

UC Davis

UC Davis Previously Published Works

Title

Developmental dynamic transcriptomics reveals multiple effectors and transcription factors critical for *Ditylenchus destructor* parasitism

Permalink

<https://escholarship.org/uc/item/7526k551>

Journal

BMC Biology, 24(1)

ISSN

1478-5854

Authors

Chen, Yangyang

Zhang, Shurong

Wang, Xueyu

et al.

Publication Date

2025-12-18

DOI

10.1186/s12915-025-02482-7

Peer reviewed

RESEARCH

Open Access



Developmental dynamic transcriptomics reveals multiple effectors and transcription factors critical for *Ditylenchus destructor* parasitism

Yangyang Chen^{1†}, Shurong Zhang^{1†}, Xueyu Wang¹, Boyan Hu¹, Dexin Bo¹, Yucheng Liao¹, Yali Zhang^{1,2}, Noha Mohamed Ashry^{1,3}, Yayi Zhou¹, Donghai Peng¹, Ming Sun^{1*} and Dadong Dai^{1,2*}

Abstract

Background Plant-parasitic nematodes (PPNs) pose a major threat to global agricultural production, yet fundamental research on their biology remains limited. The origin and evolutionary trajectory of PPNS remain elusive, largely due to the scarcity of chromosome-level genomic data. Among them, migratory PPNS are considered a key transitional form between free-living and obligate parasitic lifestyles, as they exhibit both plant parasitism and fungal feeding behaviors.

Results In this study, we assembled a chromosome-level genome of the sweet potato rot nematode *Ditylenchus destructor* and confirmed the presence of four chromosomes through Hi-C scaffolding and karyotype analysis. Comparative genomic analysis with two other migratory PPNS, *Bursaphelenchus xylophilus* and *Aphelenchoides besseyi*, revealed that the Nigon elements in *B. xylophilus* are largely conserved with those of the model organism *Caenorhabditis elegans*, while *D. destructor* and *A. besseyi* exhibit extensive Nigon element rearrangements. These rearrangements were strongly correlated with patterns of protein sequence collinearity. Moreover, transcriptomic profiling across five developmental stages of *D. destructor* identified numerous stage-specific candidate secreted proteins, including putative effectors, and transcription factors. Functional analysis via RNA interference demonstrated that many of these genes play important roles in either embryonic development or parasitism.

Conclusions Together, our results provide valuable genomic and transcriptomic resources for studying PPNS, uncovering critical insights into their genome evolution and parasitism-related gene functions, and laying a crucial foundation for advancing the understanding of PPN biology and their impact on agricultural systems.

Keywords *D. destructor*, Transcriptomic profiling, RNAi, Parasitism

[†]Yangyang Chen and Shurong Zhang contributed equally to this work.

*Correspondence:

Ming Sun

m98sun@mail.hzau.edu.cn

Dadong Dai

daidadong@mail.hzau.edu.cn

Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Background

Plant-parasitic nematodes (PPNs) pose a significant threat to global agriculture, causing substantial crop losses and economic burdens [1]. These different types of plant parasitic nematodes can damage the roots, stems, leaves, flowers and fruits of plants, resulting in reduced plant production, a decrease in product quality and yield, as well as co-infection with other pathogens and ultimately plant death [2]. Among them, *Ditylenchus destructor* is a migratory endoparasitic nematode that infects a wide range of host plants, including sweet potatoes and other economically important crops. Unlike sedentary nematodes, *D. destructor* does not induce and maintain permanent feeding sites and is infectious from juveniles to adults [3]. Understanding the genomic and molecular basis of its parasitism is crucial for developing effective nematode management strategies.

The effector of a pathogen is an important weapon for it to infect its host [4]. Previous studies on *D. destructor* have found many effectors that are closely related to parasitism. For example, RNAi of alpha-amylase can reduce the parasitic ability of nematodes by 76.6% [5]; the allergen-like proteins can promote the infection of *D. destructor* nematodes [6]; Dd-cpl-1 RNAi not only leads to a significant decrease in the nematode's ability to infect, but also causes delayed development of embryos and a decrease in the amount of eggs laid by female [7]. However, no studies have been reported on the function of *D. destructor* transcription factors to date. Transcription factors are central regulators of eukaryotic gene expression, orchestrating diverse biological processes by modulating the transcriptional activity of target genes, and they often play critical roles in the development, environmental adaptation, and pathogenicity of various organisms, including parasites and pathogens [8]. *Meloidogyne incognita* harbors multiple DAF-16- and SKN-1-like genes that are regulated during oxidative stress or host invasion, and host-delivered RNAi targeting these genes significantly impairs nematode infection [9]. In addition, in fungi, the bZIP transcription factor MeaB plays a crucial regulatory role in multiple aspects of *Aspergillus fumigatus*, including virulence, nitrogen metabolism, biofilm formation, and cell wall integrity [10–12]. Therefore, through high quality genome annotation, exploring the role of these virulence genes and transcription factors in the process of nematode infection of the host will be a meaningful work for analyzing the mechanism of nematode parasitism of the host.

Chromosomal organization and transposable elements (TEs) play pivotal roles in shaping genome architecture, influencing gene expression, and driving genetic diversity [13]. TEs not only contribute to genome size variation and structural rearrangements but also serve as a source

of novel regulatory elements, such as promoters, enhancers, and silencers, which can rewire gene expression networks. Through insertional mutagenesis, they can disrupt coding sequences or alter gene regulation, thereby impacting organismal development, adaptation, and evolution. Moreover, TE mobilization is often triggered by environmental stress or biotic interactions, potentially providing rapid genomic responses that facilitate adaptation to new ecological niches, including host–parasite dynamics in PPNs [14, 15]. However, the genomic evolution of migratory parasitic nematodes remains largely unexplored, necessitating a detailed investigation into their chromosomal dynamics and TE landscape. Despite the increasing availability of genomic data, the chromosomal evolution of PPNs remains poorly understood. A key question in nematode genome evolution is how chromosomal rearrangements and transposable elements contribute to adaptation and parasitism. Previous studies have suggested that free-living nematodes, such as *Caenorhabditis elegans*, maintain relatively conserved chromosomal structures, whereas PPNs exhibit extensive rearrangements [16]. To provide a common framework for comparative studies, nematode chromosomes have been classified into seven ancestral linkage groups, known as Nigon elements, which are largely conserved across diverse nematode lineages [13]. Analyses of Nigon elements have revealed both conserved synteny and lineage-specific chromosomal fusions, fissions, and translocations, offering valuable insights into genome evolution, adaptation, and speciation in nematodes. However, the extent to which Nigon element rearrangements have occurred in migratory PPNs, and how such changes relate to parasitic adaptations, remain unclear. Complete, chromosome-scale genome assemblies are therefore essential to resolve these structural dynamics, assess their functional impacts, and advance our understanding of chromosome evolution in PPNs.

In the early stage of our study, we assembled the genome of *D. destructor* through second-generation sequencing [17], and on this basis, the related functional genes of *D. destructor* were studied [5, 7]. At the same time, we established the transcriptome landscape of 11 time points in *D. destructor* embryonic development [18]. Advancements in sequencing technologies have enabled the construction of high-quality chromosome-level genomes, providing unprecedented insights into nematode evolution and host adaptation. Among this, we utilized third-generation sequencing and High-throughput Chromosome Conformation Capture (Hi-C) to assemble the unzipped genome of the polyploid root-knot nematode, providing novel insights into nematode genomics [16]. In this study, we present a chromosome-level genome assembly of *D. destructor* and perform a

comparative genomic analysis with other PPNs, including *B. xylophilus* and *A. besseyi*. Our long-term goal is to address fundamental questions about genome plasticity and adaptability in PPNs by examining chromosome evolution, transposon activity, and the functional roles of secreted proteins and transcription factors in nematode development and parasitism. In this study, we integrate transcriptomic and RNAi analyses to uncover novel insights into the genetic mechanisms underlying nematode infection, thereby identifying potential molecular targets for nematode control strategies. Ultimately, this work advances our understanding of the genomic and evolutionary foundations of PPNs adaptations.

Results

Chromosome-level genome assembly and annotation of *D. destructor*

The chromosomal evolution of plant-parasitic nematodes remains a relatively understudied field, and the construction of chromosome-level genomes is crucial for advancing research in this area. Firstly, we performed Illumina short-read sequencing on the genomic DNA of *D. destructor* and assessed its ploidy using smudgeplot, which indicated that *D. destructor* is diploid (Additional file 1: Fig. S1A) with a haploid genome size of 109 Mb (Additional file 1: Fig. S1B). To further determine the chromosome number of *D. destructor*, we conducted karyotype analysis, which revealed that *D. destructor* possesses 8 chromosomes (Fig. 1A), i.e., $2n=8$. We utilized 25 GB of PacBio Sequel I data to assemble the genome of *D. destructor*, resulting in a genome size of 105 Mb, an N50 of 899 kb, and a total of 344 contigs. Following polishing with second-generation sequencing data, we generated 40 GB of Hi-C sequencing data. Leveraging the Hi-C data, we successfully anchored 99.92% of the contig sequence length onto four chromosomes (Fig. 1B). Our assembled genome is approximately 30 Mb smaller than the recently published version [19] but has similar BUSCO values (72.2% vs 73.8%).

