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ORIGINAL ARTICLE OPEN ACCESS

In Silico Comparative and Evolutionary Analysis of the β-1,4-Endoglucanase Gene in Plant-Parasitic Nematodes

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Abstract

Plant-parasitic nematodes are among the most economically significant agricultural pathogens affecting crop productivity worldwide. Root-knot nematodes belonging to the genus *Meloidogyne* are particularly destructive because of their broad host range and ability to establish long-term parasitic interactions within plant roots. Successful parasitism in these nematodes largely depends on the secretion of cell wall-degrading enzymes that facilitate host penetration and tissue migration. Among these enzymes, β-1,4-endoglucanase plays a major role in cellulose degradation during plant infection. The present study was designed to investigate the comparative sequence conservation and evolutionary relationships of the β-1,4-endoglucanase gene in selected plant-parasitic nematodes using computational bioinformatics approaches. Nucleotide sequences corresponding to the β-1,4-endoglucanase gene were retrieved from the National Center for Biotechnology Information (NCBI) database for *Meloidogyne incognita*, *Meloidogyne javanica*, and *Globodera rostochiensis*. Sequence similarity analysis was performed using the Basic Local Alignment Search Tool (BLASTn) to verify gene identity and identify homologous sequences. Multiple sequence alignment was carried out using Clustal Omega in order to identify conserved regions and sequence divergence among selected nematode species. Evolutionary relationships were further examined using the Phylogeny.fr platform. BLAST analysis demonstrated significant sequence similarity among cellulase-associated genes of the selected nematodes, confirming evolutionary conservation of the β-1,4-endoglucanase gene. Multiple sequence alignment revealed several highly conserved nucleotide regions, particularly among *Meloidogyne species*, indicating strong functional conservation. Percent identity matrix analysis demonstrated higher similarity values within root-knot nematodes, whereas *Globodera rostochiensis* exhibited comparatively lower similarity values. Phylogenetic reconstruction further revealed close clustering among *Meloidogyne* species and evolutionary divergence of cyst nematodes. The findings of the present study demonstrate the usefulness of computational bioinformatics tools in comparative genomics and molecular evolutionary analysis of agriculturally important nematodes. The identified conserved regions within the β-1,4-endoglucanase gene may contribute toward future functional studies and sustainable nematode management strategies.

Keywords: *β-1,4-endoglucanase, plant-parasitic nematodes, Meloidogyne incognita, BLAST, Clustal Omega, phylogenetic analysis, comparative genomics, in silico analysis*.

Introduction

Plant-parasitic nematodes represent one of the most destructive groups of agricultural pathogens affecting global crop production (Perry & Moens, 2011). These microscopic roundworms infect roots, stems, leaves, seeds, and other plant tissues, leading to reduced nutrient uptake, impaired physiological processes, and significant yield losses. Global economic damage caused by plant-parasitic nematodes has been estimated to exceed billions of dollars annually, particularly in tropical and subtropical agricultural systems where climatic conditions favor rapid nematode multiplication (Jones *et al.,* 2013).

Among the different groups of plant-parasitic nematodes, root-knot nematodes belonging to the genus Meloidogyne are regarded as the most economically important because of their broad host range and high reproductive potential. *Meloidogyne* incognita infects numerous economically valuable crops including tomato, brinjal, potato, soybean,

cotton, cucumber, and tobacco. Infection by root-knot nematodes results in the formation of characteristic root galls that interfere with water and nutrient absorption, ultimately causing stunted growth and yield reduction.

The pathogenic success of plant-parasitic nematodes depends largely on their ability to invade host tissues and establish feeding sites (Davis *et al.,* 2004). During host invasion, nematodes secrete a variety of parasitism-associated molecules including cellulases, pectinases, expansins, and effector proteins that facilitate plant cell wall degradation and suppression of host defense mechanisms (Haegeman *et al.,* 2012). Among these enzymes, β-1,4-endoglucanases are considered highly important because they hydrolyze β-1,4-glycosidic bonds present within cellulose, which is the primary structural component of plant cell walls (Danchin *et al.,* 2010).

