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1. Nematode Identification and Characterization Techniques

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Abstract:

The production of important crops around the world is constrained by plant parasitic nematodes. They worldwide are thought to reduce crop yields by an average of 10-15% per year. The sustainable production of food across the globe is put to test by this. Nematode related issues have become more problematic due open area crop extension, intense cropping and crop rotation. Thus, it is currently necessary to discover sustainable means of containing these infections. To select effective management strategies and conduct insightful research, nematode species must be correctly identified. Because characteristics can vary within a single species, morphology based nematode classification has proven difficult. But utilizing methods based on genetic markers and biochemical has been effectively used to diagnosis a range of nematode species. Even though this new technique has been helpful because of their practicality, speed, accuracy and cost effectiveness, the use of integrative diagnosis combining morphology, biochemical and molecular data are more appropriate when it comes to strengthening diagnosis defining species boundaries, and having a more suitable molecular database for nematode species. Several molecular techniques have been applied with varying degrees of success to support morphology-based techniques and/or avoid these tissues. These techniques include anything from fingerprinting to protein and/or DNA based information sequence analysis. Moreover, the use of image analysis tools has helped this success. In this article, we present a review of the existing approaches and equipment's for locating plant parasitic nematodes.

Keywords:

Nematode identification, Nematode identification techniques, nematode diagnosis based on biochemical and molecular

1.1 Introduction:

Nematodes, boasting a staggering diversity and overwhelming abundance, reign as the dominant metazoans in both soil and aquatic sediments, comprising a staggering one million species (Abad *et al*. 2008). Nevertheless, nematodes remain one of the least studied organisms, with less than 0.01% of their species diversity having been documented to date (Abebe *et al*. 2011). Out of the vast array of more than 26,000 documented species, over 4,100 of them pose a significant threat, leading to substantial economic losses in the agricultural sector due to the damage they inflict on crop (Jones *et al.* 2013).

Nematodes play a pivotal role in the realms of medicine, veterinary science (Blaxter 2011), and environmental nutrient recycling. Their accurate identification is a cornerstone for understanding the vast diversity within the nematode world and crafting efficient strategies for their management and control. Historically, nematode identification relied on tangible characteristics like body dimensions, reproductive organ shapes, mouth and tail structures, and other physical attributes. However, this conventional approach faces challenges. Notably, it can fall short when distinguishing closely related species due to limited apparent variations. Moreover, the dearth of proficient taxonomists, whose numbers are dwindling, further compounds the issues associated with morphology-based classification (De Oliveira *et al.* 2011). Morphology-based identification can be challenging, particularly when there are many samples involved.

Various sub-organismal techniques, primarily focused on proteins and DNA, have been employed to address the limitations associated with morphology-based nematode classification. One significant milestone in this regard was the utilization of nematode ribosomal DNA (rDNA) sequencing in the groundbreaking research conducted by Blaxter *et al.* in 1998.

This pioneering work greatly advanced our understanding of nematode evolutionary relationships and species identification. While delving into the intricate details of worm evolution and their evolutionary connections is beyond our scope here, it is imperative to grasp the significance of accurately identifying nematode species and to appreciate the trade-off between a pragmatic species definition and one grounded in rigorous philosophical principles. In nematode identification, there exists a delicate balance between a practical, functional species definition and one that adheres strictly to philosophical ideals (Adams, 2001).

While it is undeniably important to place nematode species within their correct evolutionary lineages, operational species definitions are more commonly employed in nematode identification techniques. These operational definitions are primarily aimed at assessing potential threats to the well-being of plants and animals, ensuring the health and safety of ecosystems, without resorting to undue philosophical complexities.