We annotated 93,855 transposon elements in the genome, with a total length of 23.9 Mb, accounting for 15.9% of the genome size. Since both the juveniles and adults of migratory parasitic nematodes have parasitic abilities, current studies have used transcriptome data from mixed stages to annotate their gene expression. To comprehensively study the changes in gene expression of *D. destructor* during development, we used stage synchronization (see method) to obtain 18 transcriptome data from six stages (three biological replicates per stage): egg, J2, J3J4, male, female, and mixed stage. We identified a total of 20,494 protein coding genes, 563 tRNA, and 48 rRNA in *D. destructor's* genome. We then compared our assembled genome with the previously published

one [19], they share the same chromosome number ($n=4$). However, the genome assembled in this study is smaller in size (105 Mb vs. 133 Mb), has a higher proportion of sequences anchored to chromosomes (99.96% vs. 96.74%), contains fewer unanchored contigs (14 vs. 76), and possesses a comparable number of protein-coding genes (20,494 vs. 19,165) as well as a similar GC content (37.07% vs. 38.25%). Our analysis revealed a high degree of collinearity at the gene level between the two genomes (Additional file 1: Fig. S2). Subsequently, we compared the whole genome sequences of the two assemblies and confirmed their high collinearity (Fig. 1C). Additionally, we found that the Dd-A1 genome was larger, with each chromosome containing sequences absent from our assembly (Fig. 1C). In addition, the comparison of the two genomes identified a total of 151,874 SNPs, 26,146 insertions, 23,368 deletions, 93 inversions, 60 translocations, and 42 copygains.

We analyzed various genomic features, including GC content, transposable element (TE) abundance, gene density, and gene expression, and visualized them using circular diagrams (Fig. 1D). To further investigate chromosome organization within the nucleus, we utilized Hi-C data to reconstruct the *D. destructor* chromosome structure and their spatial relationships (Fig. 1E). These findings may offer valuable insights for future studies on gene expression differences and the interplay between stage transitions and chromatin dynamics.

Comparison of genomes among migratory parasitic nematodes

Comparison of chromosomes among PPNs provides valuable insights into their evolutionary trajectories. We conducted a comparative analysis of chromosome sequences among *D. destructor*, *B. xylophilus*, and *A. besseyi*, focusing on their Nigon elements. To facilitate comparison with *Panagrolaimus*, a free-living nematode more closely related to PPNs of the Tylenchomorpha clade [20], we annotated the genome of *Panagrolaimus* sp. ES5 [21]. Nigon element analysis revealed that its autosomes are primarily composed of single Nigon elements, whereas the X chromosome represents a fusion of four major elements—NigonA, C, D, and X—suggesting that it originated through fusion events involving two autosomes (Fig. 2A). The Nigon originals of *D. destructor* and *A. besseyi* were fragmented (Fig. 2B, C), suggesting that multiple chromosome divisions and fusions had occurred. To explore the potential association between variations in Nigon elements and chromosomal rearrangements, we performed a synteny analysis of protein sequences across the chromosomes of these four nematode species. Syntenic blocks were identified only when more than ten adjacent protein sequences

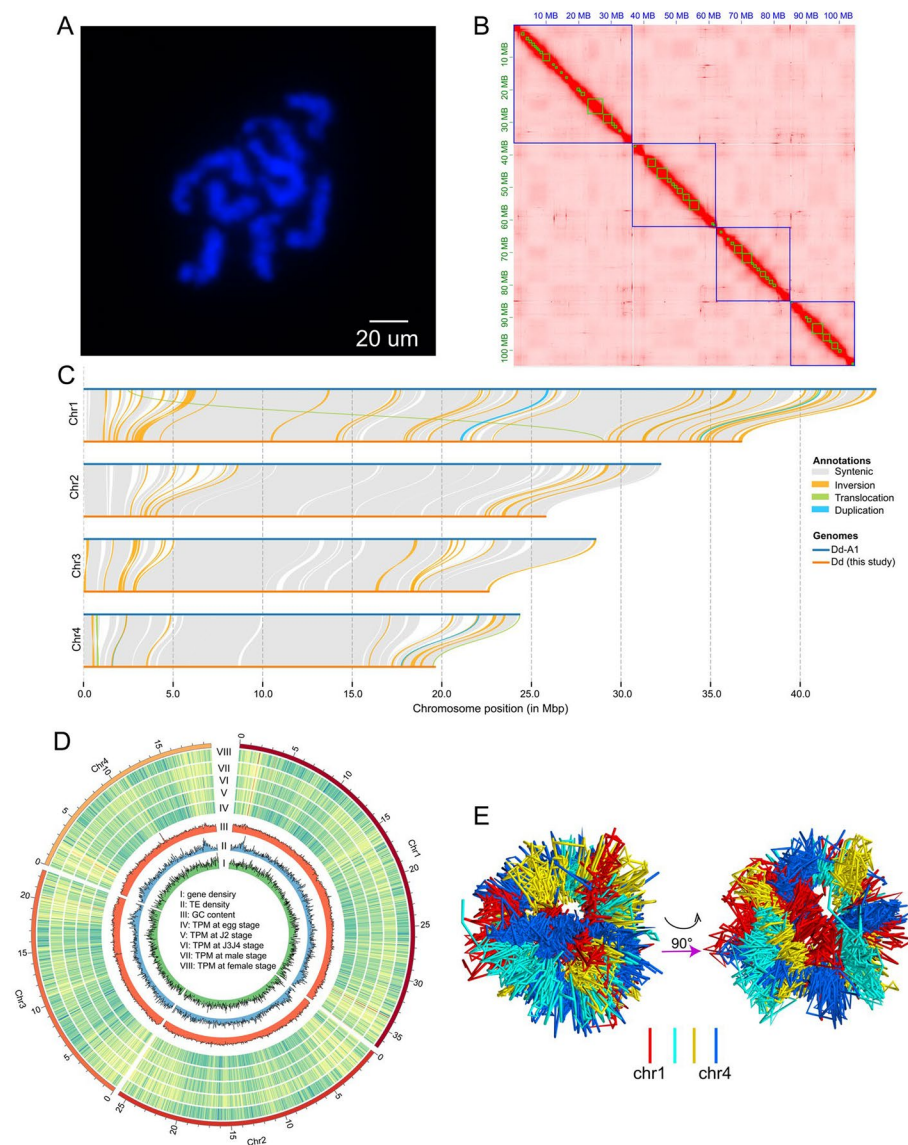


Fig. 1 The genome landscape of *D. destructor*. **A** The karyotype photo of *D. destructor* shows that it has 4 pairs of chromosomes. **B** Chromatin interaction signals mount the *D. destructor* genome onto four chromosomes (haploid). **C** Syntenic analysis of *D. destructor* genome between this study and the published Dd-A1 genome [19]. **D** Circos plot of *D. destructor* haploid genomic features. **E** Reconstructed 3D chromatin structure within the nucleus of *D. destructor* at the mixed developmental stage based on Hi-C data

exhibited similarity across species. Our findings demonstrated a strong concordance between the syntenic blocks of protein sequences and the relative positions of Nigon elements (Fig. 2D). For example, the A Nigon element, which is primarily located on *B. xylophilus* chromosome 1 [22], is dispersed across four chromosomes in *D. destructor* but is concentrated on chromosome 1 in *A. besseyi*. This pattern is consistent with the distribution of syntenic blocks and their chromosomal locations. For each of the remaining chromosomes, the positional relationships between Nigon elements and syntenic blocks of

protein sequences also exhibit a one-to-one correspondence (Fig. 2). Synteny analysis between *Panagrolaimus* sp. ES5 and *D. destructor* also revealed a high degree of concordance between Nigon element composition and patterns of protein-coding gene collinearity (Additional file 1: Fig. S3). Based on this evidence from both Nigon and collinearity, we infer that chromosome 3 of *D. destructor* represents its X chromosome. Our comparative analysis reveals that chromosomal rearrangements, including fusions and fragmentations, have shaped the Nigon structures of PPNs, with *B. xylophilus* retaining a

