Molecular Insights into Migratory Plant-Parasitic Nematodes

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Annu. Rev. Phytopathol. 2025. 63:403–30

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

https://doi.org/10.1146/annurev-phyto-112624-103056

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Keywords

molecular plant–nematode interactions, effector biology, functional adaptations, phenotypic diversity

Abstract

Migratory plant-parasitic nematodes (PPNs) pose significant threats to global agriculture and forestry. Recent advances in next-generation sequencing on migratory endoparasitic nematodes have revealed substantial genomic diversity, enhancing our understanding of their evolutionary adaptations and molecular mechanisms of pathogenicity. Their genomic plasticity also reflects functional adaptations for an endoparasitic lifestyle (i.e., detoxification and antioxidant defenses, anhydrobiosis or cryptobiosis, and environmental stress tolerance). Key findings highlight an expanding array of parasitism proteins, suggesting a more complex network of effectors than was previously recognized. This review provides an updated overview of relevant aspects of the biology and parasitic strategies of migratory endoparasitic nematodes, with a focus on species within clades 10 and 12. These molecular insights underscore the importance of ongoing research into lesser-studied species, which will ultimately contribute to the development of targeted strategies for nematode control and crop protection.

1. INTRODUCTION

Nematodes are remarkably ubiquitous and the most abundant animals on the planet. This group of multicellular organisms, mostly microscopic, can thrive in diverse and extreme environments (2). The Phylum Nematoda is currently divided into twelve clades (158), with plant parasitism evolving independently at least four times (9). Plant-parasitic nematodes (PPNs) are distributed across clades 1 (Triplonchida), 2 (Dorylaimida), 10 (Aphelenchoididae), and 12 (Tylenchida) (158) and represent nearly a sixth of currently known nematode species (60). PPNs represent a considerable challenge to global food security and forest ecosystems, causing substantial economic losses in major crops, estimated to exceed 157 billion dollars annually worldwide (98).

The coevolution of PPNs with their host plants resulted in remarkable synchronization between life cycles, developing diverse strategies to become successful plant pathogens (2). Most of our understanding of the molecular basis of these interactions has been focused on sedentary PPNs [i.e., cyst nematodes (CNs) and root-knot nematodes (RKNs)] because of their significant impact on agriculture (3, 40, 114). Migratory PPNs are also critical for agricultural and forest ecosystems worldwide, and recent research has expanded our knowledge of the biology of these nematodes (76, 115). The discovery of a previously unknown migratory PPN, *Litylenchus crenatae* (family Anguinidae), affecting beech forest ecosystems (i.e., beech leaf disease) in North America (166) serves as a prime example of a new species invading and threatening a non-native ecosystem, further illustrating the importance of migratory PPNs.

2. BIOLOGY OF MIGRATORY ENDOPARASITIC NEMATODES

Migratory endoparasitic nematodes present different strategies for how they interact with their host plants and exploit their resources. These nematodes exhibit diverse lifestyles based on their feeding behavior, which can categorize them as either facultative or obligate PPNs (**Figure 1**). For example, the pinewood nematode *Bursaphelenchus xylophilus* and the foliar nematode *Aphelenchoides besseyi*, both from clade 10, are examples of facultative PPNs, switching between fungal and plant-cell feeding based on nutrient availability. In clade 12, although some species are also facultative PPNs (e.g., the fungivorous *Aphelenchus avenae*), the majority are obligate, meaning that they rely on living plant tissues to complete their entire life cycle (e.g., root lesion *Pratylenchus* spp.) (76, 115). Some migratory species employ a relatively simple feeding strategy by acquiring the cell contents as they move through the host tissues (e.g., Pratylenchidae), whereas others develop a more intricate and specialized network of feeding cells as they proliferate within the host, such as hyperplasia and hypertrophy of infected host tissues (e.g., Anguinidae) (124, 166).

Migratory endoparasitic nematodes spend most of their life migrating in the plant tissues, causing physiological changes in belowground (e.g., the root-lesion nematodes *Pratylenchus* or the burrowing nematode *Radopholus similis*) or aboveground host plant parts (e.g., the stem and bulb nematodes *Ditylenchus* spp. or the chrysanthemum foliar nematode *Aphelenchoides ritzemabosi*) (**Figure 1**) (76). The majority of this group infects angiosperm plant species, whereas *B. xylophilus* infects solely gymnosperm coniferous trees with the help of its phoretic partner, the insect vector *Monochamus* spp. (**Figure 1**). Thus, the host specificity of migratory endoparasitic nematodes diverges depending on the species. For example, beech leaf (*L. crenatae*), red ring (*Bursaphelenchus cocophilus*), and pine wilt (*B. xylophilus*) diseases (145, 160, 166) impact forestry trees worldwide, specifically targeting beech, palm, and pine trees, respectively. Other species are cosmopolitan PPNs, able to infect almost all crop categories (i.e., staple, forage, ornamental, and tree crops), like *Pratylenchus penetrans* (21) or *Ditylenchus* spp. (151).

The molecular mechanisms driving the adaptation and specialization of migratory PPNs to specific host plants offer critical insights into parasitism and adaptation, presenting a fascinating

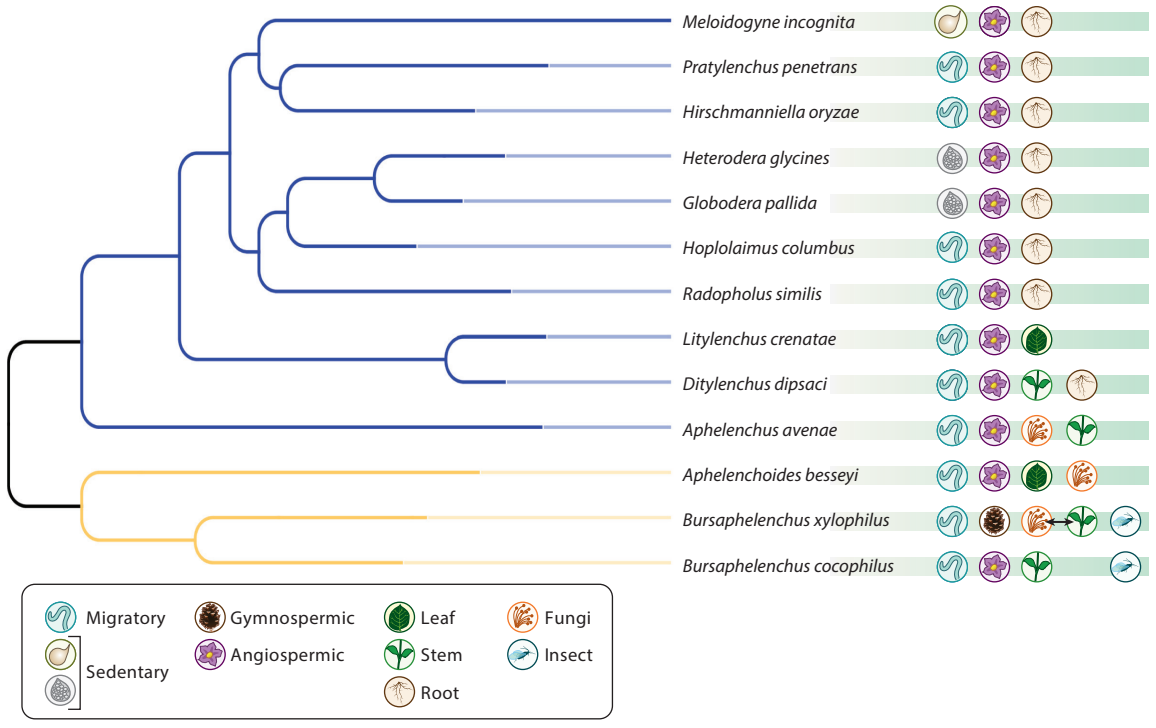

Figure 1

Schematic representation of relevant plant-parasitic nematode species belonging to clade 10 (*orange*) and clade 12 (*blue*) (adapted from 158). The phylogenetic inference was based on 28S rRNA sequences.

area of research. In this review, we focus on migratory endoparasitic PPNs, specifically on species within clades 10 and 12, which include many of the most economically significant species. It is noteworthy that, despite the economic importance of these species, they represent only a small fraction of the total number of migratory PPNs described worldwide.

3. GENERAL FEATURES OF MIGRATORY NEMATODES' MOLECULAR DATA

A significant number of omics data sets has been generated for PPNs, including multiple species of migratory endoparasitic nematodes (82, 118). The use of next-generation sequencing (NGS) facilitates a fast accumulation of data, laying the groundwork for novel and unparalleled integrated comparative analyses across divergent nematode species. Most sequencing efforts have predominantly centered on economically significant species within the Tylenchida and Aphelenchida orders (118). The development of these new large genomic sets provides new perspectives on the evolutionary mechanism driving the establishment of these nematodes. In addition, it enhances the comprehension of the genetic and mechanistic foundations of their pathogenicity and their adaptation to host plants (118).

