> <p>&gt; Non-electrophoretic real-time new DNA sequencing approach, widely applicable for the detailed characterization of nucleic acids</p>	<p>&gt; Used to examine soil microbial diversity [159], plant pathogenic fungal communities [160,161], and nematode diversity analysis from metagenomic samples [28]</p>	<p>• Identification of a suitable marker to provide good taxonomic coverage and species resolution is still an issue</p>

markers benefit from a higher multiplex ratio, which is favorable when the targeted DNA areas have little sequence divergence [84]. The RFLP was employed to discover polymorphism in *Haemonchus contortus* [85]. It is a reliable taxonomic technique [86,87]. Smith [88] utilized this method to distinguish between two peach root-knot nematode species in Florida: *M. floridensis* and *M. arenaria*. In Korea, eight endonucleases were employed to identify four cyst nematodes (*Heterodera schachtii*, *H. sojae*, *H. trifolii*, and *H. glycines*) using PCR-RFLP [89]. The approach includes designing primers, choosing restriction enzymes, amplifying and creating products, and electrophoresis of digested products. The root-knot nematode *Meloidogyne arenaria* has a single inherited nematode dominant resistance gene [90]. These markers aid in breeding programs and detect parasitic nematode resistance genes.

### 3.2. PCR-denaturing gradient gel electrophoresis (PCR-DGGE)

The PCR-DGGE is a molecular fingerprinting technology that differentiates PCR products. DNA fragments of the same length but different sequences can be separated on a denaturing gradient acrylamide gel. As a result of this sequence diversity, various domains melt at different temperatures, and molecules migrate to different places in the gel [91]. Because each amplicon has a unique melting profile, once the melting point is achieved for one domain of an amplicon, that segment of DNA becomes partially melted, resulting in branching "breaking" pieces [92]. Thus, the molecules' mobility is reduced, resulting in various migration profiles. As it contains highly conserved areas, it allows the construction of primers that can amplify varied regions of distinct species. This strategy worked well for the taxonomic classification of soil nematode communities [93]. The PCR-DGGE was used to identify soil nematodes and other microorganisms in the field [92]. Due to the diversity of nematode species, detection sensitivity may be reduced [93,94]. Using the SSU rRNA gene, Hieu [95] discovered 15 soil nematode species, other nematodes, and non-nematode species using the PCR-DGGE approach. It is used in microbial ecology and food microbiology. The presence of many copies of this marker with minor sequence heterogeneity impedes the research of microbial communities using PCR-DGGE. The method can overestimate the microbial community [92].

### 3.3. Amplified fragment length polymorphism (AFLP)

The AFLP is a DNA fingerprinting technology that uses PCR amplification to differentiate DNA restriction segments. As with RFLP, this approach necessitates the use of restriction enzymes. Two restriction enzymes are used to digest the DNA, one that cuts frequently and another that cuts less frequently. It does, however, need to target PCR amplification of the desired fragments. The number of fragments amplified will be smaller than the total digested during the restriction step. The AFLP, on the other hand, is based on the notion of precise and selective amplification followed by restriction digestion of genomic DNA and ligation with adaptors [62]. Gel electrophoresis analysis reveals a distinctive pattern of DNA fragments. The AFLP has been extensively used to characterize genetic variation in cattle nematode parasites, including lungworms [20]. While RAPD and AFLP share several characteristics, the AFLP produces more consistent results due to the stricter

reaction conditions. The AFLP approach uses a tiny amount of DNA and does not require prior sequence knowledge [20].

### 3.4. Random amplified polymorphic DNA (RAPD)

In order to identify genes in different organisms, molecular markers evolved. Because of its precision, simplicity, and high reproducibility, PCR amplification has replaced in vivo cloning in molecular biology. For genetic polymorphism, highly informative DNA markers have been produced. RAPD using PCR has been one of the most widely used molecular techniques in the previous decade [96]. First, it can screen populations for genetic variations that lack specific markers. Second, it is less expensive, requires less equipment, and does not require DNA sequencing. It has been used to assess and discover molecular genetic diversity in nematodes, including *Meloidogyne* sp. Orui [97] employed the RAPD amplification to distinguish eleven *Meloidogyne* species using nematode DNA. Adam [98] described continuous amplification patterns in *M. javanica* J2 males and females. The RAPD is an excellent tool for studying species relationships using the complete genome, although it is vulnerable to procedure modifications [62]. This method determines inter- and intra-specific connections among *Meloidogyne* species [99]. Amplification of nanograms of whole genomic DNA using short synthesized oligonucleotides of random sequences. The amplification products are then separated on agarose gels.