1.2 Traditional or Morphometric Identification Methods:

1.2.1 Traditional or Morphometric Identification:

Traditional nematode identification methods have long relied on microscopic image analysis to discern differences in morphology and anatomy among various nematode species. Among these methods, morphological identification has been a cost-effective approach that seeks to establish a connection between physical characteristics and potential functions. However, this approach encounters challenges when attempting to differentiate nematodes that share subtle morphological and morphometric differences, such as body length, the presence and shape of a stylet, tail morphology, and other features. This difficulty is particularly pronounced when dealing with nematodes that exhibit limited morphological distinctions (De Oliveira *et al.* 2011). For instance, the identification of root-knot nematodes (RKN), scientifically known as *Meloidogyne* spp., initially relied on the examination of adult female perineal patterns (Karssen and Van Alest, 2001; Eisenback and Hunt, 2009). These patterns encompassed the posterior region, including the vulva-anus area (perineum), tail terminus, phasmids, lateral lines, and the surrounding cuticular striae (Eisenback *et al.* 1980). These characteristics were initially proposed as a means to distinguish among RKN species like *Meloidogyne incognita*, *M. javanica, M. arenaria*, and *M. Hapla* (Chitwood 1887). However, as new nematode species were discovered, it became evident that perineal patterns, along with other morphometric features, often overlapped between these species (Brito *et al*. 2004; Villae-Luna *et al*. 2016; Maurya *et al*. 2020). Consequently, relying solely on these morphological traits ceased to be sufficient for accurate species identification (Ye *et al.* 2019; Da Chunha 2018).

Today, the identification of RKN species has evolved to incorporate a combination of both morphological and molecular traits. This approach is necessary to address the limitations of relying solely on morphological characteristics, as it allows for a more precise and comprehensive understanding of nematode diversity. Molecular techniques, such as DNA analysis and genetic markers, have become invaluable tools in elucidating the genetic variations that underlie species distinctions. By integrating these molecular insights with traditional morphological observations, researchers can now more confidently and accurately identify RKN species and distinguish them from newly discovered nematode species, marking a significant advancement in nematology.

Nematode species identification relies heavily on intricate morphological features, encompassing traits such as head shape, annual count, total height, stylet length, stylet knob morphology, lateral field structure, spermatheca presence and form, female tail terminus shape, spicule characteristics, and gubernaculum attributes. Unfortunately, the scarcity of taxonomists with the requisite expertise poses a significant obstacle to measuring these traits and analyzing samples effectively (Handoo *et al*. 2008).

Moreover, the morphological characteristics of nematodes can undergo alterations in response to diverse environmental factors, including habitat variations, host plant interactions, nutritional conditions, and other influences (De Oliveira *et al*. 2011). Consequently, accurately discerning nematode species solely through morphological examination can be formidable, especially for those lacking specialized knowledge.

Integrating sub-organismal data, such as DNA sequencing, may become imperative. Recent strides in high-performance computing, however, hold the promise of improving the precision of human-assisted image assessments in nematode taxonomy (Carneiro *et al.* 2017).

1.2.2 The Use of Technology:

Artificial intelligence, encompassing deep learning and machine learning techniques, has revolutionized the identification and quantification of nematodes through image analysis. This approach proves particularly valuable for efficiently managing large sample volumes and detecting elusive and minuscule entities like nematode eggs amidst complex backgrounds. The automated detection of nematode phenotypes involves several stages of machine learning. To minimize subjectivity, a substantial dataset of nematode images, including their eggs and cysts, is initially amassed and independently annotated by a panel of experts. This annotated dataset serves as the foundation for developing an algorithm that learns, in a layered hierarchy, the salient characteristics of these objects from images while filtering out background noise. Subsequently, a network model, employing a supervised learning algorithm, reconstructs the specific patterns of interest from input images. Addressing variations in background noise across samples from diverse sources, Akintayo *et al.* (2018) introduced a novel end-to-end Convolutional Selective Auto encoder (CSAE) designed for the identification of soybean cyst nematode (SCN) eggs amidst varying backgrounds. Through the utilization of numerous annotated image patches, smaller segments within the overall image, the CSAE is trained to recognize SCN eggs. Determining the presence of an egg within a specific patch involves combining information from several overlapping local patches to reconstruct the entire image. The model's correlation of pixel intensity values with reconstructed images reflects the confidence level in predicting whether the object in the image is indeed an SCN egg. In validation tests conducted with two sample sets collected from regions with different soil conditions, egg counts performed by human experts and those generated using this AI technique were found to be statistically equivalent at the 95% confidence level.