3.1. Genomic Data

The currently available genome assemblies for migratory endoparasitic PPNs, belonging to clades 10 and 12, are shown in **Figure 2**. The genome sizes varied greatly between species so far

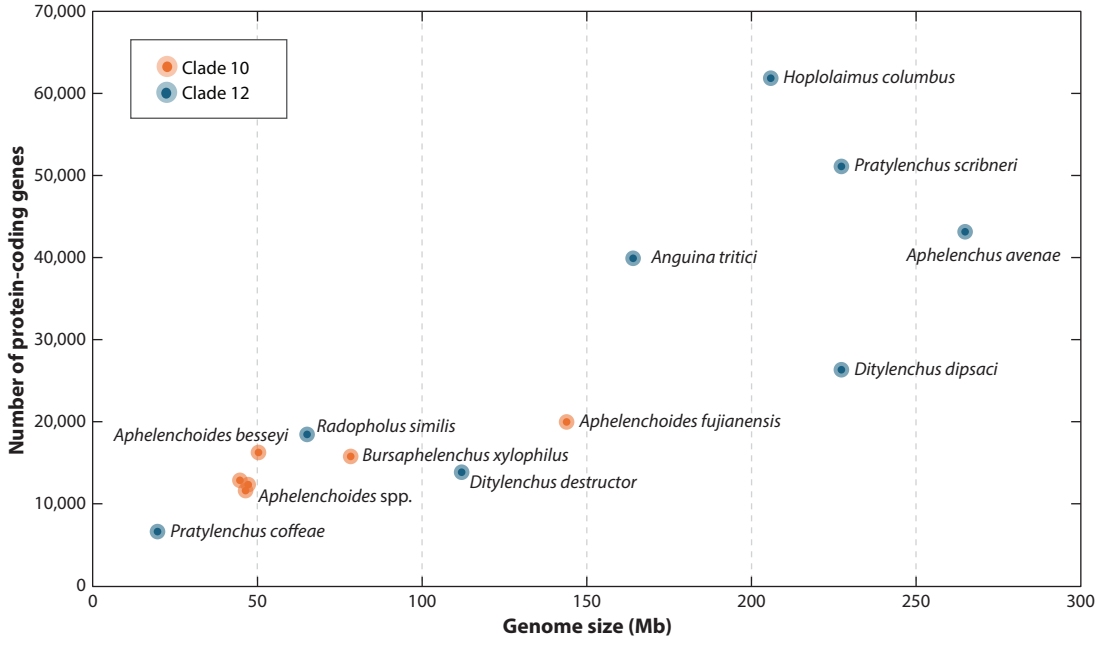

Figure 2

Genome size distribution in relation to the number of protein-coding genes of the most representative migratory plant-parasitic nematodes. The available genomes for species in clade 10 are represented by *Aphelenchoides besseyi* (PRJNA901680) (74), *Aphelenchoides fujianensis* (PRJNA834628); (89), *Aphelenchoides* spp. (PRJNA834627–8) (89), and *Bursaphelenchus xylophilus* (PRJEB40022) (29). In clade 12, the genome assemblies are represented by *Anguina tritici* (PRJNA659265) (147), *Aphelenchus avenae* (PRJNA236621–2) (172), *Ditylenchus dipsaci* (PRJANA498219) (113), *Ditylenchus destructor* (PRJNA312427) (192), *Hoplolaimus columbus* (PRJNA659263) (106), *Pratylenchus coffeae* (PRJNA276478) (14), *Pratylenchus scribneri* (PRJNA932437) (1), and *Radopholus similis* (PRJNA522283) (110).

studied, ranging from 19.6 Mb (*Pratylenchus coffeae*) (14) to 417 Mb (*Hoplolaimus galeatus*) (105), exceeding the model nematode *Caenorhabditis elegans* (100.3 Mb) (15). The diversity of this group is further highlighted by the number of protein-coding genes, which spans from 6,712 in *P. coffeae* (14) to the predicted 61,855 genes in *Hoplolaimus columbus* (106), reflecting the diversity of the lifestyles of these nematodes.

3.1.1. Aphelenchoididae. One of the first genomes of PPNs to be sequenced was the clade 10 migratory nematode *B. xylophilus*. The initial genome version was assembled using 454 and Illumina technologies (74.6 Mb) (81), followed by two updated versions based on Illumina and Nanopore sequencing with genome sizes of 75.9 Mb (153) and 78.3 Mb (29), respectively (**Figure 2**). The first draft genome of *B. xylophilus* (81) revealed the same karyotype as *C. elegans*, and the highest GC (guanine-cytosine) content of any PPN examined (82). The *B. xylophilus* genome exhibits a high level of genetic diversity and significant genomic variations (single nucleotide polymorphisms or indels) between virulent and avirulent populations (126). These variations may be associated with the multiple introductions of this species into new geographic areas or may reflect the pathogenic or ecological traits of the nematode (126). Recently, a chromosome-level assembly comprising six chromosomes with a total size of 77.1 Mb revealed more genetic variations driving genome evolution in different populations of *B. xylophilus* and provided more insights into key protein families potentially associated with pathogenicity and host adaptation, e.g., glycoside hydrolase (GH) families (36).

Several other genomes have been released for the *Aphelenchoides* genus, a sister group of *Bursaphelenchus* (74, 89). With the exception of *Aphelenchoides fujianensis*, which has a genome size of 144 Mb, the genomes of other *Aphelenchoides* species range between 45 Mb for *Aphelenchoides pseudobesseyi* (89) and 50.3 Mb for *A. besseyi* (74). The significant reduction in genome sizes is attributed to the loss of repetitive elements, including the loss of entire gene families, as seen in *Aphelenchoides bicaudatus*, which has lost DNA and LINE (long interspersed nuclear elements) transposable elements (89).

3.1.2. Pratylenchidae. The genome of *P. coffeae* is one of the most compact metazoan genomes to date, with a very low prevalence of repetitive DNA, a reduction in the number of gene family members, and a reduced average number of introns (14). These distinctive features offer valuable insights into the minimum essential gene set required for a functional multicellular organism, shedding light on the evolutionary mechanistic route(s) underlying plant parasitism (14). Two additional partial genome sequences were made available using the genome-skimming technique for *Pratylenchus neglectus*, *Pratylenchus penetrans*, and *Pratylenchus thornei* (31). More recently, the genome assembly of *Pratylenchus scribneri* revealed a much larger genome of 227.2 Mb (**Figure 2**), which could reflect high gene duplication and likely repetitive gene content for this species (1). The significant variation in *Pratylenchus* genome sizes suggests a widespread genome heterogeneity, accompanied by considerable differences in chromosome numbers (e.g., 2n ranging from 10 to 26 chromosomes) observed among amphimictic and nonsexually reproducing species within this genus (135). Interestingly, *Pratylenchus* species are regarded as closely related to RKNs. Therefore, detailed phylogenomic analyses could offer significant insights into the adaptation and evolutionary plasticity across various *Pratylenchus* genomes and, potentially, into the evolution of the more specialized parasitism of RKNs (8).

Although the burrowing nematode *R. similis* is classified as a member of the Pratylenchidae family, its inclusion in this group is primarily based on convergent morphological features (55). Additional molecular and phylogenetic evidence reinforced the idea that *R. similis* is evolutionarily closer to CNs (62, 110, 168). Three draft genome assemblies are currently available for different populations collected from plantain and banana in Costa Rica, ranging from 50 to 65 Mb, with relatively smaller sizes than other migratory nematodes (**Figure 2**) (182). Noteworthy mentions are the two draft genomes generated using single specimens for lance nematodes, namely *H. columbus* (106) and *H. galeatus* (105). Detailed comparative analyses and genome annotation are still lacking.

3.1.3. Anguinidae and Aphelenchidae. Genome data available for the Anguinidae include the potato rot nematode *Ditylenchus destructor* (192), the stem and bulb nematode *Ditylenchus dipsaci* (113, 129), and the weed plant nematode *Ditylenchus weischeri* (129). *D. destructor* revealed the smallest genome assembly for this genus so far (111.1 Mb), whereas the genome of *D. dipsaci* is double the size with 227.2 Mb and double the number of genes (**Figure 2**). *D. destructor* stands out for having a higher number of orthologous genes with *C. elegans* than other sedentary PPNs, suggesting an intermediate evolutionary status between free-living and sedentary forms (192). The draft genome of the seed gall nematode *Anguina tritici* is estimated to be 164 Mb (147). This nematode shows an impressive capacity to survive in an anhydrobiotic state for several decades while retaining its parasitism capacities (99). A partial genome skimming for *Anguina agrostis* has also been released (31) as well as a draft genome assembly for *Subanguina moxae*, indicating a genome size of 90.2 Mb (152).