Three RAPD markers, OPB-06, OPA-01, and OPA-12, have been identified for the root-knot nematode species *M. incognita*, *M. javanica*, and *M. arenaria*. For more complicated DNA fingerprinting profiles, arbitrarily primed primers were roughly 15 nucleotide lengths (AP-PCR), while primers shorter than ten nucleotides are called DNA amplification fingerprinting (DAF). Both AP-PCR and DAF use specific PCR-RAPD conditions. While the length of random primers, amplification settings, and visualization methods vary, they differ from typical PCR conditions. An oligonucleotide with a random sequence is utilized; no prior information of the DNA to be examined is required. A proper annealing temperature allows primers of random sequence to bind multiple priming sites on complementary template DNA sequences and create specific products. The amplified DNA profile is based on nucleotide sequence homology between the oligonucleotide primer and template DNA. Because priming sites alter, nucleotide diversity between different template DNAs causes bands to appear or disappear. The RAPD profile will show the presence or lack of bands from identical alleles in a heterozygote. This method is similar to AFLP but without the restriction step. It has been used to assess genetic variation within and between species [100–102]. Bandi [103] examined the RAPD patterns with allozymes. They discovered that both approaches revealed the polyspecific structure of *Trichinella*, but the RAPD could detect cryptic species. Not all random primers performed equally well in the RAPD study. These markers are also important in plant breeding and genetics [104]. In a study of genetic polymorphism of some entomopathogenic nematode species, simple sequence repeats (SRP) outperformed the RAPD [105].

### 3.5. Microsatellites/simple sequence repeats (SSR)

Microsatellites, or SSRs, are small nucleotide sequences frequently

found in tandem repetitions in the eukaryotic genome [106]. The SSR markers have a high degree of polymorphism and can be resolved on high-resolution sequencing gels. They are polyallelic and codominant and may aid in the resolution of intraspecific species variation. They can aid in identifying several alleles at a single locus in the genome and provide comprehensive insight into population genetic investigations. They have a wide range of uses in forensic science, including paternity determination and criminal prosecution. They are used to determine the precise geographic origins of plant-parasitic nematodes [107] and the sexual reproductive mode of nematodes [108]. Due to the availability of sequences, they are less expensive and easier to score.

### 3.6. Microarrays

Microarrays are made up of DNA molecules such as complementary DNAs (cDNAs) or oligonucleotides that have been produced on glass slides or silicon chips. DNA, cDNA, or mRNA taken from organisms, cells, or tissues are fluorescently tagged and hybridized to the microarrayed DNA. Fluorescent spots are seen and scanned for quantitative analysis using a confocal scanner [109]. It is based on combining DNA amplification with hybridization to many particular oligonucleotide probes. A single experiment can monitor a large number of genetic characteristics. This technique offers a high throughput capability and a high level of specificity and sensitivity for organism identification. It is an excellent technique for identifying single or multiple parasites that appear in all habitats [110]. This technique has mainly been used to investigate gene expression variation in *Caenorhabditis elegans* under various situations [111] and host-pathogen interactions, primarily from the hosts' perspective [112]. Ahmed [113] described how DNA microarrays might be used to identify gastrointestinal nematodes. The microarrays enable the uniform identification of many pathogens, and future advancements are anticipated [99].