In a study conducted by Hakim *et al.* (2018), they developed an innovative artificial intelligence method cantered around the use of *Caenorhabditis elegans*, a nematode worm, to create a comprehensive platform known as WorMachine. This platform leverages the functionalities of various image processing software to enable automated and simultaneous analysis of informative phenotypic traits within a unified framework. The WorMachine platform's image processing component takes static input images obtained through bright-field acquisitions, which may or may not have overlapping regions, and performs tasks such as binarization, identification, and cropping of specific worms. Following this initial step, a feature extractor is employed to separate morphological and fluorescence characteristics from the isolated worm masks. These distinct attributes are then analyzed individually, facilitating the labeling of different worms within the images. To further enhance its capabilities, the machine learning component of WorMachine utilizes techniques like Principal Component Analysis (PCA) and t-distributed Stochastic Neighbor Embedding (t-SNE). These methods enable the platform to perform binary classification or scoring of intricate phenotypes based on the extracted features and assigned labels, employing t-SNE for the visualization of these multi-dimensional data points. To demonstrate the platform's efficacy, the authors conducted experiments using fluorescent reporters to discern sex-

specific expression patterns in mutant *C. elegans* strains. This allowed them to distinguish between males (XO) and hermaphrodites (XX), as well as various intermediate phenotypes. Notably, the research employed a strain with mutations in the sex-determination gene, CB5362, as a case study. WorMachine successfully quantified continuous morphological phenotypes, including measurements of tail shape, gonad width (with a focus on mid-width in egg-bearing worms), body length, and area (where males exhibit smaller dimensions). Additionally, the brightness of the head and tail regions (with darker tails in males under bright-field conditions) was assessed. Utilizing the extracted data and employing PCA and t-SNE analyses, the authors were able to estimate the extent of masculinization for each individual worm. Their findings reinforced earlier research observations, indicating a correlation between higher temperatures and increased masculinity in the studied nematode population. This research showcases the potential of WorMachine as a valuable tool for quantitative analysis in the field of phenotypic trait research.

1.2.3 Auto Florescence:

Utilizing the inherent auto fluorescence of microorganisms offers a valuable enhancement to traditional light microscopy techniques. In a study conducted by Bhatta *et al.* 2006, they illuminated the distinctive emission and excitation spectra of bacterial genera like Lactobacillus and Saccharomyces. Notably, they emphasized the potential utility of these spectroscopic fingerprints for distinguishing various fungal species within the Saccharomyces genus, all without the need for fluorescent labeling. Building upon this research, Qazi *et al.* 2020 explored the auto fluorescence characteristics of different helminth eggs across a range of wavelengths, from visible white light to the infrared spectrum.

Qazi *et al.* (2020) asserted that aspects of Raman spectroscopy and parameters related to fluorescence lifetime values offer promising avenues for taxonomic classification within nematode organisms. Their work demonstrated that variations in fluorescence lifetime values, representing the decay in fluorescence intensity over time, served as diagnostic markers for distinguishing between species such as *Ascaris lumbricoides* and *A. suum.*

1.3 Techniques of Molecular Identification:

Molecular techniques have improved over traditional or classical taxonomic methods for nematode characterization (Ahmed *et al*., 2015). The widely used and effective Polymerase Chain Reaction (PCR) is used to categories nematodes (Blok 2005; Reslova *et al*. 2021).

1.3.1 PCR-Based Techniques:

PCR-based markers have revolutionized the categorization and characterization of new species within nematode taxa, such as *Rhabditid, Meloidogyne, Pratylenchus, Globodera,* and *Heterodera*. These molecular tools have proven to be both reliable and significant in advancing our understanding of nematode diversity (Ibrahim *et al*. 2017; Madani *et al*. 2005; Shah and Mir 2015). In the realm of agricultural animal health, the use of DNA-based technologies like real-time polymerase chain reaction (PCR) and multiplexed tandem PCR has transformed the initial screening for strong lid nematodes. This modern approach has

replaced traditional larval culture techniques and offers numerous advantages, including heightened sensitivity, specificity, rapid results, ease of use, and cost-effectiveness compared to conventional diagnostic procedures. Furthermore, PCR-based detection methods allow for the efficient production of numerous in vitro clones of a specific DNA template, facilitating research and taxonomy studies. Over the last decade of the 20th century, several studies recommended integrating these efficient molecular techniques with traditional methods to gain a more comprehensive understanding of nematode taxonomy (Handoo *et al.* 2008; Gasser *et al.* 2008). Molecular analysis unveils specific target DNA sequences crucial for identifying nematode species, thus advancing our knowledge of nematode systematic and biology (Mattiucci *et al.* 2008). By providing enhanced sensitivity, accuracy, and time savings, PCR-based molecular approaches represent a significant leap forward in this field, complementing traditional descriptive methods. Importantly, these methods have proven capable of highlighting polymorphism differences among closely related worm species, further contributing to our understanding of nematode diversity and evolution (Thevenoux *et al.* 2020).