A genome of 255 Mb was estimated for *A. avenae* (Aphelenchidae), the only genome sequenced so far for this genus (**Figure 2**) (172). The average number of genes is twice that of other nematode genomes, probably due to high levels of gene duplication. The expansion of protein kinases identified in the genome is likely to result from an evolutionary adaptation to desiccation (172).

The growing number of PPN genomes can enhance our understanding of the complexities that differentiate PPNs from their free-living relatives. Moreover, as large-scale genomic studies have progressed, new features are revealed at the species level. However, single reference genomes may not capture the genetic diversity of the species analyzed. Pangenomic analysis, combined with long-read data and transcriptomics, can provide insights at both the individual level (variable genome features) and species level (core genome features), which is highly relevant for discovering important genes associated with pathogenic traits.

3.2. Transcriptomic and Proteomic Data

The increasing availability of transcriptomic data generated for migratory endoparasitic PPNs makes a substantial contribution to existing genomic resources and, in some cases, compensates for the lack of available genomic data sets for various species across multiple clades (**Table 1**). These transcriptome resources are crucial for addressing important biological questions (see Section 3.3) and have expanded our ability to identify parasitism-related genes and genetic adaptations (103). Initially, transcriptomic data relied on small-scale data sets of expressed sequence tags or Roche 454 sequencing (**Table 1**). With the advent of NGS technologies, substantial transcriptome data sets have been generated, allowing large-scale comparative studies (103). Perhaps not surprisingly, many predicted genes lack functional annotation. Moreover, as some of these sequences originated from single species across multiple distant families, a significant number are species- or genus-specific, without any homology in currently available sequence databases (e.g., 38, 57, 128, 164).

Integrating additional omics techniques, like proteomics, along with computational modeling, can offer a more thorough insight into the biological interactions between plants and pathogens. So far, few proteomic studies are available for migratory PPNs. For instance, the profile of the surface coat (SC) proteins of *B. xylophilus* revealed a diverse array of proteins associated with the host immune response [such as regulators of reactive oxygen species (ROS)] and highlighted the binding patterns of several lectins to the SC proteins from different life stages of virulent/avirulent *B. xylophilus* isolates (143, 144). The secretome of *B. xylophilus* stimulated with pine extracts identified several cell wall–degrading enzymes (CWDEs), detoxification enzymes, and peptidases (142). Further studies performed an in-depth characterization of the proteomic changes between the secretomes of *B. xylophilus* and the nonpathogenic *Bursaphelenchus mucronatus* (17) as well as the secretomes from susceptible and nonsusceptible pine extracts (146). In both studies, a large range of proteins were identified, which included peptidases, hydrolases, and proteins with antioxidant activities. More recently, a comparative proteomic study of the secretomes of different *B. xylophilus* populations from distinct geographical locations identified a set of proteins associated with peptidase, cellulase, and lipase activities common among the isolates (18, 19).

3.3. Relevant Adaptations to Migratory Lifestyle

The genetic makeup of PPNs has evolved under the selective pressures imposed by their unique lifestyles, resulting in distinctive gene compositions within their genomes. Despite the modest number of genome assemblies for migratory PPNs, their annotation highlights important aspects of their exclusively motile life cycle compared to other sedentary species (82). Comparative genomics augments the prediction of novel PPN proteins, some of which are found to be acquired by lateral gene transfer (LGT) from other microorganisms, such as bacteria or fungi (9). Furthermore, important biological questions are now revealed and can be seen as a prelude to the significant genomic diversity within this group of nematodes, which remains largely unexplored within the Phylum Nematoda.

Table 1 Representative transcriptomic studies for migratory endoparasitic plant-parasitic nematodes

Clade	Family	Species name	Transcriptome study	Sequencing technology	Accession (GenBank, EMBL, and DDBJ databases)	Reference(s)
ORDER RHABDITIDA						
10	Aphelenchoididae	*Aphelenchoides besseyi*	Mixed-stage population	Illumina GAIIX	SRR1040470	175
		Aphelenchoides ritzemabosi	Mixed-stage population	Illumina HiSeq 2000	SRR3999959	183
		A. besseyi	Mixed-stage population	Illumina HiSeq 2000	SRR4002929-SRR4002930	174
		Aphelenchoides fragariae	Response to anhydrobiosis	Illumina HiSeq 2000	SRP148503	49, 50
	Parasitaphelenchidae	*Bursaphelenchus xylophilus*	Nonparasitic versus parasitic stage	EST	CJ975765-CJ992284	80
		B. xylophilus	Pine and medium-grown propagative mixed stages	EST	NA	78
		B. xylophilus	Nonparasitic versus parasitic stage	Illumina HiSeq 2000	PRJEB9165	39
		B. xylophilus	Nonparasitic versus parasitic stage	Ilumina HiSeq 2000	NA	157
		B. xylophilus	Gland cells	Illumina Nextseq 500	PRJEB24347	38
		B. xylophilus	Life-stage specific	Illumina HiSeq 2000	PRJDB3458	153
		B. xylophilus	Parasitic stage	Illumina HiSeq	PRJNA397001	63–65
ORDER TYLENCHIDA						
12	Anguinidae	*Ditylenchus africanus*	Mixed-stage population	EST	FE920352-FE925198	57
		Ditylenchus destructor	Mixed-stage population	EST	JZ125157–JZ134956	128
		D. destructor	Response to cold and desiccation	Illumina HiSeq 4000	SRR5234500-SRR523504	104
		Anguina tritici	Parasitic stage	Illumina HiSeq (NovaSeq 6000)	PRJNA905225	88
	Aphelenchidae	*Aphelenchus avenae*	Mixed-stage population	EST	GO479265-GO484340	79
		A. avenae	Response to anhydrobiosis	EST	SAMN00167835	133

(*Continued*)

Table 1 (*Continued*)

Clade	Family	Species name	Transcriptome study	Sequencing technology	Accession (GenBank, EMBL, and DDBJ databases)	Reference(s)
	Pratylenchidae	*Pratylenchus thornei*	Mixed-stage population	Roche 454 Gs FLX DNA	JO845319-JO845338; JL859810-JL866456	121
		Pratylenchus penetrans	Gland cells	Roche 454 Gs FLX DNA	NA	108
		P. penetrans	Nonparasitic versus parasitic stage	Illumina HiSeq	PRJNA304159	164
		P. penetrans	Gland cells	Roche 454 Gs FLX DNA	PRJNA432986-PRJNA304159	167
		P. penetrans	Gland cells	Ilumina NextSeq 500	PRJNA512537	169
		Pratylenchus zeae	Mixed-stage population	Roche 454 Gs FLX DNA	PRJNA268047	47
		Hirschmanniella oryzae	Parasitic stage	Roche 454 Gs FLX DNA	SRA048498	4
		Radopholus similis	Mixed-stage population	EST	NA	72
		R. similis	Gland cells	Roche 454 Gs FLX DNA	NA	108
		R. similis	Life-stage specific	Illumina HiSeq2000	SRR6425989-SRR6425988	68
		R. similis	Mixed-stage population and parasitic stage	Illumina HiSeq	PRJNA427497	169

Abbreviations: DDBJ, DNA Data Bank of Japan; EMBL, European Molecular Biology Laboratory; EST, expressed sequence tag; NA, not available.

3.3.1. Detoxification and antioxidant defenses. Migratory PPNs have developed genomic adaptations to manage plant defenses and sustain their homeostasis, both within the host tissues and under challenging environmental conditions. The xenobiotic metabolism of nematodes involves the biotransformation of exogenous plant compounds to facilitate their excretion (reviewed in 100). A prime example is the multilayer detoxification strategy found by studying the genome/transcriptome of *B. xylophilus* that allows nematode evasion of a complex cocktail of ROS products, toxins, and other plant metabolites (39, 46, 81, 161, 189). In addition, some of these detoxification genes (e.g., flavin monooxygenase, short-chain dehydrogenase, aldo/keto reductase, aldehyde dehydrogenase, UDP-glucuronosyl transferases, and ATP-binding cassettes) were found in expanded gene families in *B. xylophilus*, possibly due to gene duplication and divergence of paralogs (81, 82, 189). The importance of ROS-related genes in migratory PPNs and their role in the interaction with plant hosts is detailed below (see Section 4).