### 3.7. Multiplex-PCR

Multiplex-PCR is a PCR variant that enables the simultaneous amplification of several target regions by using multiple pairs of primers in a single reaction. Numerous species-specific forward primers targeting the ITS region were combined with a universal reverse primer that anneals to all species' DNAs. The resulting species-specific amplified products are not identical in size, which aided in identifying later coinfection by several species [114,115]. The multiplex-PCR assisted in identifying nematode species belonging to the *Contraecum*, *Pseudoterranova*, *Anisakis*, and *Hysterothylacium* genera identified in commercial fish species [114,115]. The multiplex-PCR saves time by screening many *Anisakid* species in a single run. This approach, however, is still somewhat slow in terms of sample output. Despite their widespread use, PCR-based technologies are difficult to standardize. Additionally, PCR-based techniques are limited to low-throughput identifications and are therefore time-consuming. This technique has been used in various DNA testing applications, including species identification, mutation and deletion analysis, quantitative tests, polymorphism detection, and reverse transcription PCR [35,116]. While a single PCR enables the simultaneous detection and identification of many plant-parasitic nematodes, each species requires its amplification reaction. DNA sequences are a prolific source of novel taxonomic characteristics. Sequenced characterized amplified region (SCAR) markers can be amplified using DNA from females, egg masses, and second-stage juveniles. The SCAR markers have aided in the resolution of *Meloidogyne* combinations that have caused widespread damage to coffee in Brazil [117].

### 3.8. Quantitative PCR

Quantitative PCR (qPCR) inserts a fluorescent tag into each amplified DNA product, allowing real-time quantification of the number of copies

created per PCR cycle. This method uses a TaqMan probe to detect and quantify *Pseudoterranova* spp. and *Anisakis* spp. parasitizing fishes by targeting ITS-1 and 18S rRNA genes [118]. The qPCR [119] used ITS-2 amplification to identify *A. pegreffii* in fish. The post-qPCR high-resolution melting has been used to confirm the profile of a hybrid species of *A. pegreffii* and *A. simplex* [120]. The presence or lack of PCR products or the size of specific PCR products due to specific primers has traditionally been used to identify organisms. Fleming [121] reported the first PCR nematode quantification. The qPCR was utilized to identify mixed populations of *Globodera rostochiensis* and *G. pallida* in potato samples [122]. Plant-parasitic nematodes are identified mainly by this PCR approach [123,124]. The qPCR was also used to estimate the risk of *Meloidogyne* spp. causing tomato damage [125] and detecting harmful nematodes in host tissues [126].

Real-time PCR was used to detect and quantify sugarcane parasites such as dagger nematode (*Xiphinema elongatum*), root-knot nematode (*Meloidogyne javanica*), and lesion nematode (*Pratylenchus zeae*) [127]. The melting temperatures and peak heights of the PCR results are compared between the two species to reflect their relative DNA amounts. The qTR-PCR has been used extensively in many studies, including the detection of beet cyst nematode (*Heterodera schachtii*) and potato cyst nematode (*G. pallida*) [128], stem nematode (*Ditylenchus dipsaci*) [129], *Meloidogyne* spp. from tomatoes [125], pinewood nematode (*Bursaphelenchus xylophilus*) [130] and enumeration of *Pratylenchus neglectus* and *Pratylenchus thornei* [131]. To detect *Meloidogyne incognita*, qPCR was enhanced utilizing fluorescent molecular probes and a particular primer [132]. The qPCR with taxon-specific probes was employed for high-throughput nematode screening. However, qPCR employed specific primers and TaqMan probe sets to detect and count *Trichodorus similis* and *Paratrichodorus pachydermous*, respectively [133]. A similar method has been used to count ruminant gastrointestinal nematodes [134], *Meloidogyne* species [135], and entomopathogenic nematode species [136]. This approach determined *Heterodera glycines* in soil samples from actual agricultural fields [137].