Recognizing a specific nematode species within the diverse community residing in soil represents a significant breakthrough in scientific research. Initially, this feat was achieved through the utilization of limited quantities of pure DNA, followed by subsequent confirmation through the identification of individual worms within a robust soil matrix (Carneiro *et al.,* 2004). This process leverages either the actual nematode organisms themselves or their DNA as templates for Polymerase Chain Reaction (PCR) amplification (Seesao *et al.,* 2014). Several researchers have put forth updated methodologies for categorizing and identifying nematodes by revising the 18S rRNA sequence comparison-based approach, with renewed emphasis on PCR techniques (Dawkins and Spencer, 1989). In response to the evolving landscape of molecular biology and the need for more efficient taxonomic identification, a variety of emerging techniques have been developed. These encompass PCR and sequence-based methods such as ITS and COX, as well as probe-based techniques like qRT-PCR and multiplex PCR. Additionally, fingerprint-based approaches, including RFLP, AFLP, and RAPD, have been designed to cater to the diverse demands of nematode taxonomy and identification.

1.3.2 Fingerprint-Based Techniques:

A. RFLP (Restriction Fragment Length Polymorphism):

One of the initial molecular approaches employed to differentiate between various worm species relied on the use of different restriction enzymes to digest complete genomic DNA or specific amplified products. This technique produced distinctive banding patterns based on the degree of sequence divergence among various isolates. It operates on the principle of sequence polymorphism, where distinct cleavage sites for restriction enzymes are provided due to genetic variation, resulting in fragments of varying sizes. For instance, in a study involving the lungworm *Metastrongylus*, the H1 gene and the second intergenic spacer were analyzed using this straightforward method. It was able to confer resistance to *Globodera rostochiensis*, a parasite of the potato cyst nematode, and distinguish between three populations of the *Meloidogyne arenaria* race (Anderson, 2000). Additionally, a study investigated 15 nematode isolates from six different *Trichostronglus* species, revealing the

diversity within morphologically similar filarial parasites through Restriction Fragment Length Polymorphism (RFLP). Another application involved the use of the restriction endonucleases Mbo I and Tag I in combination with probes pBM103 and rDNA from *C. elegans.* This combination generated fragments that enabled differentiation between six filial species (Bogale e*t al.,* 2020). This approach was also applied to categorize various nematodes effectively, such as in the case of *Bursaphelenchus*, where it allowed identification up to the species level. Furthermore, ITS-RFLP has proven valuable in distinguishing between pathogenic and non-pathogenic isolates of *B. xylophilus*. The technique has been utilized to investigate the phylogeny and molecular differentiation of cereal cyst nematodes (CCNs) in several Heterodera and Gotland strain species. By employing the restriction enzyme TaqI, this experiment successfully differentiated between *H. avenae, H. lapitons, H. filipjevi,* and the Gotland strain. These results highlight the versatility of RFLP-based characterization as a valuable method for studying nematodes and elucidating their lineage (Castagnone-Sereno 2011).

B. Polymorphism in Amplified Fragment Length (AFLP):

AFLP, or Amplified Fragment Length Polymorphism, stands out as a robust DNA fingerprinting method for organisms lacking prior sequence information. This technique involves amplifying restriction fragments generated from fully digested genomic DNA, typically using a combination of two restriction enzymes. In the realm of positional gene cloning and molecular breeding, researchers have harnessed the power of AFLP to construct high-density linkage maps. For instance, in a study by Höglund *et al.* (2004), this method was instrumental in identifying genetic variations in lungworms and other parasitic nematodes, as previously demonstrated by Pinedo *et al.* (1993). The AFLP method was developed to overcome challenges associated with adaptor ligation and endonuclease digestion of genomic DNA. Its core concept revolves around selective and precise amplification (Subnotin *et al.* 2000). Utilizing this technique, scientists have been able to delve into gene expression profiles, aiding in the detection of potential parasitic disorders, such as the potato cyst nematode (*Globodera rostochiensis*), as explored by Cameron *et al.* (1988). Moreover, the AFLP approach has shed light on the tobacco cyst nematode (TCN) complex (Mulis *et al.* 1986). While AFLP and RAPD (Random Amplified Polymorphic DNA) procedures share similarities, AFLP tends to yield more dependable results when rigorous experimental guidelines are followed. Unlike RAPD-PCR, AFLP focuses on minute amounts of DNA and does not necessitate prior sequence knowledge, making it a valuable tool in genetic analysis and research.