3.3.2. Anhydrobiosis and environmental adaptations. Some PPNs can survive low temperatures and desiccation for long periods of time without the presence of a host. The molecular mechanisms involved in nematode cold resistance or anhydrobiosis tolerance start to reveal core molecular information about the plasticity of some migratory PPNs. *A. avenae* is considered a model species for studying anhydrobiosis in PPNs. Early studies have shown that one of the unique features of this species is the accumulation of large amounts of trehalose, a nonreducing sugar that protects membranes and proteins from structural damage under anhydrobiosis (54, 107). The trehalose metabolism of other PPNs has also been related to the ability to withstand harsh environments, such as in *A. besseyi* for anaerobic environments (24) or *B. xylophilus* for cryptobiosis (25, 127). Later, other factors were also related to *A. avenae* desiccation tolerance (11, 12, 22), in particular, the hydrophilic protein Aav-LEA-1, a member of the group-3 subclass of the late embryogenesis abundant (LEA) proteins commonly accumulated by plants under water-deficit conditions (11). Transcriptome data uncovered several gene pathways (including LEA and heat-shock proteins) found to be associated with cold or desiccation stress in *D. destructor* (104). Curiously, homologs of LEA genes were also found in *B. xylophilus* in association with dauer larvae biology (80).

The recent genome assembly of *A. avenae* sheds light on its evolutionary adaptations and comprehensive mechanisms for surviving under anhydrobiosis (172). For example, the finding of 15 LEA proteins, phylogenetically closer to LEA proteins from plants, and 74 species-specific intrinsically disordered proteins has been considered a major molecular strategy for extreme desiccation resistance, as is the presence of a large family of desiccation-resistant heat-shock 70 proteins (172). The analysis of the *A. avenae* transcriptome during its ametabolic state unveiled an increase in ATP levels, facilitating the global recycling of macromolecules and enhancing autophagy during the initial phases of anhydrobiosis (172).

The transcriptomic analysis of the anhydrobiotic response of *Aphelenchoides fragariae* (49) revealed an upregulation of the detoxification-related proteins, multiple genes encoding for molecular chaperones, and unfolded protein response enzymes underlining strategies to cope with cellular toxification and protein misfolding/aggregation (50).

3.3.3. Life stages development. The life cycle of PPNs includes six stages: egg, four juvenile stages (J1–J4), and the adult stage. Some nematodes, such as *B. xylophilus*, present two additional stages (D3 and D4) related to dauer development, which is a nonfeeding stage and highly associated with harsh survival conditions and nematode dispersal (103, 153). Life-stage transcriptome analyses have revealed a core expression pattern of genes related to nematode development (103). In the particular case of *B. xylophilus*, several studies focused on the gene profile dynamics of nematode development, including the formation of the dauer larvae (e.g., 103, 153, 157), analyzed

the whole life-cycle transcriptome of *B. xylophilus* and showed differential gene regulation among both dispersal (D3–D4) and propagative cycles. Genes involved in basic biological functions were found conserved between *C. elegans* and *B. xylophilus*, such as structural constituents of cuticle-, collagen-, and lipid-binding genes, and *C. elegans* orthologs on molting functions (153). CWDEs were differentially expressed between stages (e.g., highly expressed in J3 and D4), emphasizing their stage-specific regulation (153). Peptidases or chitinases (GH18) were also differentially expressed among developmental stages, reinforcing their distinct role function in pathogenicity (153). Zhang et al. (188) analyzed the transcriptomic changes in *B. xylophilus* during the dauer molting process induced by the pine volatile β-pinene, revealing a significant enrichment of differentially expressed genes in the metabolism of xenobiotics, fatty acids, and carbon of the J4 stage. A stage-specific transcriptome data set generated for *R. similis* showed differential gene expression across various developmental stages of this species (69).

3.3.4. Cross-phyla interactions. Migratory endoparasitic PPNs that feed on both plants and fungi serve as excellent model organisms for studying genomic plasticity related to their interaction with species from different phyla. For example, the higher number of digestive proteases (e.g., serine, aspartic, metallo, and cysteine families) found in the *B. xylophilus* genome, in comparison with other PPNs, may indicate the diverse range of potential food substrates (81). For example, the presence of the GH18 chitinase family in this nematode supports their mycetophagous phase (153). Different gene expression patterns have also begun to reveal the complexity of the nematode phase transitions (i.e., mycetophagous versus phytophagous). For instance, many genes associated with xenobiotic detoxification were linked with the phytophagous *B. xylophilus*, accompanied by physiological and morphological changes attributed to adaptation and stimulated by the plant environment (157). Some of these morphological changes were potentially associated with the differential regulation of collagen genes and rearrangements of the nematode cuticle (157). Moreover, Ekino et al. (37) showed that *B. xylophilus* presents a different body structure between the mycetophagous and phytophagous phases, suggesting that nematode ultrastructure phenotypic plasticity is a key strategy for nematode survival inside the hostile plant environment.

Another example of these complex biological interactions is evidenced by the genomic adaptation to phoretic relationships between nematodes and insects. The nonrelated nematodes *B. xylophilus* and the animal parasitic nematode (APN) *Brugia malayi* have evolved life-stage larval forms independently driven by the need for dispersal via an insect vector (103). These distinct larval forms, tailored to the insect vector, evolved separately, suggesting a convergence in their transcriptomes (103). Additionally, these insect-vectored nematodes possess a reduction in the size of C-type lectin family genes compared with self-dispersing ones, whereas their functional domains are more diverse, which may have contributed to the evolutionary transition (122).

4. EFFECTORS OF MIGRATORY PLANT-PARASITIC NEMATODES

Plants and pathogens have shared a complex relationship over the course of their evolutions (9). The development and successful parasitism by PPNs rely on a continuous molecular dialogue between the nematode and the host plant. To successfully colonize their hosts, PPNs secrete cocktails of effector proteins, which suppress plant immunity defenses or interfere with other host cellular and physiological processes to facilitate nematode parasitism (117, 119, 136, 165). To withstand pathogen attack, plants have developed a two-layered immune system composed of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) (75). Although in some cases PTI might be a sufficient defense response, many pathogens have evolved effector proteins that can be deployed to inhibit or suppress PTI-related defenses. Plants have adapted to recognize specific effectors through resistance proteins, activating their

second layer of immunity. This often results in a hypersensitive response (HR) and cell death, which help block pathogen development.

The secretions produced by PPNs are the main pivotal components of the different molecular processes involved in the suppression of host defenses, modulating complex changes in morphology, function, and gene expression in interacting host cells (28, 40, 114, 136). The main sources of these nematode effectors are three large and complex esophageal secretory gland cells, two subventral and one dorsal (40, 114, 165) (**Figure 3**). These gland cells produce and secrete the effectors into the host through the stylet, a mouth spear. Other nematode tissues may also contribute to parasitism-related secretions, such as the amphids, hypodermis, or excretory/secretory system as seen in APNs (**Figure 3**) (42, 139, 156, 159, 191). Building on our knowledge of effectors in sedentary nematodes, secreted proteins are delivered into the apoplasm (42, 163) or cytoplasm (42, 114). Moreover, some effectors, after being secreted into the cytoplasm, can be transported into the apoplasm (e.g., CLE-like peptides from CN) (53) or target other cell compartments, such as the cell nucleus (132). Thus, the diverse host responses and the distinct strategies implemented across migratory nematodes seem to be influenced by the specific set of effectors that each nematode delivers into the plant tissues.

4.1. Identification of Effectors in Migratory Plant-Parasitic Nematodes

The identification of effectors in PPNs has relied on well-established bioinformatic pipelines, which typically involve criteria like the presence of a signal peptide and absence of transmembrane domains, common features of effector proteins. Large amounts of NGS data are being generated, yielding extensive lists of putative secreted proteins, many of which lack predictive domains or known functions (58, 165). Previous attempts to identify effector genes in migratory PPNs have relied on comparative analyses based on sequence homology, revealing conserved genes among many PPNs. The presence of a common repertoire of effectors, excluding LGT-acquired genes, suggests the conservation of specific genetic elements across multiple PPNs, despite their distinct parasitism strategies (168). Although this approach is valuable, it may overlook the effector composition of certain species, particularly when studying the effector repertoires of phylogenetic taxonomically unrelated species.

4.1.1. Target esophageal gland cell transcriptomes. To enhance the accuracy of genuine effector identification, significant progress has been made through the implementation of target cell transcriptomics. This approach involved sequencing of RNA extracted from the isolated gland cells collected via microaspiration (108). This methodology has been successfully applied across various species, providing unprecedentedly accurate repertoires of candidate effectors that encompass both sedentary (108, 117) and migratory PPNs (38, 167, 168, 170). Contrary to previous assumptions that migratory nematodes are less specialized PPNs with a limited number of effectors, recent data have uncovered substantial evidence of a more intricate and extensive network of effectors in these migratory species (38, 39, 167, 168, 170). These gland cell transcriptomes not only revealed an over-representation of homologs of known effectors but, more significantly, have yielded sets of hundreds of reliable pioneer candidate effectors (38, 167, 168, 170).