## 4. DNA sequencing methods

The amplification and sequencing of diagnostic sections of nematode DNA have become the primary source of new information [138,139]. Many years of DNA sequencing have resulted in public DNA sequence databases for BLAST match searching [140]. Finding a gene that works in all nematode groups and taxonomic levels can be complex. Choosing a DNA locus that describes a species remains unresolved [140]. Several diagnostic markers, including LSU (28S rRNA), SSU (18S rRNA), ITS-rDNA, COI gene, and heat shock protein 90 (hsp90), have proven helpful for nematode identification and molecular phylogenetic analysis [141,142]. These are multi-copy genes that facilitate amplification. These zones help distinguish phylogeny across phyla, populations, and taxa [11,55,143]. However, newer sequencing technologies, including high throughput sequencing (HTS) and third-generation sequencing (TGS), have grown more inexpensive for more comprehensive applications [144]. The 28S LSU and 18S SSU rRNA D2 and D3 expansion segments are widely utilized as standard molecular markers for most plant-parasitic nematodes. The existence of multiple conserved primers that amplify DNA from many species and phylogenetically informative sections make these regions particularly successful in detecting plant-parasitic nematodes [55,145]. The GenBank genomes help identify most plant-parasitic nematode species [146–148]. However, DNA sequencing has been used to identify marine nematodes and determine population genetic structure [9,27,149]. Bhadury [27] investigated mitochondrial and nuclear genomic areas for marine nematode identification. Their research found that the 18S SSU rRNA gene increased most consistently across taxa and can identify unknown worm species. It is only feasible when most members are sequenced and publicly available data.

Using DNA sequences, Bhadury [150] investigated the spread of

*Terschellingia longicaudata* across habitats and locations. They found conspecific cryptic species based on morphology and morphometry. Due to the inability to recognize species complexes, classic morphological approaches may underestimate biodiversity in numerous marine nematode groups. *Pellioiditis (Rhabditis)* contains ten sympatrically distributed cryptic species, which are not detectable by morphology [9]. Five cryptic taxa were discovered using two molecular markers (ITS and COI) [151]. Many nematode species have global distributions, indicating that poor species identification methods may disguise higher diversity. The use of various molecular markers is required to characterize marine nematode biodiversity better. Different types of sequencing methods have been employed to study the taxonomy of nematodes which are described as:

#### 4.1. Sangers method/chain termination method

Fredrick Sanger invented the chain termination method for DNA sequencing [152]. It was used to analyze single genes or segments of genes. For three decades, researchers relied heavily on Sanger's sequencing. Morise [153] used Sanger sequencing to analyze the nucleotide sequences of the 18S small subunit ribosomal RNA (SSU) and mitochondrial cytochrome oxidase subunit I (COI) genes as DNA barcodes from individual nematodes isolated from two distinct environmental soils, an unmanaged flowerbed, and an agricultural field. Their research found that each soil's nematode community had a specific feeding habitat: plant-parasitic and predatory nematodes were numerous in flowerbed soil while bacterivorous nematodes were abundant in field soil. Thus, taxonomy analysis based on DNA sequences effectively investigates soil nematodes in various habitats. The rapid improvement of DNA sequencing technology has resulted in a paradigm shift from one-by-one DNA barcoding using Sanger sequencing of a PCR amplicon from a single organism to DNA metabarcoding utilizing NGS-assisted extensive sequencing of an amplicon from a complex community. Later, with the advent of NGS, biologists and clinicians faced new obstacles and opportunities in genomic research and clinical diagnosis [144,154,155].

#### 4.2. Pyrosequencing of metagenomic samples

Pyrosequencing is a newer sequencing tool commonly used to characterize nucleic acids [28]. It involves the production of inorganic pyrophosphate with ATP creation and the ATP-dependent conversion of luciferin to oxyluciferin. Oxyluciferin synthesis results in light pulses whose amplitude is linked to the presence of one or more nucleotides. Enzymatic reactions produce a detectable signal precisely proportionate to the number of incorporated nucleotides [156,157]. The ability to detect many species in complicated populations is an essential application of pyrosequencing. It has been used to sequence complicated environmental samples' [157,158]. This strategy has been applied in soil microbial diversity [159] and plant pathogenic fungal communities [160,161]. It has shown promising results in evaluating metagenomic nematode diversity [28]. Previously, high-throughput sequencing was used to analyze nematode diversity in simulated metagenomic samples, including varied reference worms in known abundances [140]. The usage of both LSU rRNA and SSU rRNA loci improved species detection from 90% to 95%, indicating that multiple loci are a significant factor in obtaining a complete community profile. However, pyrosequencing failed to offer a complete picture of community structure and differentiate the proportional abundance of each taxon in the artificial community [140]. The fact that this technology can provide a basic nematode community profile is a big step forward from our current single nematode sequencing efforts and a big step towards leveraging environmental DNA data to research nematode community structure.