1.3.3 Sequence-Based Detection Method:

In sequence-based molecular techniques, researchers often analyze nucleotide sequences from specific segments of nuclear DNA, mitochondrial DNA (mtDNA), or even the entire genome (Fang *et al.,* 2010). For diagnostic purposes, many studies rely on ribosomal DNA (rDNA) and the mitochondrial cytochrome C oxidase subunit I (COX1) genes because they contain variable sections that are well-preserved. These genes exist in multiple copies within the worm genome, making identification and PCR amplification relatively straightforward (Umeharo *et al*., 2008). Subsequently, the sequencing data generated is utilized to determine the phylogeny of the taxa (Handoo *et al.,* 2008). Ribosomal DNA (rDNA) is composed of

tandem repeats that include variable non-coding sections like the internal transcribed spacer (ITS) and external transcribed spacer (ETS), along with conserved coding regions such as the 28S, 18S, and 5.8S subunits (Sint *et al.,* 2012). These repeating units are interspersed with intergenic spacers. Notably, the presence of the 5.8S coding region in the rDNA cistron effectively divides the ITS sequence into ITS-1 and ITS-2, providing a source of sequence variability in rDNA that is valuable in molecular systematics, especially for distinguishing closely related or sister species (Mossali *et al.,* 2010). For the diagnosis of *Caenorhabditis* spp., genetic crosses are necessary with unidentified biological species, and these crosses have primarily relied on ITS-2 markers for identification (Fang *et al.,* 2011).

Furthermore, in the context of livestock parasitic nematodes, nuclear rDNA ITS-1 and ITS-2 have consistently proven to be reliable genetic markers. They have been instrumental in distinguishing various strongylid nematodes, including species such as *Haemonchus, Teladorsagia, Ostertagia, Trichostrongylus, Cooperia, Nematodirus*, and *Bunostomum.* It's worth noting that when comparing ITS sequences from different Strongylid nematodes, ITS-1 (ranging from 364 to 522 bp) typically appears larger than ITS-2 (ranging from 215 to 484 bp). A unique feature is observed in the *Ostertagia ostertagi* and *O. lyrata* ITS-1 region (801 bp), which stands out among *Trichostrongylids* due to the presence of an internal 204 bp region that repeats twice (Sapkota *et al.,* 2016).

1.3.4 Probe-Based Detection Techniques:

Two approved probe-based detection techniques for nematode species found in fish populations, such as *Anisakis, Pseudo terranova, Hysterothylacium*, and *Contracaicum*, have been widely utilized in research. These techniques are multiplex PCR and quantitative polymerase chain reaction (qPCR) (Sedlak *et al*. 2004; Correa *et al.* 2014). Multiplex PCR is a versatile method that allows the simultaneous amplification of multiple DNA fragments within a single reaction. This approach has found extensive applications in various biological and medical research fields (Castagnone-Sereno *et al.* 2011). In the case of the ITS region, researchers employed up to seven distinct forward primers in combination with universal reverse primers compatible with all nematode species. This approach enabled the detection of various species even when they co-infect the same host (Correa *et al*. 2014).

In the context of detecting parasitic nematodes *Anisakis* spp. and *Pseudo terranova* spp. in fish-based products, a TaqMan-based qPCR targeting the ITS-1 and 18S rRNA genes was employed, allowing for both detection and quantification (Randing *et al.* 2001). For identifying A. pegriffi in fish, researchers turned to qPCR targeting the ITS-2 gene. In a different study, *Meloidogyne* spp. were utilized to investigate the risk of tomato damage (Hoglund et al. 2004). Li *et al.* (2014) devised a technique to determine the levels of *Heterodera glycine* in soil samples from agricultural fields. This method paved the way for a real-time PCR assay to detect *M. hapla* in soil, particularly around root galls. Notably, this assay allowed differentiation of *M. hapla* DNA from the other 14 Meloidogyne species. Researchers were able to detect M. hapla DNA in soil samples, with as little as a third of an egg's worth of soil, approximately 250 mg. Furthermore, the TaqMan qPCR technique has been instrumental in detecting and quantifying several nematode species, as observed in various studies (Marché *et al.* 2001).