4.1.2. Promoter motifs. The NGS advances and computational methods have enabled a genome-scale identification of effectors in PPNs, capitalizing on the recognition of *cis*-regulatory elements within the effector gene promoter regions. For example, the pivotal work conducted on the dorsal gland cell of the CN *Globodera rostochiensis* revealed the motif DOG box identified in the promoter region of effector genes (41). Likewise, new *cis*-regulatory motifs have been found for phylogenetically distant migratory PPNs, such as the pinewood (38), root lesion (167, 170), and burrowing nematodes (168) (**Figure 3**). The *B. xylophilus* STATAWAARS motif is a noncanonical

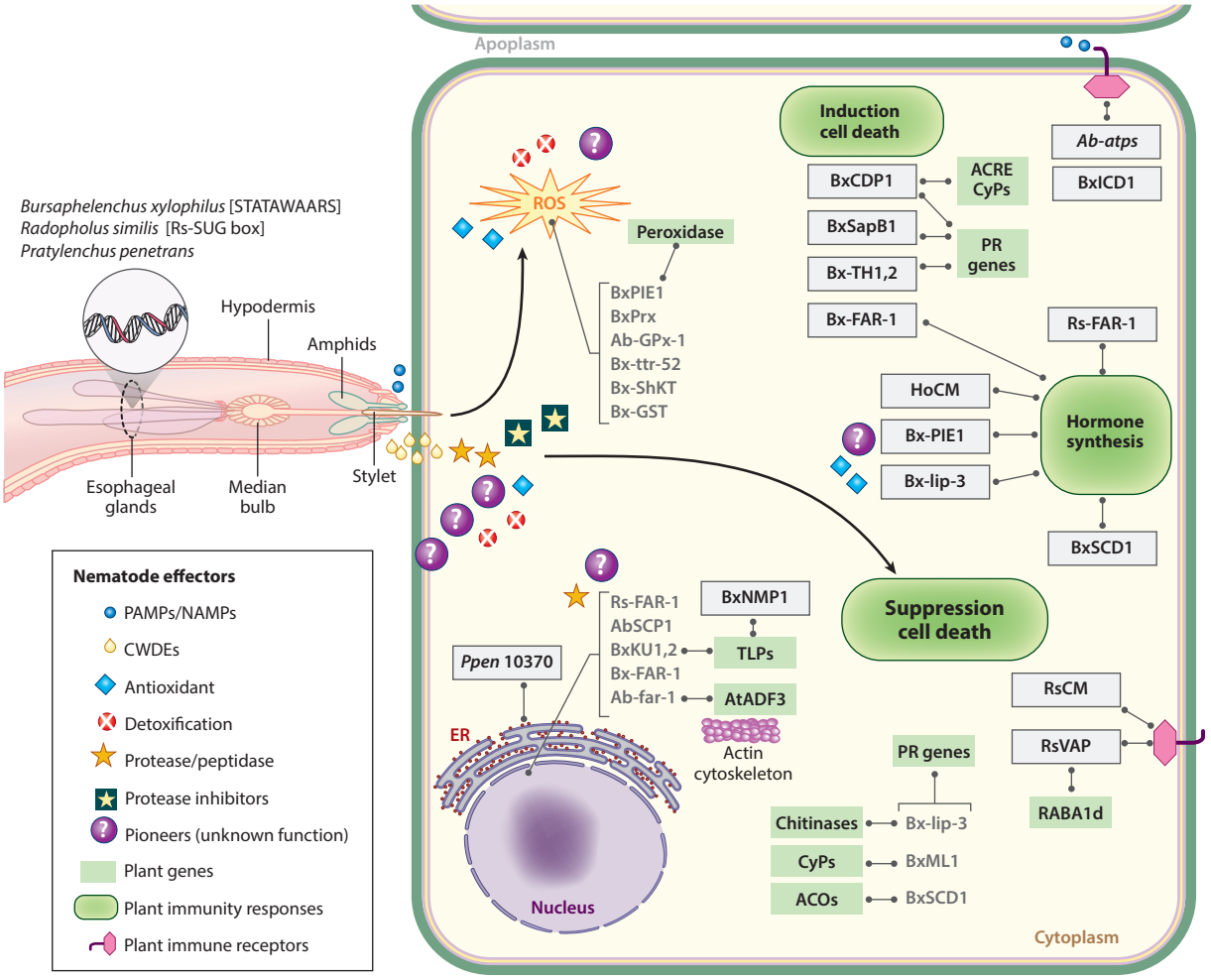

Figure 3

A schematic representation of the interactions between migratory endoparasitic nematodes and their host target cells. Promoter DNA motifs have been found associated with highly expressed effector genes in the esophageal gland cells of some migratory nematodes, such as *Bursaphelenchus xylophilus*, *Pratylenchus penetrans*, and *Radopholus similis*. These effectors can be secreted directly into the cytoplasm and apoplast. The nematode cell wall–degrading enzymes (CWDEs) degrade plant cell walls and contribute to nematode penetration and migration within plant tissues. To withstand pathogen attack, plants have developed a two-layered immune system composed of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). To evade plant defenses, a large set of effectors are secreted, targeting different host molecular pathways, including the suppression of reactive oxygen species (ROS) or cell death. A significant number of nematodes have been identified as inducers of cell death, whereas others may interact with plant hormone pathways (i.e., salicylic acid, jasmonic acid, and ethylene pathways). Abbreviations: ACOs, 1-aminocyclopropane-1-carboxylate oxidase; ACRE, Avr9/Cf-9 rapidly elicited genes; CyPs, cyclophilin proteins; ER, endoplasmic reticulum; HoCM, *Hirschmanniella oryzae* chorismite mutase; NAMP, nematode-associated molecular pattern; PR, pathogenesis-related; RsCM, *Radopholus similis* chorismate mutase; RsVAP, *Radopholus similis* venom allergen-like protein; TLPs, thaumatin-like proteins.

DNA motif overrepresented in the promoter region of a large number of genes encoding secreted proteins and abundantly expressed in the gland cells (38). A preliminary enriched motif of *P. penetrans* with the consensus sequence CAA[A|G|T|C]TG[T|G]C was identified as associated with several genes localized in the subventral glands (167). In the case of *R. similis*, a promoter

motif was identified as associated with effectors localized in the subventral esophageal gland cells, representing the first subventral gland-specific motif (Rs-SUG box) (168).

High-quality genome assemblies are essential to streamlining future effector prediction efforts. The identification of a promoter motif element(s) associated with a proportion of (nonrelated) effector genes implies a coordinated regulatory mechanism governing the expression of these genes (117). A new bioinformatic tool, MOnSTER, has been developed to identify clusters of motifs in effector protein sequences in both sedentary and migratory PPNs (16).

4.1.3. In situ hybridization assays. Although these predictions can provide accurate lists of candidate effectors, experimental validation remains an indispensable step. In situ hybridization (ISH) assays are commonly employed to determine gene spatial localization within the nematode tissues (30). Transcripts of effector candidates have been validated by ISH in the gland cells of migratory PPNs belonging to both clade 10 (e.g., 38, 39, 68) and clade 12 (167, 168, 170). A new whole-mount nematode preparation, the Sperling prep, provides a more sensitive method for localizing and enhancing the visualization of nematode transcripts. This advanced technique utilizes ISH chain reactions to achieve improved resolution in the detection of specific transcripts within PPNs (150).

4.2. Molecular Characterization of Migratory Nematode Effectors

Migratory endoparasitic PPNs can cause significant host cell damage (e.g., roots) while evading recognition and avoiding HR from the plant (148). This suggests that these nematodes allocate resources toward subverting host defense mechanisms. Interestingly, some species may possess a distinct set of effectors to manipulate the cell cycle machinery of their host plants (e.g., Anguinidae) (166). The variability of parasitic strategies among migratory PPNs, coupled with their wide host range, suggests a completely novel and unexplored realm in the effector biology of these species.

Detailed molecular analyses of PPNs reveal a diverse and evolving repertoire of effectors, shaped by various evolutionary processes such as LGT (27), neofunctionalization (97), and de novo gene birth (168). Despite evolving distinct parasitic strategies, migratory and sedentary PPNs share a common set of effector genes and likely target similar host molecular pathways. Grouping effectors into functional classes has underscored the significance of specific genes, or gene families, including distinct sets of CWDEs, genes involved in suppression of host immunity, detoxification, or modulation of ROS, among others.