The discovery of endogenous cellulase genes in plant-parasitic nematodes represented a major breakthrough in molecular nematology. Earlier scientific theories suggested that animals lacked endogenous cellulases and depended entirely on microbial symbionts for cellulose degradation. However, Smant *et al.,* (1998) demonstrated that nematodes possess their own cellulase genes expressed within esophageal gland cells, fundamentally changing the understanding of nematode evolution and plant parasitism.

Comparative sequence studies further suggested that cellulase genes in plant-parasitic nematodes may have originated through horizontal gene transfer from bacteria (Keeling & Palmer, 2008). Such evolutionary acquisition likely provided ancestral nematodes with the ability to degrade plant cell walls efficiently and adapt to plant-associated lifestyles.

Recent advances in molecular biology and bioinformatics have significantly improved the ability to investigate nematode genes computationally (Khan *et al.,* 2016). Public databases such as the National Center for Biotechnology Information (NCBI) provide extensive nucleotide and protein sequence repositories that facilitate comparative genomics and evolutionary studies (Lesk, 2019). Bioinformatics tools such as BLAST, Clustal Omega, and Phylogeny.fr enable researchers to analyze sequence similarity, identify conserved motifs, and reconstruct evolutionary relationships rapidly and cost-effectively.

The present study was therefore designed to perform comparative and evolutionary analysis of the β-1,4-endoglucanase gene in selected plant-parasitic nematodes using computational bioinformatics approaches. Particular emphasis was placed on identifying conserved nucleotide regions and investigating phylogenetic relationships among selected nematode species.

Historical Review and Evolutionary Paradigms

The empirical foundation of plant nematology was established through macroscopic field observations and foundational alpha-taxonomic descriptions of host tissue anomalies, notably by Needham (1743) on Anguinatritici and Göldi (1887) on the root-knot genus Meloidogyne. By the mid-twentieth century, advancements in histopathology shifted investigative boundaries toward the cellular dynamics of the host-parasite interface. Early cellular assays demonstrated that infective second-stage juveniles (J2s) of sedentary endoparasites do not rely purely on mechanical force to traverse roots. Instead, they actively secrete complex salivary mixtures from specialized esophageal glands to manipulate host ontogeny, forcing the development of complex nutritional sinks known as giant cells or syncytia (Bird, 1996; Jones *et al.,* 2013).

A major biochemical paradigm shift occurred when Smant *et al.,* (1998) cloned functional, endogenous β-1,4-endoglucanase genes directly from the subventral esophageal glands of cyst nematodes (*Globodera* and *Heterodera spp.*). This discovery dismantled the long-standing biological dogma that metazoans lacked the metabolic machinery to synthesize endogenous cell-wall-degrading enzymes (CWDEs) without obligate microbial symbionts. Because these nematode cellulases exhibit no sequence homology with traditional animal glycosyl hydrolases but share deep structural conservation with specific soil bacteria, subsequent evolutionary mapping proved that plant parasitism within the phylum Nematoda was accelerated by ancient, lateral Horizontal Gene Transfer (HGT) events (Danchin *et al.,* 2010). Over evolutionary time, these acquired genes underwent adaptive radiation, becoming critical pathogenicity factors that cleave the rigid β-1,4-glycosidic bonds of host cell walls during early tissue migration.

The arrival of high-throughput sequencing and computational biology transformed modern nematology into a data-driven genomics discipline. The assembly of the first complete Meloidogyne incognita genome by Abad *et al.,* (2008) together with subsequent nematode genomic studies (Opperman *et al.,* 2008), exposed a massive multi-gene expansion of these cellulolytic sequences. In the modern in silico

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era, public repositories like the NCBI database permit rapid, cost-effective cross-taxa comparisons. By leveraging robust multiple sequence alignment tools like Clustal Omega/MView and automated curation pipelines like Gblocks, contemporary researchers can precisely map position-specific structural homologies and trace lineage-specific insertion-deletion (indel) dynamics (Dereeper *et al.,* 2008; Sievers & Higgins, 2018). Characterizing these structural constraints across diverse geographic isolates is highly critical, as these immutable consensus regions reveal the strict evolutionary boundaries of the enzyme, offering ideal molecular targets for next-generation RNA interference (RNAi) crop protection strategies.