1.3.5 Techniques Based on Protein:

Protein sequences, mass-to-charge ratios, and immunological techniques, such as DNA-based approaches, focus on utilizing unique protein compositions and structures to classify nematode species. Unlike DNA, proteins have a more limited vocabulary due to the redundancy of the genetic code, but their alphabet is significantly more complex, consisting of over 20 amino acids compared to the four DNA bases. The utilization of protein structures and post-translational modifications offers a broader range of diversity to define nematode species and aid in their identification. However, the need for specialized knowledge in protein-based methods often presents a significant barrier.

A. Isozyme Analyses:

One of the earliest methods for nematode identification that did not rely on morphology was the utilization of enzyme phenotypes. Essentially, this technique involved extracting soluble proteins from entire nematodes using buffer solutions, separating these extracts through starch or polyacrylamide gel electrophoresis, and subsequently staining them to detect specific enzymes. This electrophoretic approach, commonly referred to as Multi-Locus Enzyme Electrophoresis (MEE), relies on the migration patterns of isozymes, which exhibit variations in electrical charge, molecular weight, and conformation due to subtle differences in amino acid compositions. While various enzymes like malate dehydrogenase, superoxide dismutase, and glutamate-oxaloacetate transaminase were used to different extents (Esbenshade and Triantaphyllou, 1990; Esbenshade and Triantaphyllou, 1985), esterases emerged as the most frequently employed enzymes (Esbenshade and Triantaphyllou, 1990). In addition to traditional morphological methods, this approach offered valuable insights into the evolutionary relationships, particularly among the primary species within the Meloidogyne genus. Nevertheless, it's important to note that this method was labor-intensive and time-consuming. One limitation was the necessity of including known samples for reference purposes (Esbenshade and Triantaphyllou, 1990).

B. Two-Dimensional Gel Analysis:

In the realm of nematode taxonomy, the utilization of two-dimensional gel electrophoresis (2-DGE) has proven to be a valuable tool. This method enables the separation of complex protein mixtures based on their charge and mass characteristics. It achieves this by first employing isoelectric focusing to resolve proteins by charge in one dimension, followed by mass-based resolution in the orthogonal dimension. The resulting patterns of protein resolution are then used to assess similarities and differences among isolates, which can be transformed into binary data for use in phenetic and cladistic analyses. A noteworthy advantage of 2-DGE in nematode taxonomy is its capacity to provide insights into the evolutionary history of the nematode species being studied. Researchers, such as Navas *et al.* in 2002, have successfully demonstrated how this method can reveal not only species-specific protein differences but also potential evolutionary links between different species. Additionally, 2-DGE can be coupled with mass spectrometry to isolate and investigate species-specific polypeptides, enabling researchers to draw conclusions about the underlying encoding genes. It's important to acknowledge that the effectiveness of 2-DGE in nematode taxonomy depends on various factors, including the specific procedures

employed and the quantity of samples analyzed. These factors influence the number of polypeptides that can be resolved and the degree of polymorphism observed. For instance, Navas *et al.* (2002) reported a range of polypeptide counts, spanning from 73 to 203, among the 18 isolates they examined. Therefore, while 2-DGE offers valuable insights into nematode taxonomy, its outcomes can vary based on experimental conditions and the diversity of samples under investigation.

The authors acknowledged the occasional difficulty in scoring the spots, primarily due to the challenge of distinguishing between genuine variations and potential distortions in the gel. Consequently, they focused on analyzing the 95 locations that consistently exhibited expression in both replicates for each nematode. Within this set, they identified that 37 locations were monomorphic, rendering them uninformative for their study. Notably, two of the nematode species under investigation were represented by only a single isolate. It can be inferred that if the authors had access to a more extensive pool of isolates, the total number of locations analyzed and the informative spots identified could have diverged from their reported findings.