4.2.1. Cell wall–degrading enzymes. The migratory nature of PPNs implies a continuous movement through the plant tissues. To penetrate the host tissues, nematodes need to overcome the plant cell wall, which consists of a rigid barrier to most pathogens (116). Consistent with previous findings, migratory nematodes exhibit a significant array of CWDEs involved in the degradation or modification of the host cell wall (**Figure 3**): GH gene families, such as cellulases (GH5), polygalacturonases (GH28), xylanases (GH30), arabinases (GH43), and arabinogalactan galactosidases (GH53), among others (7, 10, 79, 81, 89, 128, 169).

Most genes encoding CWDEs are found in the gland cells of species from clades 10 and 12, highlighting their role as genuine candidate effectors. In clade 12, the following species have been validated so far: *A. avenae* (79), *A. tritici* (147), *Ditylenchus africanus* (57), *D. destructor* (128, 192), *Hirschmanniella oryzae* (4), *P. coffeae* (6), *P. penetrans* (162, 167), *Pratylenchus vulnus* (43, 44), *Pratylenchus zeae* (47), and *R. similis* (55, 56, 168). In clade 10, the following species have been validated so far: *B. xylophilus* (83–85) and *A. besseyi* (175). In contrast to the wide occurrence of GH5 cellulase genes within clade 12, GH45 cellulase genes have been exclusively identified in members of clade 10 (48, 81, 83, 125, 175).

Ectopic expression of different CWDEs (e.g., GH16, pectate lyases, and expansins) from *R. similis* using a potato virus X (PVX)-based system led to plant phenotypes consistent with the anticipated roles of these proteins (168). Likewise, PVX-expression of *Pp-pme* in *Nicotiana benthamiana* caused lesion-like spots, chlorosis, vein clearing, and yellowing of leaves post-inoculation (162), whereas expression of *Pp-exp1* led to severe dwarfing, chlorosis, and HR-like lesions on leaves and roots, along with the activation of defense responses in *N. benthamiana* (169). The nature of these responses, whether direct or indirect recognition by the host defense system, remains to be elucidated.

Employing RNA interference (RNAi) assays via nematode soaking or plant-mediated silencing, targeting genes of these CWDE families has significantly hindered root invasion and nematode development and reproduction. Several studies have focused on members of the GH5 family, including *Aphelenchoides fragariae* and *A. ritzemabosi* (48, 183), *D. destructor* (128), *P. coffeae* (6), *P. vulnus* (43, 44), *P. zeae* (47), and *R. similis* (120). Additionally, targeting other CWDEs, such as xylanase-encoding genes from *R. similis* (56) and *P. coffeae* (6), has proven effective in reducing nematode infectivity, most likely because of impaired root penetration and migration.

The significant similarity between analogous genes in bacteria and fungi strongly suggests that some of these CWDEs were acquired through LGT from microbial ancestors, followed by gene duplication to enhance their pathogenicity (27, 83, 125, 138). For example, a new putative β-mannanase, previously undocumented in nematodes, was identified for *H. oryzae* (4). More recently, two new instances of putative LGT have been documented for *P. penetrans* and *R. similis*. In the former, a pectin methylesterase gene marked the initial discovery of this family within Nematoda (162). In the latter, the detection of an α-L-arabinofuranosidase (GH62) in *R. similis* represented the first gene of this family ever found in any metazoan (168).

Analyses of stage-specific transcriptomes have also uncovered unique expression patterns of specific CWDEs (153, 167, 168). In *P. coffeae*, the expression of specific CWDEs was found to adjust to the composition of cellulose or xylan of different plant species, suggesting the ability of the nematode to detect and react to specific root exudate components (6). These findings propose that the ability to modulate gene expression in response to different host plants may represent a common adaptive strategy among PPNs (6).

4.2.2. Reactive oxygen species modulation-related effectors. A key feature of PTI is the strong generation of ROS that mediates a multitude of plant defense mechanisms and related metabolic processes (61, 123, 155). PPNs possess catabolic enzymes known to counterattack the effects of ROS [e.g., superoxide dismutases (SODs), ascorbate peroxidases, catalases (CTLs), glutathione peroxidases, or thioredoxins] (52). Although some of these proteins can protect cells from ROS damage within the tissues, others can be part of the nematode effectorome and be secreted into the surrounding environment or directly into the plant (52). Various antioxidant and detoxification proteins (see Section 3.3.1) that are indeed produced in the nematode gland cells, such as *P. penetrans* CTL (167) or *Ab-GPx-1* (173), ultimately participate in parasitism (**Figure 3**). The gland cell Bx-GST exhibited enzymatic activity against α-pinene, β-pinene, and hydrogen peroxide, suggesting a potential adaptation of the nematode to tackle the range of host-derived compounds (39). The 2-cysteine peroxiredoxin of *B. xylophilus* (BxPrx) with expression in gland cells and other tissues showed antioxidant activity, suggesting protection against free-radical exposure in all life stages (96). The gland cell Bx Prx3-interacting effector 1 (BxPIE1), a small cysteine-rich effector and novel virulence factor, specifically targets the *Pinus thunbergii* PtPrx3–1 (type-III class haem-peroxidase), leading to suppression of ROS metabolism and inactivating jasmonic acid (JA) and ethylene (ET) pathways (137). When silenced, *siBxPIE1* reduced nematode reproduction and disease progression and simultaneously interfered with the upregulation of the

host pathogenesis-related genes *PtPR-3* (class IV chitinase) and *PtPR-9* (peroxidase) (**Figure 3**) (137).

Another category of effectors that are not antioxidant enzymes but have been demonstrated to influence ROS scavenging and potentially assist in managing oxidative stress are nematode-specific transthyretin-like proteins (TTLs). In *B. xylophilus*, a few genes encoding TTLs were found to be expressed mainly in the esophageal glands (38, 180). *Bx-ttr-52* has the ability to degrade hydrogen peroxide, and it has been shown to suppress the activity of several key antioxidant enzymes of pine (e.g., CTL, SOD, PRX) (180). Similar to TTLs, several putative secreted proteins containing ShK domains have been localized in the gland cells of *B. xylophilus* (111), *P. penetrans* (170), and *R. similis* (168). The gland cell–expressed *Bx-ShKT* gene of *B. xylophilus* revealed significant upregulation when exposed to hydrogen peroxide, suggesting a potential role in modulating oxidative stress (111) (**Figure 3**).

4.2.3. Effectors targeting plant immunity responses. Evasion or suppression of the plant immune responses is a hallmark of successful plant pathogens (119). There is growing evidence for the role of secreted effector proteins from migratory PPNs in regulating and targeting key plant molecular pathways involved in immunity to enhance nematode fitness. For example, calreticulin, previously identified in *Meloidogyne incognita*, has been shown to inhibit PTI by suppressing the activation of PTI defense-marker genes and callose deposition (73). Following this work, calreticulin transcripts were also confirmed in the esophageal glands of *P. penetrans* (167), *P. zeae* (47), *R. similis* (94), *D. destructor* (128), and *A. besseyi* (45). Although the function of calreticulin in migratory PPNs is not yet clear, exposure of *R. similis* to RNAi-induced gene silencing, either through soaking assays or tomato transgenic plants expressing *Rs-crt* dsRNA, reduced its reproductive ability and pathogenicity (94). Similarly, knockdown of the *crt-1* gene in both *A. besseyi* (*Ab-crt-1*) and *B. xylophilus* (*Bx-crt-1*) led to reduced nematode reproduction (92), and affected nematode stress adaptation and behavioral patterns (45).

In the second level of defense, plants employ nucleotide-binding and leucine-rich repeat (NB-LRR) proteins, which are produced by disease resistance (R) genes, to detect effectors and activate enhanced innate immunity (ETI) (75). This defense mechanism is characterized by its strength and specificity, aimed at effectively countering the invading pathogens. PPNs have evolved a suite of specialized effectors designed to counteract and suppress host cell death triggered in response to infection. An expanding set of *B. xylophilus* effectors have been identified that specifically suppress cell death. For instance, the BxSCD1 has the ability to suppress cell death and immune responses (ROS and PTI marker genes) in *N. benthamiana* and *P. thunbergii*, by targeting *P. thunbergii* PtACO1 (1-aminocyclopropane-1-carboxylate oxidase 1) (177) (**Figure 3**). Silencing *BxSCD1* led to a delay of the disease in *P. thunbergii* seedlings, thus showing a role in nematode virulence. In addition, this effector also shows some level of interaction with ET biosynthesis (177). Two additional BxSCD have been characterized, namely BxSCD3 (66) and BxSCD5 (63). BxSCD3 is related to host immunity suppression, showing that when secreted into the intracellular space of *N. benthamiana*, it can inhibit cell death triggered by the PAMPs PsXEG1 and INF1 from *Phytophthora sojae* and *Phytophthora infestans*, respectively (66). Silencing of *BxSCD3* impacted the defense responses of *P. thunbergii* while enhancing nematode infection by downregulating the expression of *PtPR-3* and *PtPR-6* genes (66). Additionally, *BxSCD5* is highly upregulated at early stages of infection and suppresses PsXEG1-triggered cell death in *N. benthamiana* when it is secreted into the apoplast (63).