Materials and Methods

Retrieval of Nucleotide Sequences

Nucleotide sequences corresponding to the β-1,4-endoglucanase gene were retrieved from the NCBI nucleotide database. Sequences from selected plant-parasitic nematodes including *Meloidogyne incognita*, *Meloidogyne javanica*, and *Globodera rostochiensis* were selected based on sequence completeness, annotation quality, and biological relevance.

The following steps were performed during sequence retrieval:

- Accessing the NCBI nucleotide database
- Searching for β-1,4-endoglucanase gene sequences
- Downloading FASTA-formatted nucleotide sequences
- Recording accession numbers for analysis
- Verifying sequence quality and completeness

Sequence Similarity Analysis Using BLAST

Sequence similarity analysis was performed using the Basic Local Alignment Search Tool (BLASTn) available through the NCBI BLAST server (Altschul *et al.,* 1990). Each retrieved nucleotide sequence was individually compared against the nucleotide collection database to identify homologous cellulase sequences.

The following parameters were evaluated during BLAST analysis:

- Query coverage
- Percentage identity
- Alignment score
- E-value
- Sequence homology

Sequences showing high query coverage and low E-values were considered highly homologous and evolutionarily conserved.

Multiple Sequence Alignment

Multiple sequence alignment (MSA) was performed using Clustal Omega available through the EMBL-EBI web server (Edgar, 2004). Curated FASTA sequences were uploaded to the platform and aligned using default alignment parameters.

The alignment analysis enabled:

- Identification of conserved regions
- Detection of sequence variation
- Comparison of homologous nucleotide stretches
- Identification of insertions and deletions

Aligned sequences were downloaded in Clustal format for further analysis and interpretation.

Percent Identity Matrix Analysis

A percent identity matrix generated during Clustal Omega alignment was used to evaluate pairwise sequence similarity among selected nematode sequences. The matrix provided quantitative information regarding evolutionary conservation and divergence.

Phylogenetic Analysis

Phylogenetic reconstruction was performed using the Phylogeny.fr online platform. Aligned nucleotide sequences generated through Clustal Omega were uploaded to the "One Click" analysis workflow.

The integrated workflow included:

- MUSCLE for alignment refinement (Edgar, 2004)
- Gblocks for removal of poorly aligned regions (Castresana, 2000)
- PhyML for maximum-likelihood tree generation (Guindon & Gascuel, 2003)
- Tree Dyn for phylogenetic visualization (Chevenet *et al.,* 2006)

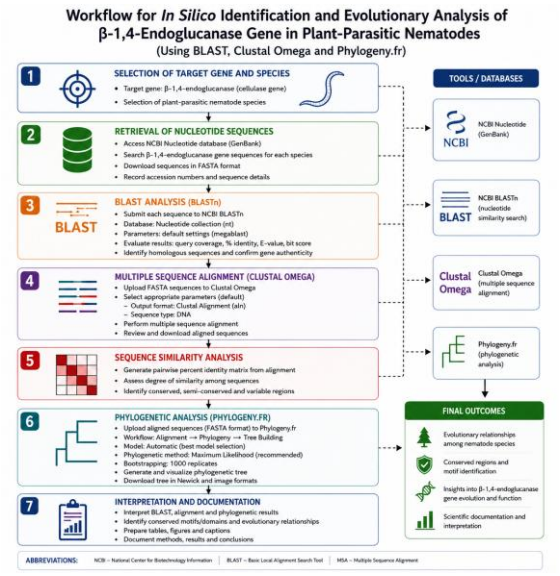

Fig. 1: Schematic workflow of the integrative bioinformatics pipeline utilized for the comparative and evolutionary analysis of the nematode β-1,4-endoglucanase (eng) gene.

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The resulting phylogenetic tree was analyzed to investigate evolutionary relationships among selected plant-parasitic nematodes.

Results and Discussion

Retrieval and Characterization of Sequence Datasets

A targeted dataset consisting of six high-quality coding sequences (CDS) optimizing the cell-wall-degrading enzyme β-1,4-endoglucanase (eng) was successfully compiled from the NCBI GenBank database. The dataset strategically represented two distinct economic groups of sedentary endoparasitic nematodes: the cyst-forming lineage (Genus: *Globodera*) and the root-knot lineage (Genus: *Meloidogyne*). For genomic sequences characterized by fragmented exon-intron architectures (*Globodera* entries), all intervening non-coding introns were computationally excised to isolate unbroken, continuous reading frames. The complete molecular profile of the selected isolates, including accession numbers, functional gene designations, and nucleotide sequence lengths, is comprehensively documented in Table 1.