#### 4.3. Next-generation sequencing/ deep sequencing

Next-generation sequencing uses advanced techniques to sequence billions of nucleotides in a single run. 454 ([www.454.com](http://www.454.com)) generates 200–300 nucleotide reads, while Illumina ([www.illumina.com](http://www.illumina.com)) provides 20–50 nucleotide reads. These approaches have been used to sequence entire bacterial and eukaryotic genomes (DNA and RNA) [144]. NGS is used in scientific, clinical, and applied research. Compared to Sanger's approach, NGS can offer data from numerous samples, each run at a far lower cost [144]. In addition to whole-genome sequencing, researchers may now sequence complete exomes and methylomes [162–165] and perform ChIP-sequencing [166,167]. A closer look at gene family evolution may also be aided by the data obtained by sequencing technology [168,169]. Deep sequencing of SSU-derived amplicons has been used to metabarcoding marine [170,171] and terrestrial nematodes [172–175]. The best SSU gene for NGS-based DNA metabarcoding has yet to be determined.

Next-generation sequencing has revolutionized the sequencing of organellar genomes [176]. A genome with high sequencing depths can be assembled quickly using this method, but not on worms [176]. Using Illumina technology, tens of millions of small DNA segments (100 bp reads) can be generated [177]. The Illumina technology used *M. graminicola* to create a root-knot nematode mitogenome. The mtDNA genome sequence was compared to *M. javanica* and other *Haplolaimoidea* species. Kenmotsu [178] employed Illumina-based amplicon sequencing to identify suitable locations for nematode barcoding from the copse, uncultivated field, and cultivated home garden soils. Their research found that Rhabditida and Dorylamida were most prevalent in the copse soil, Rhabditida (mainly bacterivores) in the house garden soil, and Dorylamida, Mononchida, and Rhabditida in the field soil. Amplicon sequencing can also be used to metabarcoding soil nematode groups.

Several creatures, including nematodes, can now be identified quickly and precisely using DNA sequencing. However, sample collection to molecular analysis and identification takes days and requires both fixed and mobile lab equipment. In comparison to Sanger sequencing, Knot [179] recognized both free-living and parasitic nematodes (*Caenorhabditis elegans*, *Panagrellus redivivus*, *Turbatrix aceti*, and *Anisakis simplex*) with 96–100% accuracy by this approach. This approach is critical for field genetic nematode identification in soil, marine sediments, and feces. Oxford Nanopore Technologies (ONT) introduced MinION in 2014 [180]. It is a portable USB-powered sequencer that generates lengthy readings in real-time [181]. The nanopore is placed into the biological membrane and drives DNA fragments, causing electrical current variations to be detected and converted to DNA bases [182]. As demonstrated by sequencing efforts, MinION's portability makes it excellent for field study in severe environments like the Arctic [183,184] and Antarctic [185].

#### 4.4. Internal transcribed spacer sequences (ITS)

The 5.8S coding region in the rDNA cistron divides the internal transcribed spacer (ITS) into ITS1 and ITS2 [186], which helps identify closely related nematodes taxa [22,187,188]. For *Caenorhabditis* [26], ITS2 was used for species identification by genetic crossing freshly collected isolates with recognized biological species. However, identical ITS2 sequences in different species make ITS2 a poor criterion for species identification. The fact that rDNA and COX1 are found in numerous copies in worm genomes is a plus. Single nematode DNA templates can be used for PCR amplification. The resulting sequence data is then used in character-based or phylogenetic analysis to identify the taxa. rDNA has become a prominent molecular marker for comparative studies among nematodes of various genera and species. It comprises conserved coding (5S, 18S, and 28S subunits) and variable non-coding regions (ITS and ETS; external-transcribed region) structured as tandem repeats with spacer regions [186]. Because rDNA allows for universal primer construction, distinct portions have been proposed as DNA barcodes in

different organisms to identify each taxon. rDNA provides phylogenetic resolution across taxonomic levels. The recommended DNA sections for bacteria, fungi, and nematodes include 16S [189], ITS [190], and 18S [11,191], respectively. The COX1 region is employed in mammals [192]. Intraspecific changes should be small compared to interspecific variations. While looking for nematodes in deep-sea sediment from the equatorial Pacific Ocean, typical 18S primers frequently amplified fungal sequences rather than the intended target nematode genes [27].