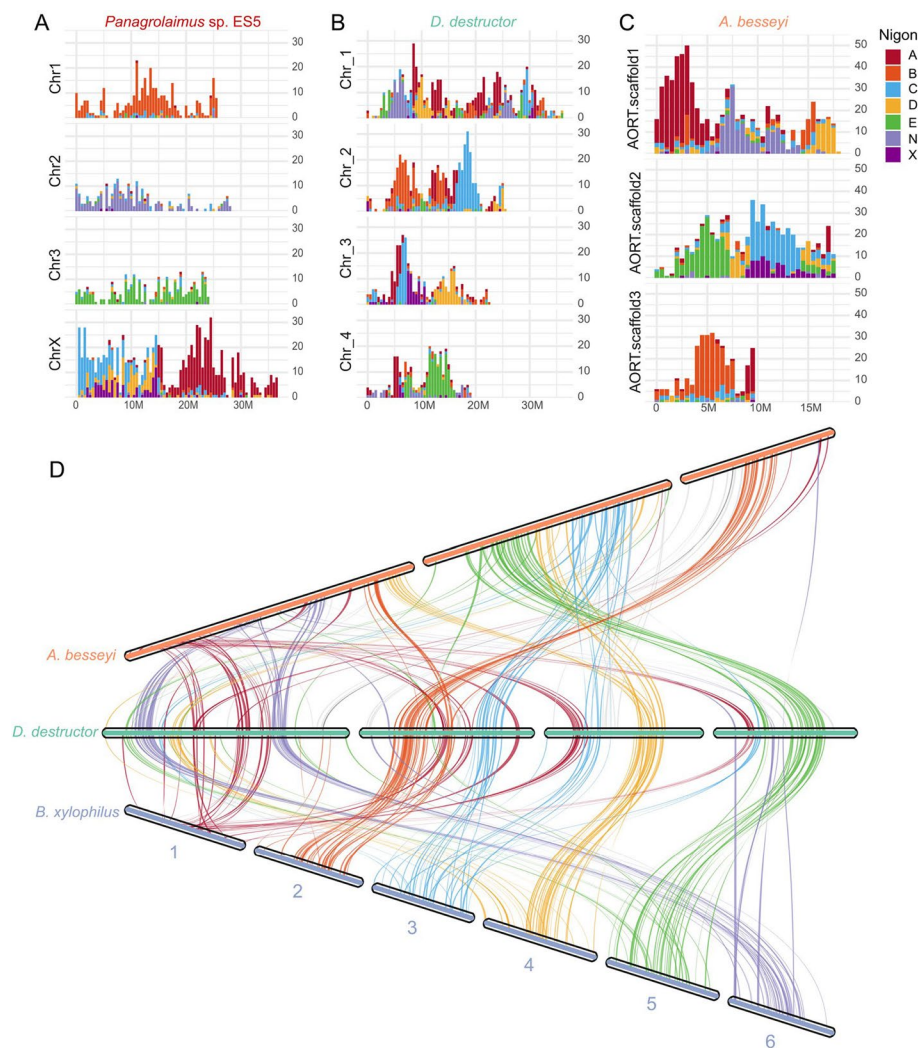


Fig. 2 Comparative genomic features of three migratory plant-parasitic nematodes. **A** The distribution characteristics of Nigon elements on the *Panagrolaimus* sp. ES5 genome shows that each element is generally concentrated on one autosome. **B** The distribution characteristics of Nigon elements on the *D. destructor* genome. **C** The distribution characteristics of Nigon elements on the *A. besseyi* genome. **D** Synteny of protein sequences among three migratory plant-parasitic nematodes. The results showed that the collinearity of protein sequences was highly consistent with the distribution characteristics of Nigon elements

more conserved architecture, while *D. destructor* and *A. besseyi* exhibit extensive chromosomal reorganization.

Transposons elements (TEs) are important drivers of genome dynamics, promoting genome rearrangement, chromosome structural changes, and the evolution of gene regulatory networks [13]. To investigate the role of TEs in migratory PPNs genome evolution, we conducted a comparative analysis of TEs in these three nematode species. First, we annotated the TE elements in their genomes, revealing that, compared to *B. xylophilus*, *D. destructor*, and *A. besseyi* possess a greater diversity of TE families, particularly the LINE transposon family (Additional file 1: Fig. S4A; Additional file 2: Table S1–3). By

analyzing the age distribution of TEs using Kimura substitution rates, we found that the peak age distribution of retrotransposons (LTR/LINE) in *D. destructor* and *A. besseyi* is closer to 0 compared to *B. xylophilus* (Additional file 1: Fig. S4B), suggesting that these retrotransposons may have undergone recent active expansion.

Dynamic landscape of transcriptome at different stages of *D. destructor*

To study the differences in gene expression at different developmental stages of migratory parasitic nematodes, we obtained transcriptome data of *D. destructor* at five stages from egg to adult (Fig. 3A). We first performed

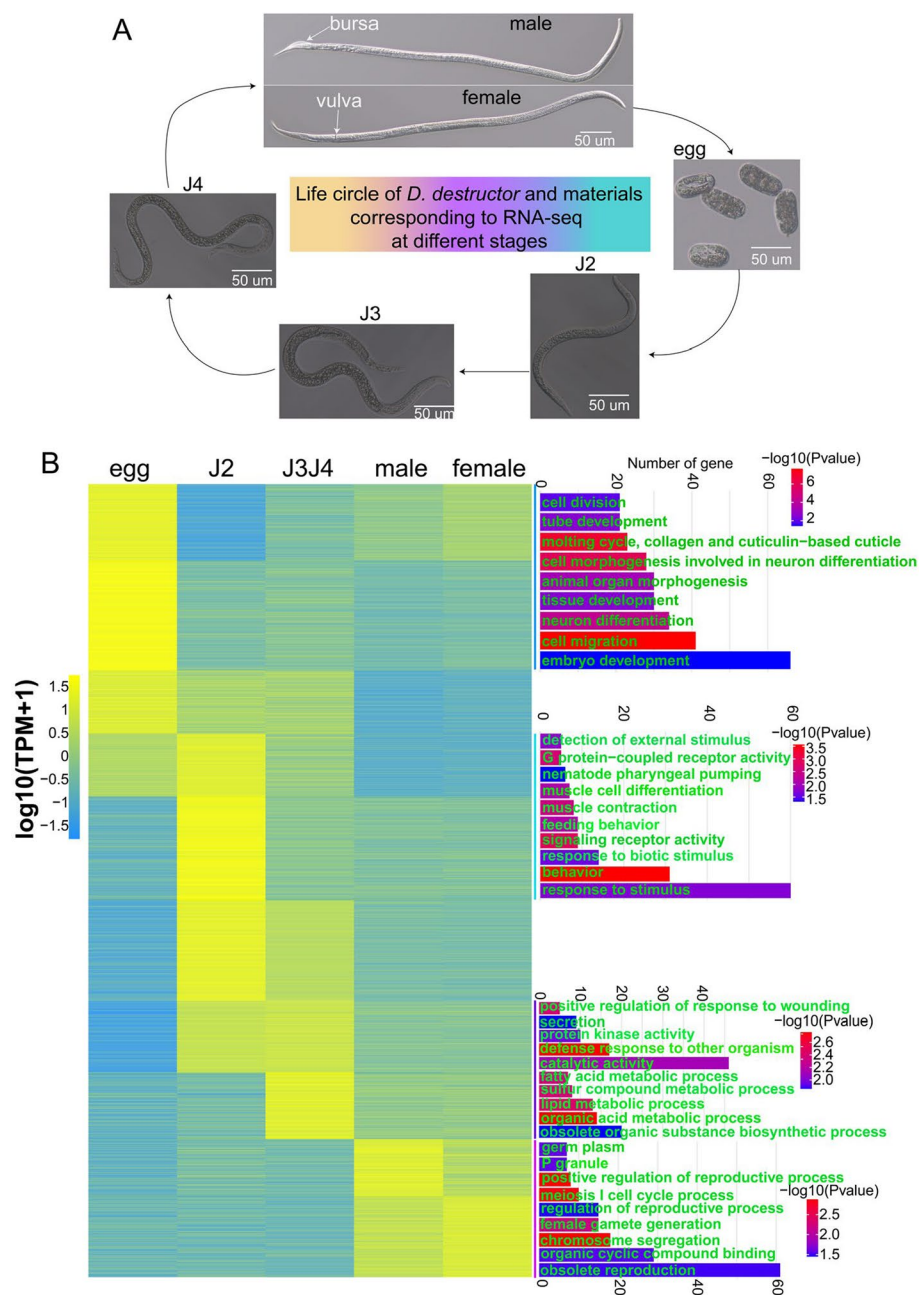


Fig. 3 Gene expression profile characteristics and functional analysis of *D. destructor* nematodes at different stages. **A** The life cycle of *Ditylenchus destructor*: Using a stage-synchronization method, we collected nematodes at different developmental stages—from eggs to adults—from mixed-stage populations cultured on sweet potato and performed RNA-seq. Scale bar = 50 μ m. **B** The heat map on the left shows the clustering of highly expressed genes in each stage after soft clustering of transcriptome data of *D. destructor* at five developmental stages. The yellow color in the same cluster, the higher the expression level. The right shows the GO functional enrichment results of genes highly expressed at each developmental stage (from top to bottom: egg, J2, J3/J4, and adult). The length of the bar plot indicates the number of genes, and the color indicates the p -value of the enrichment degree

differential gene expression analysis between adjacent developmental stages and identified 8,702, 2,881, 4,527, and 4,950 differentially expressed genes (DEGs) in egg_vs_J2, J2_vs_J3/J4, J3/J4_vs_male, and J3/J4_vs_female

(Additional file 1: Fig. S5), respectively. We conducted a functional analysis of DEGs across developmental stages, providing a valuable resource for identifying key genes involved in PPN development. The transition from the J3/

J4 stage to adult is closely linked to nematode sex determination. Pfam enrichment analysis revealed that genes upregulated in J3/J4 compared to males were primarily associated with protein degradation, particularly ubiquitin ligases such as zf-RING_2, Skp1, Cullin_Nedd8, and CSN8_PSD8_EIF3K (Additional file 1: Fig. S6A). Besides, many histone-related genes were enriched in this process, a pattern that warrants further investigation into their possible role in *D. destructor* sex determination. Moreover, these genes were significantly enriched in essential biological processes, including signal transduction, chromatin regulation, energy metabolism, transcription, DNA replication, and sperm motility (Additional file 1: Fig. S6A). Interestingly, the DEGs downregulated in J3/J4 compared to males were primarily enriched in GPCRs, collagen, molting-related genes, and glycosyltransferase families (Additional file 1: Fig. S6B). This may suggest that male nematodes enter a relatively stable mature stage, no longer undergoing intense developmental and metabolic remodeling. Instead, they focus more on reproduction while reducing investment in environmental signaling, molting, growth, and stress responses. Surprisingly, the DEGs upregulated or downregulated between J3/J4 and females showed similar functional enrichment patterns to the differential genes between males and females (Additional file 1: Fig. S6 C, D). This suggests that J3/J4 share similar genetic regulatory mechanisms during their development into both male and female nematodes. The J3/J4 life-stage appears to be a highly plastic stage, where developmental direction may be controlled by common regulatory networks, with sex determination being an additional fine-tuning of these mechanisms. Furthermore, the differentiation between males and females may rely on fewer key regulatory factors or be influenced by epigenetic regulation, as the DEGs in females did not show enrichment in histone-related genes.