Multiple Sequence Alignment and Conservation Profiles

High-fidelity multiple sequence alignment (MSA) executed via the MUSCLE algorithm on the Phylogeny.fr platform revealed a high level of structural conservation alongside informative generic variations.

Multiple sequence alignment (MSA) is an indispensable step in comparative genomics, serving to map position-specific structural homologies and trace molecular evolutionary signatures across divergent taxa. By aligning orthologous sequences across their entire length, MSA identifies highly constrained structural blocks preserved by purifying natural selection to safeguard vital enzymatic actions, while simultaneously exposing lineage-specific insertions, deletions (indels), and substitutions (Mount, 2004; Nei & Kumar, 2000).

In this study, the complete full-length multiple sequence alignment spanned an alignment baseline of approximately 1,200 to 1,500 base pairs depending on the structural architecture of the target nematode genera. To map long-range sequence conservation across the entire span of the gene, a macroscopic alignment overview was rendered using the MView/Nightingale interface (Brown *et al.,* 1998) (Figure 2). The global alignment confirmed a high degree of structural homology across all six target isolates, successfully accommodating the naturally shorter transcripts characteristic of the root-knot lineage (ranging from 870 to 936 bp) against the significantly longer coding sequences of the cyst-forming genus *Globodera* (ranging from 1,176 to 1,182 bp).

Analysis of the critical 5' terminal initiation zone revealed absolute structural synchronization across the entire dataset. Every reading frame across both distinct families initiated uniformly at the diagnostic ATG start codon (Figure 3). This uniform alignment empirically verified that the computational extraction of coding frames (CDS) was completely successful, yielding continuous open reading frames free of non-coding genomic intron text or artificial frame shifts.

Because the full-length multiple sequence alignment is structurally extensive, a highly informative mid-sequence block spanning nucleotides 481 to 720 was isolated to serve as a representative micro-level snapshot of generic divergence (Figure 4). Within this highly variable domain, the alignment clearly exposed the molecular boundaries dividing the two nematode families. The *Globodera* isolates exhibited tightly synchronized intra-generic block patterns (dense green and blue color columns), whereas the Meloidogyne isolates shared an alternate, independent cluster of conserved nucleotides.

Systematically arranged micro-gaps (dashes) within this region highlighted fixed, genus-specific indels that cleanly demarcate the two lineages. Furthermore, the position-specific consensus thresholds (70% to 100%) mapped along the base of the complete alignment confirmed that while flanking structural domains have drifted significantly over evolutionary time, core catalytic regions remain strictly constrained (Li, 1997). These highly conserved consensus blocks track the invariant structural pockets required to preserve the functional integrity of the cell-wall-degrading β- 1,4-endoglucanase enzyme across all plant-parasitic variants (Henrissat & Davies, 1997).

Automated Alignment Curation

To eliminate hyper-variable, phylogenetically uninformative regions or alignment artifacts that might introduce topological instability, automated alignment curation was implemented using Gblocks 0.91b (Castresana, 2000) (Figure 5). Out of the initial alignment layout, Gblocks successfully selected 825 highly reliable, conserved positions (underlined in blue). This algorithmic pruning removed loose flanking gaps and ambiguous single-nucleotide polymorphisms,

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leaving a highly focused, stable core dataset for downstream evolutionary modeling.

Quantitative Pairwise Sequence Identity Analysis

To quantify genetic divergence, a Percent Identity Matrix was computed using Clustal Omega (Table 2).

The matrix revealed that intra-specific identity between the two *Globodera rostochiensis* sequences stood at 87.81%, indicating minor sequence divergence within the species. Within the root-knot group, the two *Meloidogyne javanica* isolates (EU371023.1 and AM231138.1) exhibited absolute sequence identity (100.00%), indicating complete conservation across those individual isolated reads. High intra-generic identity was also observed between M. javanica and its sibling species M. incognita, ranging from 51.62% to 51.93%.