## 5. Sequence databases

An independent framework for comparing data from different studies such as morphometrical, morphological, embryological, and biological studies is provided by deducing relationships at all levels among influential groups. Developing more specific primers and gene probes to address specific taxonomic queries requires DNA sequencing and sequence databases. Once collected, gene sequences are submitted to and kept in EMBL Bank, Gen Bank, DNA Database of Japan (DDBJ), WORMBASE, and EMBASE. Large data sets from over 160,000 organisms (<http://www.ncbi.nlm.nih.gov/guide/taxonomy/>) are stored in these databases. The databases would be an invaluable resource for study and education: DNA-based databases previously targeted quickly grown and plant-parasitic nematodes [29]. The Nematol database (<http://nematol.unh.edu/index.php>) comprises images, sequences, phylogenetic trees, and Nemys (<http://nemys.ugent.be/>) that contains a massive collection of inaccessible literature on the taxonomy of nematodes. With the development of molecular methods, numerous studies were made that shared databases online. A few examples of these databases are described below:

EMBASE is a public database of sequences and meta-data. It was created in the Edinburgh-Wellcome Trust Sanger Institute project on parasitic nematode expressed sequence tags [193,194]. Researchers use this web resource for nematode biology, vaccine design, and drug discovery research ([www.nematodes.org/nembase4](http://www.nematodes.org/nembase4)). The WormBase is an international association of biologists and computer scientists who provide accurate and current information about *C. elegans* and related nematodes ([www.wormbase.org](http://www.wormbase.org)). For more specialized usage or as an aid to interpreting taxonomic literature, smaller databases like the WoRMS database (World Register of Marine Species, [www.marinespecies.org](http://www.marinespecies.org)) were created. MTDNA-Mitochondrial Directory lists mitochondrial genes. It shows nucleotide and peptide sequences, homology to similar model organisms, protein domain matches, and PSORT localization expectation data, among other things. *C. elegans* WWW Server at UTSW provides access to genome, protocols, strains, locations, photos, and *C. elegans* researchers.

The WHO-TDR funds the *Filarial Genome Network*, with additional support from The McConnell Clark Foundation. It describes *Mansonella ozzardi*, *Onchocerca volvulus*, *Mansonella streptocerca*, *Mansonella perstans*, and *Wuchereria bancrofti* are among the most well-known filarial nematodes. Access to EMBL, finished and unfinished nematode sequences, a BLAST search of nematode sequences, *C. elegans* Mitochondrial page, predicted protein sequences from the project (WORMPEP), Tc1 insertion polymorphism, and *C. elegans* Splice Sites, are supported by *C. elegans* Genome Project site at the Sanger Centre.

### 5.1. NemaGene and TremaGene

NemaGene (<http://nematode.net/NemaGene.html>) and TremaGene (<http://trematode.net/TremaGene.html>) are assemblages of genes and contigs assembled and annotated by the McDonnell Genome Institute (MGI) or published by researchers. Sequence similarity searches using WU-BLAST 2.0 [195] and InterProScan (software version 4.8, InterPro database release 32.0) [196,197] are used to assign functional annotations. They contain annotations using InterPro (IPR) identifiers, Gene Ontology (GO) words, and KEGG Orthology (KO) identifiers [198,199]. Currently, Nema Gene contains 1,456,372 entries for 73 species.

TremaGene currently contains 253,360 entries for 12 species (7 are in progress and will become accessible soon; *Fasciola gigantica*, *Fasciolopsis buski*, *Paragonimus westermani*, *P. kellicotti*, *P. miyazaki*, *P. heterotremus*, and *Opisthorchis viverrini*).