Furthermore, we calculated the transcripts per million (TPM) values of the transcriptomes at each life-stage and performed cluster analysis on the expression levels at different life-stage. The results indicate that gene expression in *D. destructor* undergoes highly dynamic changes during development (Additional file 1: Fig. S7), reflecting finely tuned regulatory mechanisms that adapt to the physiological demands of different developmental stages. To gain deeper insights into gene regulation underlying growth, development, and parasitic strategies, we performed a predictive functional analysis of clustered genes at each developmental stage. The results indicate that the functions of genes enriched at each developmental stage largely correspond to stage-specific characteristics (Fig. 3B). For example, genes highly expressed during the egg stage are primarily enriched in embryonic development, tissue and organ development, and neuronal

differentiation. In contrast, genes highly expressed during the J2 to J3/J4 stages are enriched in muscle differentiation, feeding, and responses to environmental stimuli. In the adult stage, enriched functions are related to reproduction, P granules, and gametogenesis (Fig. 3B). These findings suggest that the gene expression patterns of *D. destructor* at different developmental stages are closely linked to their specific physiological functions, reflecting precise gene regulatory mechanisms during development to accommodate the growth demands and survival strategies of each stage.

Dynamic expression profile of putative effector, HGT, and TF during development

We predicted genes encoding proteins with signal peptides but lacking transmembrane domains as secreted proteins, including the nematode effector proteins—secreted molecules that facilitate parasitism by modulating host cell structure and function—and annotated a total of 1327 secreted proteins. On the other hand, we predicted 124 horizontally transferred genes (HGTs) in the *D. destructor* genome based on heterologous feature analysis. We then analyzed the characteristics of secreted proteins and HGTs in the *D. destructor* genome. In terms of chromosomal distribution, secreted proteins are generally evenly distributed across the chromosomes, with a few regions showing localized clustering (Fig. 4A). In contrast, HGTs exhibit distinct hotspot distributions, particularly concentrated at the chromosomal termini (Fig. 4A). The enrichment of HGTs at chromosome ends may be associated with the dynamic rearrangement, high plasticity, and susceptibility of telomeric regions to foreign gene integration. This distribution pattern may facilitate the retention and adaptive evolution of HGT genes, making them more likely to be preserved by natural selection or further amplified. In addition, we found that 29 of the 124 HGT genes were secreted proteins. For example, all the pectate lyases of *D. destructor* were from HGT, and some of these HGT secreted proteins were concentrated on the chromosomes in hotspot (Fig. 4A).

Although *D. destructor* has the ability to infect at all stages except eggs, we found that most of its secretory proteins have the highest expression levels in the J2 life-stage (Fig. 4B), suggesting that the nematode faces many challenges in the initial process of establishing infection. We further conducted functional enrichment analysis on these secretory proteins highly expressed in the J2 period and found that they were mainly enriched in functions such as sugar metabolism, protein degradation, lipid degradation, and immune response (Fig. 4C). These genes play a crucial role in the process of nematodes feeding on host nutrients and resisting immune defense responses, and are the key weapons

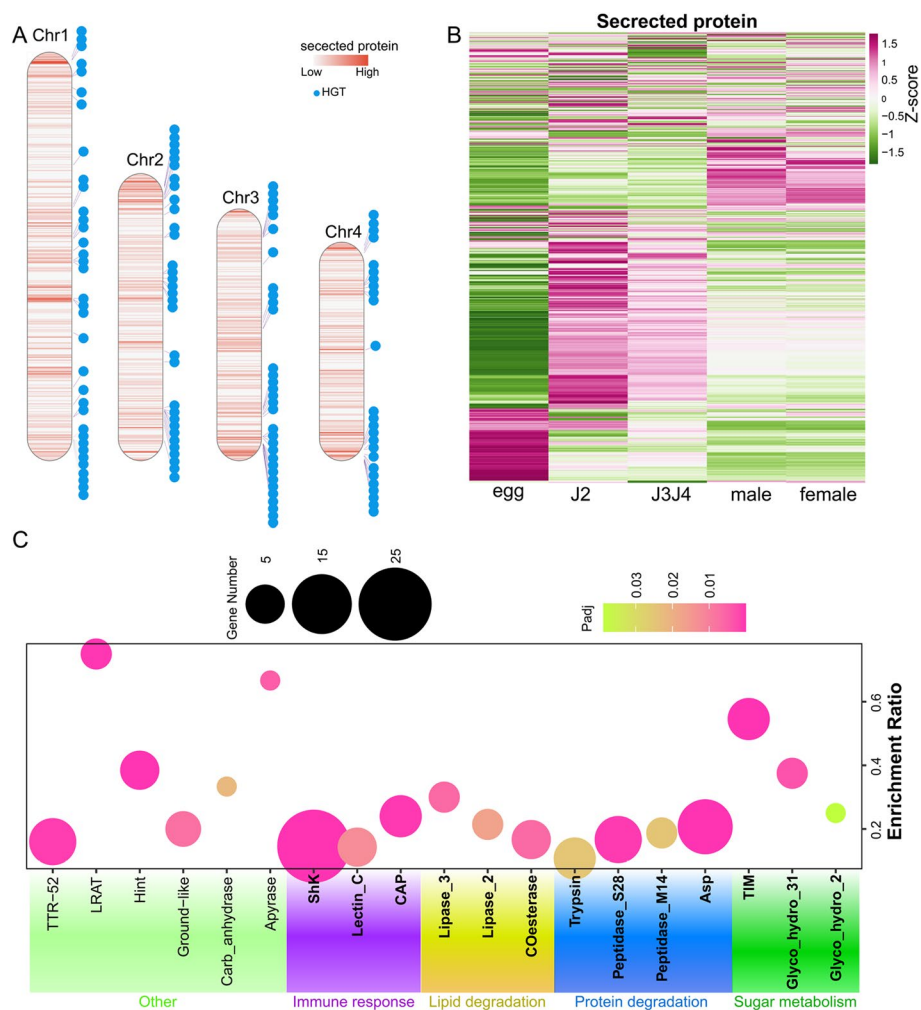


Fig. 4 Distribution characteristics of secretory proteins and HGT on the *D. destructor* genome and their expression characteristics among different developmental stages. **A** The distribution characteristics of secretory proteins and HGT on the *D. destructor* genome showed that HGT was distributed in a hotspot pattern, especially concentrated at the chromosome ends. **B** The expression of putative effector (secretory proteins) genes at different developmental ages was clustered, and most of the effectors were highly expressed in the J2 stage. A stronger magenta color indicates higher expression of the corresponding secreted protein, whereas a greener color indicates lower expression. **C** The Pfam domain enrichment analysis of putative effectors with high expression in the J2 stage. Enriched domains were mainly associated with sugar metabolism, protein degradation, and lipid degradation, highlighting their potential roles in nematode feeding and host tissue exploitation

for nematodes to first infect hosts and establish parasitism. The expression profile of HGT genes during nematode development closely resembles that of secretory proteins, with the majority exhibiting high expression in the J2 stage and relatively lower expression in the egg stage (Additional file 1: Fig. S8A). In contrast, transcription factors (TFs) display the opposite pattern, with the highest expression observed in the egg stage, followed by the J2 stage (Additional file 1: Fig. S8B). This suggests that HGT genes are more closely associated with nematode infection and parasitism, whereas TFs play a crucial regulatory role in embryonic development.