Another effector, BxML1, contains an MD-2-related lipid-recognition (ML) domain and is upregulated during the early stages of pine infection (190) (**Figure 3**). Co-expression of BxML1 suppressed BxCDP1-triggered cell death in *N. benthamiana* and specifically interacted with the

P. thunbergii cyclophilin protein (PtCyP1) (188). Similarly, the candidate effector class III lipase, BxLip-3, suppresses cell death triggered by the PAMPs PsXEG1 and BxCDP1 in *N. benthamiana* (**Figure 3**) and is involved in lipid hydrolysis (131). The expression of two *P. thunbergii* class I chitinases, *PtChia1–3* and *PtChia1–4*, are inhibited by *BxLip-3* during infection (131). In addition, this effector also shows some level of interaction with PtPR-6 (JA-/ET-responsive genes). Two newly reported gland cell–expressed Kunitz effectors, named BxKU1 and BxKU2, have been reported for their ability to suppress cell death induced by PsXEG1 in *P. thunbergii* (179). Both proteins were localized in the plant cell nucleus and cytoplasm when expressed in *N. benthamiana* and interacted with the plant thaumatin-like protein 4 (TLP4). Additionally, BxKU2 also interacts with a cell wall–related extensin-like protein (179) (**Figure 3**). Likewise, the *B. xylophilus* effector *BxNMP1* also interacts with the thaumatin-like protein PtTLP-L2 in *P. thunbergii* (185) (**Figure 3**).

Venom allergen-like protein (VAP) coding genes represent a conserved family of proteins in Nematoda and are known to play a significant role in undermining the host's immune responses (101, 102). VAPs belong to the cysteine-rich secretory protein (CAP) family and are characterized by highly conserved cysteine residues at their carboxyl terminus (181). In CNs, VAPs play a role in suppressing basal immune responses by interacting with a tomato apoplastic papain-like cysteine protease known as $Rcr3^{pim}$ (102). However, in the presence of the plasma membrane immune receptor Cf-2, the disruptions caused by Gr-VAP1 on $Rcr3^{pim}$ trigger a defense-related HR (101). Recent studies are beginning to unveil the significance of this gene family in migratory endoparasitic nematodes. *VAP* genes have been widely reported, including species of both clade 10, *B. xylophilus* (39, 77), and clade 12, *D. africanus* (57), *D. destructor* (23, 128), *R. similis* (91, 168), and *P. penetrans* (164, 167) (**Figure 3**). Interestingly, the increase in the expression patterns of *VAP* genes is correlated with most stages of *B. xylophilus* (39, 77, 184), *R. similis* (91, 168), and *D. destructor* (23), which likely reflects their role in suppressing host defense mechanisms along the nematode migration. Transient expression of *RsVAP* of *R. similis* in tobacco leaves diminished host defense responses by inhibiting the immune reaction triggered by the PAMP flg22 and inhibited cell death induced by two different immune elicitors, BAX and Gpa2/RBP-1 (91). RsVAP interacts with the tomato LeRabA1d protein (ras-related protein RABA1d) and can influence host defense responses (91). Silencing *VAP* genes via RNAi of *R. similis* and *D. destructor* compromised their infectivity and/or lowered their reproduction rates, highlighting the significance of these genes across different clades of the phylum Nematoda (23, 91).

Other effectors have been recognized for their capacity to induce cell death in host plants. For example, a distinct set of genes coding for nematode saposin-like proteins, also known as sphingolipid activator proteins, has been characterized in *B. xylophilus* (**Figure 3**). Collectively, the data indicate that BxSapB1 (65) and BxSapB2 (191) are capable of triggering cell death in *N. benthamiana*. Silencing *BxSapB1* by RNAi led to reduced expression of the pathogenesis-related genes *PtPR-1b*, *PtPR-3*, and *PtPR-5* and delayed the onset of symptoms in infected *P. thunbergii* (65).

Plant receptor-like kinases (RLKs) are transmembrane proteins composed of extracellular regions that act as sensors, perceiving external stimuli and propagating them through intracellular kinase domains (13). Rice OSRLK3 is an RLK containing a LysM domain, known to interact with PAMPs and self-defense reactions. The *A. besseyi* ATPase, *Ab-atps*, was found interacting specifically with OsRLK3 (176) (**Figure 3**). Although *Ab-atps* was found expressed in the nematode esophageal and reproductive system, it targets different subcellular locations of *Nicotiana tabacum* such as the membrane, cytoplasm, and nucleus and triggers plant cell death 5 days after leaf infiltration, suggesting a role in plant infection (176).

The novel molecular pattern BxCDP1 triggers innate immunity in *N. benthamiana*, activates defense mechanisms in *P. thunbergii*, and plays a significant role in the virulence of *B. xylophilus*

(64) (**Figure 3**). BxCDP1 induces cell death in several plant species (e.g., pine, *Arabidopsis*, tomato, pepper, and lettuce plants) and triggers ROS production and the expression of several *N. benthamiana* PTI marker genes, such as *Nicotiana benthamiana* Avr9/Cf-9 rapidly elicited 31 (*NbAcre31*), *NbPTI5*, and *NbCyp71D20* (64). In *P. thunbergii*, the pathogenesis-related genes *PtPR-3*, *PtPR-4*, and *PtPR-5* are also induced by BxCDP1 (64). Another gland cell–expressed gene, *BxICD1*, was found to induce cell death in the apoplastic space of *N. benthamiana*, dependent on *N. benthamiana* brassinosteroid-insensitive 1-associated kinase 1 (*NbBAK1*) (95). Knockdown studies showed that *BxICD1* contributes to nematode virulence and migration in 3-year-old *Pinus massoniana* seedlings (**Figure 3**) (95). These responses are mediated by the plant RLK BAK1, which forms receptor complexes with several pathogen recognition receptors to positively regulate PTI. BAK1 is involved in the recognition of nematodes and the activation of defense mechanisms. Several effectors produced by CNs and RKNs are known to manipulate BAK1 to suppress plant immune responses and facilitate parasitism by interfering with the formation of BAK1-containing receptor complexes, reducing the plant's ability to recognize the presence of the nematode (154).

Other candidate effectors, including thaumatin-like proteins (TLPs) of *B. xylophilus* such as Bx-TLP-1 (112) and Bx-TH1 and Bx-TH2 proteins (87, 141), can trigger cell death when expressed in *N. benthamiana* (**Figure 3**). Evidence suggests that TLPs are components of the nematode's secretome (141, 142). Transcripts of some thaumatin-like genes have been detected in the esophageal gland cells of *H. oryzae* (4) and *B. xylophilus* (38); however, their specific functions remain unknown. *Bx-tlp-1* is regarded as an essential component of nematode pathogenicity. RNAi knockdown of *Bx-tlp-1* decreased *B. xylophilus* reproduction and delayed disease progression in pine seedlings (112). Both *Bx-TH1* and *Bx-TH2* induced high expression levels of PR genes (*PR-2*, *PR-4*, *PR-5*, *PR-6*) at different time points post-infection in pine seed embryos, suggesting their involvement in pine wilt disease (87) (**Figure 3**).

4.2.4. Modulation of plant hormone–mediated responses. Plant hormones are key components of the immune system against PPNs (51). Among others, salicylic acid (SA), known to be produced by phenylalanine ammonia lyase and the isochorismate synthase pathways, is a critical plant hormone that can play a pivotal role as a signaling molecule in the host defense response to plant pathogen infections. Therefore, the SA pathway is frequently targeted by PPNs. In addition, pathogens can also disrupt JA and ET biosynthesis to undermine host defenses. Following the initial discovery of a chorismate mutase (CM) in *Meloidogyne javanica* (90), homolog genes/transcripts of CM have since been reported in various migratory nematodes, including *H. oryzae* (4, 5), *R. similis* (186), and several *Pratylenchus* species (58, 121, 164) (**Figure 3**). Among these species, the most well-characterized functions of CM and isochorismate mutase (ICM) proteins are derived from studies of *H. oryzae* (5). These authors demonstrated that both HoCM and HoICM could restore the enzyme activity in *Escherichia coli* strains lacking these enzymes (5). Although HoCM protein contains a signal peptide for secretion, HoICM does not, indicating that some migratory effector genes might also be secreted into the host plant through other means. Additionally, expression of various HoCM forms (either the mature protein or just the catalytic domain) in transgenic rice lines increased the plants' susceptibility to both *H. oryzae* and *Meloidogyne graminicola* (5). In contrast, HoICM only increased susceptibility to *H. oryzae*. Although SA content remained unchanged, expression of HoCM in rice lines suggested that HoCM might affect the host's immune response by decreasing the formation of secondary phenylpropanoid metabolites (5). In *R. similis*, CM transcripts were confirmed in the esophageal glands, with high expression in females and juveniles (186). Ectopic expression in transgenic tomato roots increased susceptibility, whereas RNAi silencing of *RsCM* affected nematode pathogenicity. Notably, expressing RsCM in *N. benthamiana* leaves reduced callose deposition and downregulated defense gene markers and BAX-induced cell death (186) (**Figure 3**).