### 5.2. NemaBlast and TremaBlast

Researchers can use NemaBlast (<http://nematode.net/NemaBlast.html>) and TremaBlast (<http://trematode.net/TremaBlast.html>) to search for a sequence of interest against a custom database they specify. Both programs generate alignments using WU-BLAST 2.0. NemaBlast is divided into two mapping groups. The first is a collection of Expressed Sequence Tag (EST) readings classified by the library. This set contains all EST reads generated for sequenced species, organized by species and sequencing library, allowing users to combine and match to create their own search space. NCBI does not host assembled EST contigs; instead, they supply the community with annotated Sanger-based assembled transcripts. 275,850 EST sequences from 132 libraries from 31 nematode species are included in the EST readings organized by the library collection. The second category contains assembled contigs, isotigs, and genes. This collection provides a closer look at the host species' whole gene set. Numerous species have multiple listings because users can do searches against transcript or gene datasets. TremaBlast enables visitors to do searches against TremaGene's protein sets. It now contains 221,003 protein sequences from 12 trematode species. The researcher may organize these species in whatever way he or she likes to create a search space for this alignment. The results will be delivered to the email address specified in the state in standard WU-BLAST 2.0 text output.

### 5.3. NemaBrowse and TremaBrowse

NemaBrowse (<http://nematode.net/NemaBrowse.html>) and TremaBrowse (<http://trematode.net/TremaBrowse.html>) make use of GMOD's GBrowse to visualize gene annotations and variations mapped onto genomic assemblies [200]. These occasionally contain in-progress nematode and trematode genomes and provide a practical comparative view when variant calls for specific lab and field isolates are available. Typically, visualized annotations include predictions of RNA genes and Maker protein-coding genes [201,202], tRNAs predicted using tRNA scan [203], and Single Nucleotide Polymorphism (SNP) sites predicted using Genome Analysis ToolKit (GATK) [204] and annotates using SnpEff [205]. Currently, NemaBrowse contains annotations for ten species, while TremaBrowse contains one species.

### 5.4. NemaPath and TremaPath

NemaPath and TremaPath are used to visualize the presence, absence, and total coverage of enzymatic pathways in species using knockout gene annotations [199]. Regardless of the single-organism perspective, N/Tr emaPath allows comparative perspectives between species pairs. Using real-world transcriptome data enables users to visualize and study the distinctions between enzymatic pathway entities. NemaPath contains 1,103,786 annotated genes and transcripts from 63 different species, whereas TremaPath contains 204,647 annotated proteins from 11 different trematodes. This tool can assist users in various ways, including discovering prospective therapeutic targets and comprehending the distinctions between animals that employ distinct survival strategies. Genes are annotated with KO annotations using WU-BLAST 2.0 alignments against the KEGG gene database (release 68.0). The KO identifiers of the harmed subjects are utilized to map helminth sequences to KEGG pathways. In KEGG pathway maps' enzymatic nodes are coloured to indicate the amount of supporting genes discovered in each species.

## 6. Conclusion

We are currently experiencing a biodiversity crisis. Every day, humans affect the ecosystem and destroy wildlife. While this is going on, the number of taxonomists investigating residual biodiversity is decreasing [206–208]. It is hard to find a new generation of taxonomists. This situation is likely due to identifying attitudes in society and educational institutions and species that are "invisible" from an economic and medical human perspective. Biodiversity and richness assessments are vital yet time demanding. The small size and lack of expected knowledge about the group encountered make it challenging to identify individual organisms morphologically to recognized species, notably for nematodes, whose diversity in soils and sediments is unclear.

Despite only a few thousand species being published in the scientific literature, investigations of benthic sediments reveal a million nematode species. Terrestrial nematode diversity is underreported. Many nematode species remain unclassified. So, a robust and transferrable identification system is essential for all individuals and species. The taxonomy of nematodes has always relied on morphology. Morphology is an essential component of all higher-level nematode classifications, and it may often be used to identify species quickly. Intra-specific grouping requires additional taxonomic processes to separate nematode species, subspecies, races, pathotypes, and strains. Biological inventories and biodiversity studies are in high demand, yet researchers are scarce. Using a portion of the genome as a molecular marker can help organism's identification. However, no molecular technique provides all taxonomical details; the choice of approach depends on the nature of the samples, resources, and queries. Further taxonomical research and development in the molecular approach are much needed to explore the nematode diversity for humankind.

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## Declaration of Competing Interest

No conflict of interest exists for the present manuscript.

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