Secreted proteins and TFs play crucial roles in nematode parasitism

To determine whether these highly expressed secretory proteins and transcription factors (TFs) play a pivotal role in nematode development and host parasitism, we selected four secretory proteins and four TFs that are highly expressed during the J2 stage, along with one TFs that exhibit peak expression during the egg stage (Additional file 2: Table S4). RNAi experiments were then conducted to investigate their biological functions, using both a blank control and a dsGFP control, with gene silencing efficiencies ranging from 48 to 98% across

different targets as determined by qRT-PCR (Additional file 1: Fig. S9). We first conducted RNAi experiments on the transcription factor gene *Dd_05507*, which encodes a cold shock domain protein. This type of protein is typically involved in nucleic acid binding, regulation

of transcription, and stress adaptation in eukaryotes. Knockdown of *Dd_05507* via dsRNA treatment led to a significant reduction in its transcript levels compared with the control, which in turn markedly lowered the hatching rate of eggs (Fig. 5A). These results suggest that

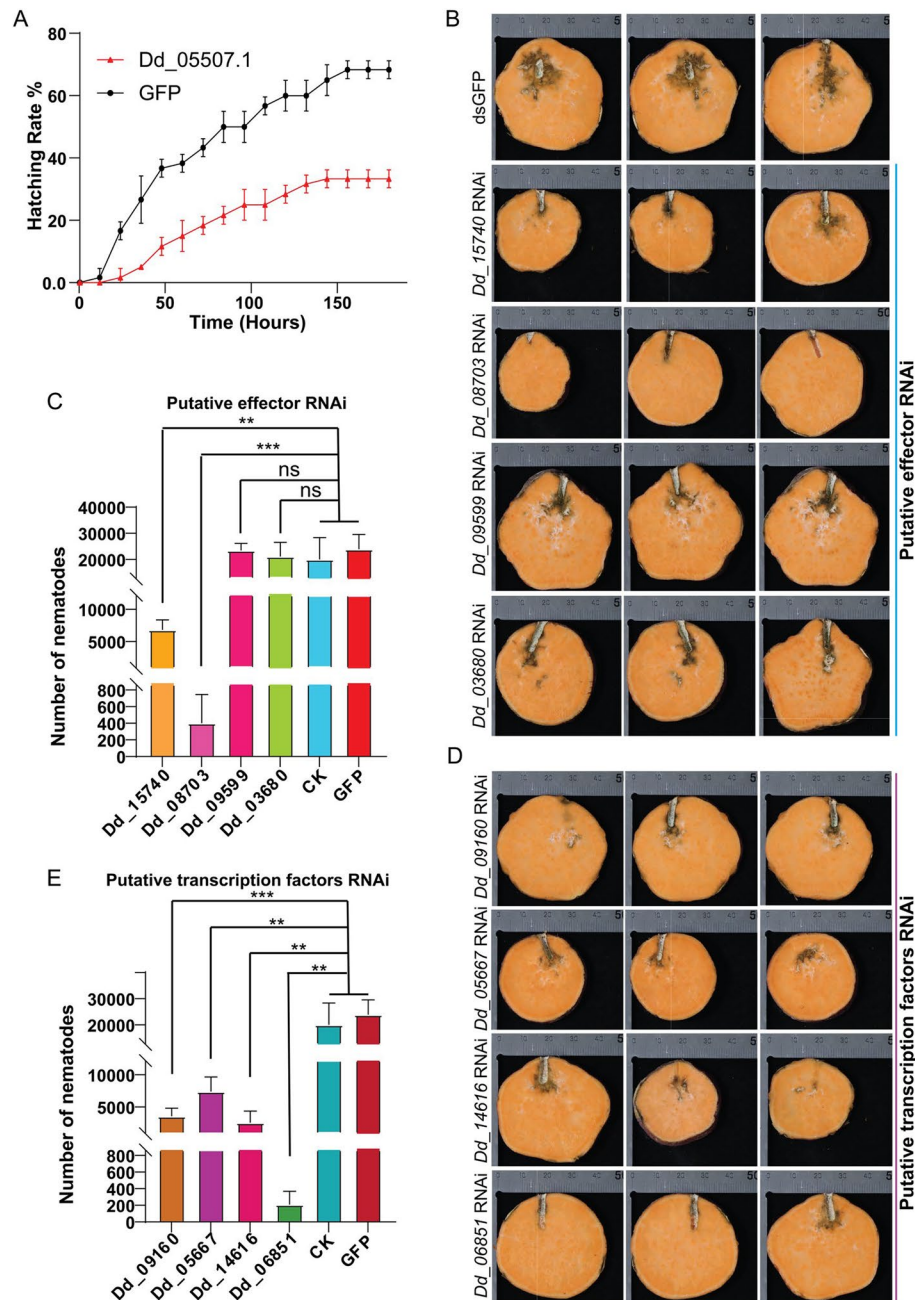


Fig. 5 The influence of effector and TF genes in *D. destructor* on its development and infection ability. **A** The hatching rate of eggs was significantly reduced after transcription factor *Dd_05507* RNAi. **B** Phenotypes of sweet potato infected with *D. destructor* after the putative effector gene RNAi. **C** Statistics of *D. destructor* reproductive ability after RNAi of putative effector genes. **D** Phenotypes of sweet potato infected with *D. destructor* after the putative TF genes RNAi. **E** Statistics of *D. destructor* reproductive ability after RNAi of putative TF genes. The number of replicates for each treatment in **C** and **E** was 4–7, and the significance was analyzed using *t*-test, ns means no significance, ** means $p < 0.01$, *** means $p < 0.001$

Dd_05507 plays a critical role in embryonic development, likely by regulating gene expression programs necessary for successful embryogenesis and hatching. We then conducted RNAi experiments on four highly expressed candidate effector genes and transcription factors during the J2 stage. Silencing two of these genes (*Dd_15740* and *Dd_08703*) significantly reduced the nematodes' ability to infect sweet potatoes, indicating that these genes may contribute to successful host invasion or establishment. However, as reduced infection could also result from the disruption of other essential functions, further studies are required to confirm their specific roles in parasitism (Fig. 5B, C). The RNAi experiments on transcription factors further confirmed that these TFs, belonging to the HMG_box, zf-C4, Homeodomain, and zf-LITAF-like families, are highly expressed during the J2 stage and play a crucial role in facilitating J2 nematodes' successful colonization of sweet potato and establishment of parasitism. (Fig. 5D, E). These findings highlight the essential role of secretory proteins and transcription factors in nematode development and parasitism. The functional validation of these genes provides key insights into the molecular mechanisms underlying host infection, offering potential targets for disrupting nematode parasitism and developing effective pest control strategies.

Discussion

Recent studies on nematode evolution have shown that *B. xylophilus* is at the root of the phylogenetic tree of PPNs [20] and has the same chromosome number as *C. elegans*, suggesting that *B. xylophilus* may not have undergone chromosomal recombination, but rather has undergone processes such as inversion, translocation, gene gain and loss, and mutation that enabled it to evolve the ability to parasitize plants. The chromosome-level assembly of *D. destructor* provides a valuable resource for studying the genome evolution of PPNs. Our comparative genomic analysis revealed significant chromosomal rearrangements among *D. destructor*, *B. xylophilus*, and *A. besseyi*, with *B. xylophilus* maintaining a more conserved Nigon structure similar to *C. elegans*, whereas *D. destructor* and *A. besseyi* exhibit extensive chromosomal reorganization. Given that Nigon elements represent ancient linkage groups in nematodes, their structural conservation or rearrangement can directly inform on the evolutionary trajectories and adaptive strategies of PPNs. These findings suggest that structural variations, including fusions and fragmentations, have played a crucial role in shaping the genome architecture of PPNs. Transposons elements play an extremely important role in the evolution of many organisms [23–26]. Moreover, TEs can not only regulate genome plasticity, but also act

as mediators of genetic variation in plant pathogens, affecting pathogenicity and adaptive evolution [27]. In the context of parasitism, TE-driven genome reorganization may facilitate the gain or modulation of effector and transcription factor genes, thereby influencing host adaptation. Our transposon analysis indicated that *D. destructor* and *A. besseyi* have undergone recent expansions of retrotransposons, particularly within the LINE family, which may have contributed to genome plasticity and adaptive evolution.

Through years of experience in PPNs, we have explored a method to synchronize the stages of *D. destructor* and are able to collect nematodes of different stages on a large scale. This work has laid the foundation for detailed studies of the gene function and expression profile of *D. destructor*. The dynamic transcriptomic landscape across developmental stages of *D. destructor* provided insights into the regulatory mechanisms underlying nematode growth and parasitism. Our differential expression analysis revealed stage-specific gene expression patterns, with embryogenesis characterized by genes involved in tissue development and neuronal differentiation, while J2 exhibited upregulation of genes associated with feeding, environmental responses, and host infection. The upregulation of ubiquitin ligases and histone-related genes in J3/J4 suggests potential epigenetic regulation of sex determination, highlighting a complex interplay between chromatin modifications and nematode development.

We identified and characterized secreted proteins and horizontally transferred genes (HGTs) in *D. destructor*, revealing their critical roles in nematode infection and adaptation. Secreted proteins were found to be highly expressed in J2 life-stage, underscoring their importance in overcoming host defenses and establishing parasitism. The chromosomal localization of HGTs at telomeric regions suggests their potential involvement in genome plasticity and adaptive evolution [16]. Notably, RNAi-mediated silencing of several highly expressed secreted proteins, including those potentially involved in digestion/feeding, led to a significant reduction in nematode infection, indicating that these proteins are important for successful parasitism. Additionally, RNAi experiments targeting transcription factors revealed their critical roles in J2 colonization and host establishment, underscoring their central position in the regulatory hierarchy of nematode parasitism. Our data confirm the important role of transcription factors in nematode infection of plants, and further raise the possibility that some of them participate in regulating effector gene expression during host invasion. These results underscore the importance of both transcription factors and effectors in the process of PPNs invasion, highlighting their critical roles in successful host infection, and emphasize that dissecting these

regulatory networks will provide valuable insights into the molecular basis of plant-parasitic nematode–host interactions.

Our findings provide a comprehensive view of the genetic and regulatory factors that may contribute to nematode parasitism, with implications for developing novel control strategies. The identification of several secreted proteins (including candidate effectors) and transcription factors that impact nematode infection offers potential targets for disrupting nematode–host interactions, which could inform the development of more effective pest management approaches.