4.2.5. Proteases and protease inhibitors. Large numbers of proteases and protease inhibitors have been identified in the secretome of certain PPNs (e.g., 142). These secreted proteases likely play various roles, such as facilitation of host tissue penetration, nutrient acquisition, and degradation of proteins, to interfere with host immune responses and enhance virulence (58, 114). Despite nematodes having hundreds of protease-encoding genes (20), only a small subset is secreted into plant tissues. For instance, the *B. xylophilus* genome contains a high number of digestive peptidases identified among the PPNs, which are highly upregulated after host infection, as well as expanded-family I25 peptidase inhibitors that inhibit C1 peptidase and legumain (C13 family) (17, 18, 81, 87). A subset of secreted proteases has been identified as relevant candidate effectors in various migratory species. A serine carboxypeptidase *AbSCP1* from *A. besseyi* belonging to the S10 family of carboxypeptidases was found expressed in the esophageal gland cells and in all developmental nematode stages (68) (**Figure 3**). When expressed in planta, this effector targeted the nucleus and impaired nematode pathogenicity when silenced via RNAi, suggesting that AbSCP1 is relevant for nematode parasitism (68). Moreover, various proteases have been localized in the esophageal gland cells, as well as other nematode tissues, of *R. similis* (69), *D. destructor* (67), and *P. penetrans* (167). Overall, silencing some of these genes revealed a significant impact on nematode invasion and pathogenicity as well as nematode hatching and development, indicating their potential as target genes for nematode control (67).

Protease inhibitors are abundant in the secretomes and transcriptomes of migratory nematodes (72), which are thought to be secreted to target host proteases and enhance virulence. Several of these genes have been localized in the esophageal gland cells of *B. xylophilus* (86), and *P. penetrans* (164, 167, 170). Recent studies have shown that specific proteases (e.g., serine proteases, as well as protease inhibitors such as trypsin inhibitor-like proteins) can be secreted via the excretory/secretory (E/S) system of *P. penetrans* (167). In APNs, the E/S system is known to play a vital role in modulating the host's immune response (59).

4.2.6. Fatty acid metabolism. Fatty acid– and retinol-binding proteins (FARs) form a unique family of α-helix-rich lipid-binding proteins with strong affinities for fatty acids, retinol, and retinoic acids. Specific to nematodes, FAR proteins are known to play important roles in the infection processes of PPNs (71, 130). In migratory PPNs of clades 10 and 12, FAR transcripts are notably abundant in the hypodermis (187) and esophageal gland cells (39, 167), suggesting their secretion and their direct involvement in promoting parasitism. In *B. xylophilus*, Bx-FAR-1 is suggested to interfere with the plant immune response mediating the expression of plant-related JA pathway genes (93, 178), similar to the roles reported for CNs and RKNs (70, 71) (**Figure 3**). *Bx-FAR-1* is upregulated in the earlier stages of infection and, when silenced, results in a reduction of the nematode reproductive ability and pathogenicity (93). In addition to targeting the nucleus and inducing cell death in *N. benthamiana* (93), Bx-FAR-1 interacts with the *P. thunbergii* Pt-F-box-1 protein, which has been reported to mediate several biotic stresses (178).

FARs have also been identified as promising gene targets for disruption of nematode development (26, 33, 34, 35, 187). In *R. similis*, the contrast between a highly pathogenic population and its less pathogenic counterpart revealed a notable difference in *Rs-far-1* gene expression levels. In addition, RNAi assays further demonstrated that *Rs-FAR-1* plays a pivotal role in modulating the levels of allene oxide synthase, a critical enzyme in the JA pathway (187). In *Aphelenchoides* spp., FAR genes showed multi-tissue localization suggesting different biological functions (26, 35). Immunofluorescence and subcellular localization showed *Ab-far-1* from *A. besseyi* targeting the nucleus and cytoplasm of *A. thaliana* (33). In addition, interaction between Ab-far-1 and *A. thaliana* actin-depolymerizing factor protein AtADF3 was confirmed, suggesting its potential relation with plant PTI inhibition and promotion of parasitism and pathogenicity of *A. besseyi* (33) (**Figure 3**).

4.2.7. Pioneer genes. With recent advancements in effector identification, the substantial prevalence of pioneer genes aligns with prior findings indicating that effectors of PPNs often encompass a high proportion of novel genes with minimal overlap among different taxa. Effectors are known to undergo rapid coevolution to manipulate their host targets efficiently and evade host defense immunity (119, 149). Validation of dozens of candidate pioneer effectors in the gland cells of *P. penetrans* (167, 170), *R. similis* (168), and *B. xylophilus* (38, 39) underscores the vast, yet unexplored, effectorome within the Nematoda. The large majority of these pioneer genes are species- or genus-specific, similar to the trend found for sedentary PPNs (109, 117, 134). However, the proportion of novel effectors may currently be overestimated due to the incomplete genome data available for many species. Furthermore, the overall absence of sequence similarity among these effectors suggests that other methods are needed to extend beyond sequence homology. For example, leveraging AlphaFold protein structural prediction can enhance the identification and evolution of effector families of sequence-unrelated proteins across all PPNs, as shown in fungal plant pathogens (140).

The genome of sedentary PPNs has unveiled a significant expansion within certain effector gene families (149). For instance, a remarkable level of variation has been observed in SPRYSEC (32) or the hypervariable (HYP) CN effector families (149). In the latter, HYPs are single-gene loci that are composed of thousands of distinct alleles (149). This extensive allelic diversity underscores the dynamic evolutionary processes within nematode populations, suggesting rapid adaptation and specialization in response to host plants (149). In the case of migratory nematodes, the large majority of the identified pioneer effectors consist of single-copy genes, with only a few instances showing variation from this pattern (167, 168, 171). Transcript profiles revealed compelling evidence that a significant fraction of these pioneer effectors have a dynamic and preponderant expression during host interaction (38, 39, 167, 171). For example, ectopic expression of three out of seven pioneer genes from *P. penetrans* led to noticeable phenotypes (171). Among these, the *Ppen10370* gene produced the most striking effects, resulting in vein necrosis and lesions in *N. benthamiana* leaves as well as small lesions in tomato and tobacco. C-terminal GFP fusion of the mature Ppen10370 protein is localized in the endoplasmic reticulum (ER) network (**Figure 3**). Transcriptome analysis linked these phenotypic changes to the differential expression of genes involved in stress response, ER protein processing, and antioxidant and detoxification activities. Although the host molecular targets of this effector are still unknown, silencing *Ppen10370* significantly reduced nematode pathogenicity, highlighting its role in parasitism (171).

Overall, our understanding of the function(s) of these pioneer effectors remains elusive. This limitation not only stems from the inherent difficulties posed by their unannotated nature but is also exacerbated by the unique challenges of generating viable transgenic PPNs (40).

5. CONCLUDING REMARKS

The growing volume of high-quality sequencing data on migratory endoparasitic nematodes is enabling us to tackle more and new biological questions, uncovering fascinating aspects of their biology and genetic adaptations. The ongoing research in this field has resulted in a substantial increase in the number of additional candidate effectors and has uncovered a remarkable evolutionary trend within the effectoromes of various economically important species. This capital of knowledge will provide valuable insights for a holistic effector computational structural biology. This includes an in-depth understanding of their host targets and pathways to gain a comprehensive knowledge of their molecular functions integrating a multidisciplinary omics approach. Understanding how effectors of these migratory PPNs interact with such a diverse range of host plants will elucidate the convergent and specific regulatory mechanisms targeted by these

nematodes. Ultimately, the translation of these findings will facilitate the development of new gene-targeted strategies to manage these nematodes and create more resilient crops.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work described in this article was partly funded by Science and Technology Foundation (FCT, Portugal) grants 10.54499/PTDC/ASP-PLA/1108/2021 and 10.54499/PTDC/ASP-PLA/0197/2020. M.E. and C.S.L.V. are supported by Science and Technology Foundation (FCT, Portugal) grants 10.54499/CEECIND/00066/2018/CP1560/CT0003 and 10.54499/CEECIND/00040/2018/CP1560/CT0001, respectively. P.V. is supported by the United States Department of Agriculture Agricultural Research Service CRIS project number 8042-22000-322-000D.

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