C. Serological Evaluation:

Since Bird's groundbreaking work in 1964, which initially proposed the development of antisera against nematodes, researchers have embarked on a journey to explore the potential of both poly- and monoclonal antibodies (mAbs) in this context. These investigations have yielded diverse outcomes. For example, in 1965, Lee's research revealed intriguing results in the Ouchterlony double diffusion assay. Lee found that when antiserum was generated against *M. incognita* and then tested against antigens from another species within the same genera, *M. hapla,* the distinct arc-shaped precipitation band, which would typically indicate cross-reactivity, was conspicuously absent. However, it's essential to consider that this apparent selectivity might have arisen due to the assay's use of a relatively limited number of nematodes. Subsequent experiments conducted by Hussy in 1972, as well as Hussy et al. in the same year, and Misaghi and McClure in 1974 confirmed the lack of specificity in the reactivity of antisera from *Meloidogyne* spp. This underscores the complex nature of nematode antisera reactivity. The situation remains intricate when dealing with cyst nematodes of *Heterodera* and *Globodera* species, as the findings have been mixed. It's important to note that polyclonal antisera produced against complete macerated nematodes, along with their associated microbiome and metabolites, commonly exhibit cross-reactivity. However, the degree of cross-reactivity and specificity can vary significantly, as evidenced by the diverse results observed in these studies.

Following the groundbreaking development of the hybridoma method by Kohler and Milstein in 1975, the Nematology community had high hopes of harnessing monoclonal antibodies (mAbs) for diagnostic purposes. This innovative approach involved isolating mature B-cells from mice previously immunized with nematode antigens. These B-cells were then fused with mouse lymphoid tumor cells, giving rise to hybridomas capable of producing antibodies indefinitely in vitro. Depending on the specific nematode antigen used for immunization, mAbs offered superior specificity in nematode detection. Through the hybridoma technique, researchers successfully generated mAbs targeting several crucial nematodes relevant to agriculture, including *Heterodera glycines* (Atkinson *et al.,* 1988),

*Meloidogyne inco*gnita (Hussy, 1989), *Globodera rostochiensis*, and *Globodera pallida* (Schots *et al.,* 1989). Notably, certain mAbs exhibited the ability to differentiate between isolates of *G. rostochiensis* and *G. pallida*, as reported by Schots *et al.* (1989). Additionally, these mAbs displayed remarkable sensitivity, enabling immunoassays to detect protein equivalents of just one nematode egg or even less. Despite these successes, the hybridoma approach had its limitations. As the number of nematode samples increased, the process became increasingly labor-intensive.

Moreover, achieving successful fusions between tumor cells and B-cells had a relatively low success rate. Recently, the emergence of single B-cell receptor sequencing (scBCR-seq) technology has opened new avenues for nematode identification. This method allows for the reconstruction of antigen-binding site sequences, facilitating comparative investigations. With the integration of next-generation sequencing technologies, scBCR-seq holds the potential to revitalize and advance nematode identification methods (Goldsrein *et al.,* 2019). This innovative approach offers promising opportunities to overcome the challenges associated with the laborious hybridoma process and enhance the precision of nematode detection in agricultural contexts.

1.4 Conclusions:

Taxonomy serves several important objectives, including the comprehension of biodiversity, species classification, and the promotion of biological knowledge exchange. Effective communication within the field of taxonomy hinges on valid naming, a process often reliant on type specimens and their associated morphological data. However, in some cases, particularly when dealing with environmental materials like eDNA, achieving this requirement can be challenging. Nevertheless, the taxonomic community has come to recognize that relying solely on morphological traits may not capture the full spectrum of biological diversity. As a result, molecular data are increasingly employed to complement or circumvent these limitations. It's important to note that a taxon gains greater significance when its members share distinctive biological characteristics beyond mere similarities in morphology or molecular profiles.

The foundation of taxonomy primarily relies on morphology-based classification. Recent advancements in image analysis have significantly enhanced this field. Leveraging artificial intelligence, we can overcome challenges arising from a shortage of highly trained taxonomists and make unbiased, swift, and accurate identifications. Additionally, assessments of auto fluorescence lifetime values and spectroscopic characteristics provide supplementary attributes for identification purposes.

The identification of taxa through molecular techniques can yield inconsistent results. This inconsistency may arise when researchers interpret sequence data from the same DNA region differently across studies or when they employ distinct DNA regions in their research. Just as taxa based on physical characteristics may not align with those determined through genomic data, and vice versa, molecular methods also exhibit variability. Consequently, there is no one-size-fits-all approach, as the choice of method(s) depends on the specific research question, the nature of the samples under investigation, and the available resources.

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