Conclusions

In summary, we generated a high-quality chromosome-level genome assembly of *Ditylenchus destructor* ($2n=8$), providing comprehensive genome annotation and structural insights. Comparative genomics revealed extensive Nigon element rearrangements in *D. destructor* and *A. besseyi*, contrasting with the conserved karyotype of *B. xylophilus*. Transposable element analysis indicated greater diversity and recent retrotransposon activity in *D. destructor*, suggesting a role in genome plasticity. Developmental transcriptomics identified stage-specific expression of secreted proteins, horizontally transferred genes, and transcription factors, with many putative effectors peaking in the infective J2 stage. RNAi experiments confirmed that several of these genes are essential for embryogenesis or host colonization, providing potential targets for nematode control.

Methods

D. destructor nematode culture and collection

The surface of sweet potatoes was sterilized using alcohol and UV light in a clean bench for 15 min. Holes were punched or triangular incisions were cut in the sweet potatoes, with an average of two holes per potato, for nematode inoculation. Counted stem nematodes were introduced into the incisions or holes, inoculating approximately 2000 nematodes per hole. Each sweet potato received two holes, totaling about 4000 nematodes per potato. The inoculated sweet potatoes were incubated at 25 °C with 40–60% humidity for 30–40 days. Nematodes were then collected in large quantities using the shallow dish method.

Stage synchronization of *D. destructor* nematodes

Sweet potatoes inoculated with *D. destructor* were incubated at 25 °C for 30–35 days, after which nematodes were collected from the tubers. The tubers were crushed using a homogenizer at 20% power, and the resulting mixture of nematodes and sweet potato slurry was transferred into a beaker. The mixture was

sequentially filtered through a series of standard sieves with mesh sizes of 10, 20, 40, 60, 100, and 200 (determined based on the sizes of nematodes and eggs), yielding a fine sweet potato particulate fraction mixed with nematodes and eggs. The obtained mixture was aliquoted and subjected to density gradient centrifugation using a 35% sucrose solution to rapidly separate nematodes, eggs, and other impurities. The supernatant containing nematodes and eggs was collected and further purified using a 3 µm membrane filter to remove bacteria and debris. The nematode and egg suspension was treated with a nematode lysis solution consisting of sodium hypochlorite (50%):sodium hydroxide:water at a ratio of 1:2:7 for 5 min, killing the nematodes while preserving viable eggs. Extending the lysis time to 45 min ensured complete degradation of nematode bodies, yielding purified, viable eggs.

For the collection of second-stage juveniles (J2), the purified eggs were incubated in a 2000-mesh sieve device at 25 °C for 72 h, allowing synchronized hatching of J2. The hatched J2 nematodes were inoculated onto *Botrytis cinerea* plates and incubated at 25 °C for 12 days. The nematodes were then collected, and adult nematodes (both male and female) were manually removed. The remaining nematodes consisted of third-stage (J3) and fourth-stage (J4) juveniles. The manually picked female and male nematodes were saved. Three replicates were collected for all life stages, and RNA was extracted for transcriptome sequencing.

DNA, RNA extraction and library construction

DNA samples used for PacBio and Illumina sequencing were obtained from egg stage of *D. destructor*. DNA extraction followed the modified CTAB method [16]. Briefly, nematodes were purified by centrifugation in a 35% sucrose solution and suspended in lysis buffer. The frozen samples were ground in liquid nitrogen, and DNA was extracted using the CTAB. Proteins and RNA were removed by phenol extraction and RNase A treatment, respectively, and DNA was precipitated with isopropanol. High-quality DNA for PacBio sequencing was further purified using magnetic beads to eliminate polysaccharide and protein contaminants. For RNA extraction, we first obtained nematodes at different developmental stages using the nematode synchronization method, collecting three biological replicates for each stage. RNA was then extracted using the TransZol Up Plus RNA Kit (TransGen Biotech, Beijing, China), followed by library construction with the VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina (NR606-01). Finally, RNA sequencing was performed on the Nova platform by NOVogene.

Hi-C library construction

The Hi-C library was constructed using *D. destructor* eggs. The construction method followed the previously established Hi-C library preparation protocol for root-knot nematodes [16]. Briefly, freshly collected eggs were crosslinked with 1.5% formaldehyde for 30 min, and the crosslinking was quenched with glycine. The eggs were then ground in a mortar to isolate nuclei. The chromatin was digested with the MboI restriction enzyme, followed by biotin labeling and ligation with T4 DNA ligase. The ligated DNA was then sheared by sonication, and biotin-labeled fragments were enriched using streptavidin magnetic beads. Library preparation was performed using the Vazyme ND606 kit, and the final Hi-C library was sequenced on the Illumina NovaSeq platform with 150 bp paired-end reads.

Chromosome-level genome assembly

For genome assembly, our workflow was as follows: First, we used Illumina paired-end sequencing data to assess the ploidy of the *D. destructor* genome using Smudgeplot [28]. After determining that the genome is diploid, we estimated the genome size using GenomeScope2 [28] with a *k*-mer size of 21, assuming a diploid model. Following genome evaluation, we performed error correction on PacBio Sequel I data using Canu (v1.9) [29], setting genome coverage at 40×. The corrected 40× data was then assembled using Smartdenovo [30]. The resulting contigs were first polished with gcpp (<https://github.com/PacificBiosciences/gcpp>) using PacBio data, followed by multiple rounds of polishing with Pilon [31] using Illumina data. Next, we used Juicer (v1.5.7) pipeline [32] and 3D-DNA (v180419) pipeline [33] to anchor the polished genome to chromosomes based on Hi-C signals. Finally, the anchored genome was manually refined in Juicebox (v1.13.01) [34], yielding the final chromosome-level genome assembly. The three-dimensional structure of the genome was reconstructed using miniMDS [35] software.

Genome annotation

We used transcriptome data from all developmental stages to annotate the genome of *Ditylenchus destructor*. First, we employed Trinity (v2.15.2) [36] to perform de novo assembly of the transcriptome data, resulting in the “Trinity.fasta” file. The assembled sequences were then used in PASA [37] for ORF prediction, generating the “pasa_assemblies.gff3” file. Next, we masked the *D. destructor* genome using RepeatMasker [38], followed by alignment of the transcriptome data to the masked genome with HISAT2 [39]. The resulting BAM files were used as input for BRAKER3 [40] to predict genes, generating the “braker.gff3” file. Additionally, the BAM files

from HISAT2 alignment were used for gene prediction with TransDecoder and StringTie (v2.1.1) [41], yielding the “stringtie.gff3” file. Finally, we merged the gene predictions from the three methods using EVIDENCEModeler, incorporating weight-based merging to produce the final annotation file, “annotation.gff3”. CDS and protein sequences were extracted from this GFF3 file using GFFRead. The genome annotation of *Panagrolaimus* sp. ES5 was performed by using the same strategy, the genome and RNA-seq data were download from NCBI with accession number GCA_964243005.1 and SRR5253562.

TE, HGT, TF, and secretory protein prediction

For TE prediction, we first used EDTA (v2.2.0) pipeline [42] to predict the genome with the parameters “-overwrite 1 -force 1 -sensitive 1 -anno 1 -evaluate 1” and obtained “TEanno.gff3” and “TElib.fa”. Then, using “TElib.fa” as a library file, RepeatMasker [38] was used to re-predict TE in the genome, and calcDivergenceFromAlign.pl was used to calculate Kimura substitution on the result file “*.align”. For HGT prediction, we use AvP pipeline [43] with uniref90 database, the candidate gene with HGT or HGT-NT label was considered as HGT genes. For transcription factors, we divided all protein sequences of *D. destructor* into two groups of no more than 9000 genes and predict the TF by using AnimalTFDB v4.0 (https://guolab.whscu.cn/AnimalTFDB4/#/TF_Predict). For secretory protein prediction, we first used signalP(v5.0) [44] to predict genes with secretory signal peptides, and then used tmhmm [45] to predict whether these genes had transmembrane domains. After eliminating genes containing transmembrane domains, they were potential secretory proteins.

Collinearity and genomic variation analysis

We used minimap2 with the parameters “-ax asm5 -eqx” to align the entire genome between the *D. destructor* genome of this study and Dd-A1 [19], and then used Syri [46] to identify collinear regions, structural variations, insertion and deletion mutations, and SNP information. The syntenic protein pairs between the *D. destructor* genome of this study and Dd-A1 were identified by using JCVI pipeline [47].

RNA-seq analysis and expression profile determination

For the transcriptomic data analysis, each developmental life-stage included three biological replicates. We individually calculated the reads count and TPM values for each biological replicate at each stage, followed by downstream analyses such as expression level comparisons. The three biological replicates from each life stage cluster together when analyzed. For the analysis of differentially

expressed genes, we first aligned the transcriptome data to the genome using HISAT2, followed by sorting with SAMtools [48] and quantifying read counts with HTSeq [49]. Differential gene expression analysis was then performed using DESeq2 [50]. For TPM calculation, we first aligned the transcriptome data to the genome using STAR [51] and then used RSEM [52] to calculate TPM (transcripts per million) values for each gene. Gene expression profile clustering across developmental stages was conducted using Mfuzz [53], while the expression pattern clustering of transcription factors, effectors, and other key genes at different stages was performed using heatmap.

Functional analysis

For the functional annotation of all predicted proteins in *D. destructor*, we first annotated GO terms and KEGG pathways using EggNOG-mapper [54] with default parameters. Subsequently, we performed Pfam domain annotation for all proteins using Pfam_scan.pl [55]. For GO enrichment analysis, we extracted all genes with assigned GO terms into a list and conducted enrichment analysis using TBtools-II [56]. KEGG and Pfam enrichment analyses were performed using in-house Python scripts, and the results were visualized with ggplot2. The enrichment analysis was performed using Fisher's exact test with Benjamini–Hochberg false discovery rate (FDR) correction, and terms with $FDR < 0.05$ were considered significantly enriched.

In vitro RNAi

RNAi was performed using an in vitro soaking method. Primers (R and F) specific to each target gene were designed to incorporate a T7 promoter sequence at the 5' end. For control treatments, dsRNA targeting an exogenous green fluorescent protein (dsGFP) sequence was synthesized in parallel. Target gene fragments were amplified from *D. destructor* cDNA, and PCR products were purified to ensure DNA concentrations exceeded 100 ng/μl. Double-stranded RNA (dsRNA) was synthesized using the Vazyme T7 RNAi Transcription Kit (TR102) according to the manufacturer's instructions, and the final dsRNA concentration was adjusted to 1000 ng/μl. For each RNAi reaction, a total volume of 300 μl was prepared, supplemented with 15 μl of 1 mol/L octopamine and 0.5 μl of 1 mol/L spermidine to enhance uptake. Approximately 6000 mixed-stage nematodes or 5000 eggs were incubated in the dsRNA mixture. The soaking was carried out at room temperature (~25 °C) with gentle rotation for 48 h. After treatment, 2000 nematodes were collected for RNA extraction and subsequent qRT-PCR analysis, while the remaining individuals were used for phenotypic assessment. The dsGFP with the

same concentration and treatment conditions was used as an exogenous gene control, while the blank control (CK) was used without the addition of dsRNA.

RT-qPCR was performed on an Applied Biosystems QuantStudio 5 Real-Time PCR Instrument (Thermo Fisher) using Vazyme SYBR qPCR Master Mix. Each 20 μl reaction contained 10 μl of 2×SYBR Green Master Mix, 0.4 μl of each primer (10 μM), 2 μl of cDNA template, and nuclease-free water to bring the volume to 20 μl. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. A melting curve analysis was performed from 65 °C to 95 °C to verify the specificity of amplification. Each sample was run in three biological replicates and three technical replicates. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with actin gene as the internal control. Primer sequences are listed in Table S4.

Experimental design

Infection assay: Sweet potatoes of uniform diameter were punctured to a standardized depth (about 1 cm). Each hole was inoculated with 500 nematodes, with 8 replicates per gene. After 30 days infection at 25 °C of dark incubator, nematodes were collected and counted. The extent of infection was examined, and photographs were taken for documentation.

Egg hatching rate assay: 30–40 early-stage embryos were selected per treatment, with 5 replicates per gene. The eggs were incubated at 25 °C for 3–7 days, hatching rates were recorded every 24 h.

Nematode quantification: Harvesting nematodes from infected sweet potatoes, a 2 cm region surrounding the infection site was excised. The infected tissue was cut into 0.5 cm×0.5 cm pieces and wrapped in a single layer of paper towel. The samples were submerged in water for 4 h to allow nematodes to migrate into the liquid. Nematode suspensions were collected and counted at least three times to estimate the total population.

Negative control group: dsGFP synthesized using GFP gene as a template was used as a negative control for RNAi. The same infection ability and egg hatching rate assays were performed for comparison.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 10 software. Each experiment was independently repeated three times to ensure reproducibility. Data are presented as mean ± SD. Statistical significance was assessed using two-tailed Student's *t*-tests.

Abbreviations

DAF-16	Abnormal Dauer Formation-16
SKN-1	Skinhead-1

bZIP	Basic leucine zipper
PPN(s)	Plant-parasitic nematode(s)
TE(s)	Transposable element(s)
HGT(s)	Horizontal gene transfer(s)
TF(s)	Transcription factor(s)
DEG(s)	Differentially expressed gene(s)
RNAi	RNA interference
RT-qPCR	Reverse transcription quantitative PCR
TPM	Transcripts per million
LTR	Long terminal repeat
LINE	Long interspersed nuclear element
BUSCO	Benchmarking Universal Single-Copy Orthologs
Hi-C	High-throughput Chromosome Conformation Capture

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12915-025-02482-7>.

Additional file 1: Figures S1–S9. Fig. S1 Ploidy and genome size estimation of *D. destructor*. Fig. S2 Colinearity analysis of gene sequences between the *D. destructor* genome in this study and the published *Dd-A1* genome. Fig. S3 Synteny analysis of protein-coding sequences between *D. destructor* and *Panagrolaimus* sp. ES5 suggests that chromosome 3 of *D. destructor* corresponds to its X chromosome. Fig. S4 The TE landscape of three migratory plant-parasitic nematodes. Fig. S5 Volcano plot showing differentially expressed genes between different developmental stages of *D. destructor*. Fig. S6 Functional enrichment analysis of differentially expressed genes between J3/J4 and adults. Fig. S7 Clustering of TPM values of gene expression between different stages of *D. destructor* shows age-specific expression profiles. Fig. S8 The expression heatmap of HGT and TF genes during the development stage. Fig. S9 RT-qPCR analysis of the relative expression levels of the candidate effector gene and transcription factor target genes following RNAi treatment.

Additional file 2: Table S1–4. Table S1 The TE class summary of *D. destructor*. Table S2 The TE class summary of *B. xylophilus*. Table S3 The TE class summary of *A. besseyi*. Table S4 Primer sequence used in this study.

Acknowledgements

We thank Romnick Latina of University of California Davis for advice on nematodes evolution and the UC Davis farm cluster server for help.

Authors' contributions

D.D. and M.S. conceived and administration the project. M.S., acquisition the funding. M.S. and D.P. manage this project. D.D. designed the experiments. Y.C. collected the data and performed the RNAi experiment. S. Z. clone the gene. S. Z. and X.W. constructed the RNA-seq libraries. D.D. performed the data analysis. B.H., D.B., Y.L., N.A., Y.Z.1, and Y.Z.2 raised nematodes. D.D. wrote the manuscript. N.M.A revised the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (32271546, U20A2040 and 31970076) and Hubei Hongshan Laboratory (2022hszd012).

Data availability

All sequencing data have been deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive. The different stages of RNA sequencing data of *D. destructor* are deposited in BioProject PRJNA1164248 [57]; The PacBio Sequel I data, Hi-C data, and Illumina data of *D. destructor* are deposited in BioProject PRJNA1236705 [58]. The genome of *B. xylophilus* and *A. besseyi* were download from wormbase parasite with accession number PRJEB40022 [59] and PRJNA834627 [60]. The genome of *Panagrolaimus* sp. ES5 was download from GenBank with accession number GCA_964243005.1 [61], and the RNA-seq data was download from SRA database with accession number SRR5253562 [62]. The genome assembly and annotation files of *D. destructor* have been deposited in the China National Center for Bioinformatics Bioproject database with accession number PRJCA051661 [63].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹National Key Laboratory of Agricultural Microbiology, Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan 430070, China. ²Department of Entomology and Nematology, University of California, Davis, CA, USA. ³Agriculture Microbiology Department, Faculty of Agriculture, Benha University, Qalubia 13736, Egypt.

Received: 12 May 2025 Accepted: 26 November 2025

Published online: 18 December 2025

References

- Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, et al. Top 10 plant-parasitic nematodes in molecular plant pathology. *Mol Plant Pathol.* 2013;14(9):946–61.
- Parrado LM, Quintanilla M. Plant-parasitic nematode disease complexes as overlooked challenges to crop production. *Front Plant Sci.* 2024;15:1439951.
- Lu Y, Yang S, Chen W, Xie H, Xu C. Advances in migratory plant endoparasitic nematode effectors. *Int J Mol Sci.* 2024;25(12):6435.
- Lanver D, Tollot M, Schweizer G, Lo Presti L, Reissmann S, Ma LS, et al. *Ustilago maydis* effectors and their impact on virulence. *Nat Rev Microbiol.* 2017;15(7):409–21.
- Chen L, Xu M, Wang C, Zheng J, Huang G, Chen F, et al. Multi-copy alpha-amylase genes are crucial for *Ditylenchus destructor* to parasitize the plant host. *PLoS ONE.* 2020;15(10):e0240805.
- Chang Q, Yang Y, Hong B, Zhao Y, Zhao M, Han S, et al. A variant of the venom allergen-like protein, Dd VAP2, is required for the migratory endoparasitic plant nematode *Ditylenchus destructor* parasitism of plants. *Front Plant Sci.* 2023;14:1322902.
- Huang G, Cong Z, Liu Z, Chen F, Bravo A, Soberon M, et al. Silencing *Ditylenchus destructor* cathepsin L-like cysteine protease has negative pleiotropic effect on nematode ontogenesis. *Sci Rep.* 2024;14(1):10030.
- Liu F, Xi M, Liu T, Wu X, Ju L, Wang D. The central role of transcription factors in bridging biotic and abiotic stress responses for plants' resilience. *New crops.* 2024;1:100005.
- Basso MF, Lourenço-Tessutti IT, Mendes RAG, Pinto CEM, Bournaud C, Gillet F-X, et al. Midaf16-like and Miskn1-like gene families are reliable targets to develop biotechnological tools for the control and management of *Meloidogyne incognita*. *Sci Rep.* 2020;10(1):6991.
- Wagner D, Schmeink A, Mos M, Morozov IY, Caddick MX, Tudzynski B. The bZIP transcription factor MeaB mediates nitrogen metabolite repression at specific loci. *Eukaryot Cell.* 2010;9(10):1588–601.
- Perez-Cuesta U, Guruceaga X, Cendon-Sanchez S, Pelegri-Martinez E, Hernando FL, Ramirez-Garcia A, et al. Nitrogen, iron, and zinc acquisition: key nutrients to *Aspergillus fumigatus* virulence. *J Fungi.* 2021;7(7):518.
- Chen Y, Gao F, Chen X, Tao S, Chen P, Lin W. The basic leucine zipper transcription factor MeaB is critical for biofilm formation, cell wall integrity, and virulence in *Aspergillus fumigatus*. *mSphere.* 2024;9(2):e00619–00623.
- Slotkin RK, Martienssen R. Transposable elements and the epigenetic regulation of the genome. *Nat Rev Genet.* 2007;8(4):272–85.
- Sundaram V, Wysocka J. Transposable elements as a potent source of diverse cis-regulatory sequences in mammalian genomes. *Philos Trans R Soc Lond B Biol Sci.* 2020;375(1795):20190347.
- Trizzino M, Kapusta A, Brown CD. Transposable elements generate regulatory novelty in a tissue-specific fashion. *BMC Genomics.* 2018;19(1):468.

16. Dai D, Xie C, Zhou Y, Bo D, Zhang S, Mao S, et al. Unzipped chromosome-level genomes reveal allopolyploid nematode origin pattern as unreduced gamete hybridization. *Nat Commun.* 2023;14(1):7156.
17. Zheng J, Peng D, Chen L, Liu H, Chen F, Xu M, et al. The *Ditylenchus destructor* genome provides new insights into the evolution of plant parasitic nematodes. *Proc Biol Sci.* 2016. <https://doi.org/10.1098/rspb.2016.0942>.
18. Wang X, Guo Z, Dai D, Xie C, Zhao Z, Zheng J, et al. High-resolution transcriptome datasets during embryogenesis of plant-parasitic nematodes. *Sci Data.* 2024;11(1):690.
19. Yang Y, Feng R, Hong B, Fang Y, Liu C, Wang K, et al. Chromosome-level genome assembly of the sweet potato rot nematode *Ditylenchus destructor*. *Sci Data.* 2025;12(1):174.
20. Qing X, Zhang YM, Sun S, Ahmed M, Lo WS, Bert W, et al. Phylogenomic insights into the evolution and origin of Nematoda. *Syst Biol.* 2025;74(3):349–58.
21. Schiffer PH, Danchin EG, Burnell AM, Creevey CJ, Wong S, Dix I, et al. Signatures of the evolution of parthenogenesis and cryptobiosis in the genomes of panagrolaimid nematodes. *iScience.* 2019;21:587–602.
22. Gonzalez de la Rosa PM, Thomson M, Trivedi U, Tracey A, Tandonnet S, Blaxter M. A telomere-to-telomere assembly of *Oscheius tipulae* and the evolution of rhabditid nematode chromosomes. *G3 (Bethesda).* 2021;11(1):jkaa020.
23. Wicker T, Gundlach H, Spannagl M, Uauy C, Borrill P, Ramirez-Gonzalez RH, De Oliveira R, International Wheat Genome Sequencing C, Mayer KFX, Paux E et al. Impact of transposable elements on genome structure and evolution in bread wheat. *Genome Biol.* 2018;19(1):103.
24. Kumar CS, Qureshi SF, Ali A, Satyanarayana ML, Rangaraju A, Venkateshwarri A, et al. Hidden magicians of genome evolution. *Indian J Med Res.* 2013;137(6):1052–60.
25. Yu Z, Li J, Wang H, Ping B, Li X, Liu Z, et al. Transposable elements in Rosaceae: insights into genome evolution, expression dynamics, and syntenic gene regulation. *Hortic Res.* 2024;11(6):uhae118.
26. Shao F, Han M, Peng Z. Evolution and diversity of transposable elements in fish genomes. *Sci Rep.* 2019;9(1):15399.
27. Mat Razali N, Cheah BH, Nadarajah K. Transposable elements adaptive role in genome plasticity, pathogenicity and evolution in fungal phytopathogens. *Int J Mol Sci.* 2019. <https://doi.org/10.3390/ijms20143597>.
28. Ranallo-Benavidez TR, Jaron KS, Schatz MC. GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. *Nat Commun.* 2020;11(1):1432.
29. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res.* 2017;27(5):722–36.
30. Liu H, Wu S, Li A, Ruan J. SMARTdenovo: a de novo assembler using long noisy reads. *GigaByte.* 2021;2021:igabyte15.
31. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE.* 2014;9(11):e112963.
32. Durand NC, Shamim MS, Machol I, Rao SS, Huntley MH, Lander ES, et al. Juicer provides a one-click system for analyzing loop-resolution Hi-C experiments. *Cell Syst.* 2016;3(1):95–8.
33. Dudchenko O, Batra SS, Omer AD, Nyquist SK, Hoeger M, Durand NC, et al. De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science.* 2017;356(6333):92–5.
34. Durand NC, Robinson JT, Shamim MS, Machol I, Mesirov JP, Lander ES, et al. Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. *Cell Syst.* 2016;3(1):99–101.
35. Rieher L, Mahony S. MiniMDS: 3D structural inference from high-resolution Hi-C data. *Bioinformatics.* 2017;33(14):i261–6.
36. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, et al. Trinity: reconstructing a full-length transcriptome without a genome from RNA-seq data. *Nat Biotechnol.* 2011;29(7):644.
37. Haas BJ, Salzberg SL, Zhu W, Pertea M, Allen JE, Orvis J, et al. Automated eukaryotic gene structure annotation using EVIDENCEModeler and the program to assemble spliced alignments. *Genome Biol.* 2008;9(1):R7.
38. Tarailo-Graovac M, Chen N. Using RepeatMasker to identify repetitive elements in genomic sequences. *Curr Protoc Bioinformatics.* 2009;Chapter 4:4.10.1–4.10.14.
39. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol.* 2019;37(8):907–15.
40. Gabriel L, Bruna T, Hoff KJ, Ebel M, Lomsadze A, Borodovsky M, et al. BRAKER3: fully automated genome annotation using RNA-seq and protein evidence with GeneMark-ETP, AUGUSTUS, and TSEBRA. *Genome Res.* 2024;34(5):769–77.
41. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. Stringtie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol.* 2015;33(3):290–5.
42. Ou S, Su W, Liao Y, Chougule K, Agda JRA, Hellenga AJ, et al. Benchmarking transposable element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome Biol.* 2019;20(1):275.
43. Koutsovoulos GD, Granjeon Noriot S, Bailly-Bechet M, Danchin EGJ, Rancurel C. AVP: a software package for automatic phylogenetic detection of candidate horizontal gene transfers. *PLoS Comput Biol.* 2022;18(11):e1010686.
44. Almagro Armenteros JJ, Tsirigos KD, Sonderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol.* 2019;37(4):420–423.
45. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J Mol Biol.* 2001;305(3):567–80.
46. Goel M, Sun H, Jiao WB, Schneeberger K, SyRI: finding genomic rearrangements and local sequence differences from whole-genome assemblies. *Genome Biol.* 2019;20(1):277.
47. Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, et al. MCS-X: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* 2012;40(7):e49.
48. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S. The Sequence Alignment/Map format and SAMtools. *Bioinformatics.* 2009;25(16):2078–2079.
49. Anders S, Pyl PT, Huber W. HTSeq—a python framework to work with high-throughput sequencing data. *Bioinformatics.* 2015;31(2):166–9.
50. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
51. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15–21.
52. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinformatics.* 2011;12:323.
53. Kumar L, M EF. Mfuzz: a software package for soft clustering of microarray data. *Bioinformatics.* 2007;21(1):5–7.
54. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, et al. Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol Biol Evol.* 2017;34(8):2115–22.
55. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer EL, et al. Pfam: the protein families database in 2021. *Nucleic Acids Res.* 2021;49(D1):D412–9.
56. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant.* 2020;13(8):1194–202.
57. Transcriptome sequencing of eggs, J2, J3/J4, males and females in *Ditylenchus destructor*. NCBI Bioproject <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1164248/> (2024).
58. *Ditylenchus destructor* PacBio, Hi-C, and Illumina sequence data. NCBI Bioproject <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1236705/> (2024).
59. *Bursaphelenchus xylophilus* complete genome. NCBI Bioproject <http://www.ncbi.nlm.nih.gov/bioproject/PRJEB40022/> (2020).
60. *Aphelenchoides* Genome sequencing and assembly. NCBI Bioproject <http://www.ncbi.nlm.nih.gov/bioproject/PRJNA834627/> (2022).
61. *Panagrolaimus* sp. ES5 genome assembly nxPanSpeb1.1 alternate haplotype. NCBI Genome https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_964243005.1/ (2024).
62. Other Sequencing of *Panagrolaimus* sp. ES5. NCBI SRA <https://www.ncbi.nlm.nih.gov/sra/SRR5253562/> (2019).
63. *Ditylenchus destructor* genome assembly and annotation. China National Center for Bioinformation Bioproject. 2025. <https://ngdc.cnc.ac.cn/bioproject/browse/PRJCA051661/>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.