In contrast, inter-generic comparisons between *Globodera* and *Meloidogyne* isolates demonstrated a marked drop in sequence identity, stabilizing between 41.81% and 52.27%. This substantial sequence divergence mathematically highlights the deep evolutionary split between the families Heteroderidae and Meloidogynidae (Futuyma, 2005).

Phylogenetic Reconstruction and Distance Modeling

Evolutionary relationships were mapped using two separate topological methods to cross-validate branching stability.

First, an initial baseline Neighbor-Joining phylogram was generated through Clustal Omega to isolate genetic divergence rates (Figure 6). The computed branch lengths directly corresponded to the estimated number of nucleotide substitutions per site (Kimura, 1983). The phylogram validated the matrix data by assigning a distance value of exactly 0 to the identical *M. javanica* isolates, a close distance of 0.023023 to the *M. incognitasubclade*, and a higher divergence value of 0.0697279 to the *Globodera* lineage, showing unequal evolutionary rates between the two groups.

To establish maximum statistical rigor, a final robust phylogenetic tree was constructed using Maximum Likelihood modeling via the PhyML pipeline on the curated Gblocks dataset (Figure 7). The tree achieved perfect topological resolution, splitting cleanly into two primary monophyletic clades.

The lower clade clustered the *Globodera* cyst nematodes together, while the upper clade grouped the *Meloidogyne* root-knot species. The tree successfully differentiated the sibling species *M. incognita* and *M. javanica* into distinct sub-branches. Crucially, all major generic intersections yielded a bootstrap support value of 1 (100%), demonstrating absolute statistical confidence in the branching configuration. This complete clustering pattern provides strong molecular evidence that while the β-1,4-endoglucanase gene is strictly conserved within individual genera to maintain its essential plant-parasitic functionality, it has undergone significant divergence during the evolutionary separation of cyst-forming and root-knot nematodes.

Biological Significance of Endoglucanase Conservation and Divergence

The structural and phylogenetic profiles of the β-1,4-endoglucanase (*eng*) gene carry profound biological implications regarding the evolutionary adaptations of plant-parasitic nematodes (PPN) to a sedentary endoparasitic lifestyle.

Mechanisms of Host Penetration and Intracellular Migration

The β-1,4-endoglucanase enzyme belongs to the Glycosyl Hydrolase Family 5 (GH5) and is directly responsible for degrading cellulose - the major structural polysaccharide of plant cell walls (Cosgrove, 2005). The absolute conservation of the ATG start codon and the presence of highly dense, unmutated nucleotide blocks across both *Globodera* and *Meloidogyne* lineages mathematically confirm the functional indispensability of this enzyme. During the early stages of pathogenesis, both cyst and root-knot nematodes must break through tough outer epidermal cell barriers. For a juvenile nematode (J2) migrating through dense host root tissue, the continuous secretion of endoglucanase from its subventral esophageal glands acts as a molecular drill, liquefying cell walls to allow intracellular passage.

Divergence Mirroring Specialized Life Strategies

While the active catalytic sites remain highly constrained to preserve enzymatic function, the significant drop in inter-generic sequence identity (41.81% to 52.27%) reveals distinct evolutionary pathotypes. This genetic divergence directly corresponds to the different feeding site architectures created by these two nematode groups:

Root-Knot Lineage (*Meloidogyne spp.*): These species must establish highly specialized, multinucleated feeding cells known as "Giant Cells" through repeated nuclear divisions without cytokinesis (Williamson & Gleason, 2003). Their endoglucanase expression must be finely modulated to soften cell

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walls without triggering massive tissue necrosis (Bohlmann & Sobczak, 2014), allowing the root cells to expand dramatically into characteristic galls or "knots."

Cyst Lineage (*Globodera spp.*): Conversely, cyst nematodes induce a "Syncytium" by systematically dissolving adjacent host root cells' cell walls, fusing their protoplasts together. This aggressive, cell-dissolving feeding strategy requires a structurally distinct endoglucanase variant, which is reflected in the distinct evolutionary branch and unique nucleotide indels observed in the Globodera clade.

Horizontal Gene Transfer (HGT) Implications

From an evolutionary standpoint, animal genomes typically lack endogenous plant cell-wall-degrading enzymes. The presence of functional β-1,4-endoglucanase genes across these isolates serves as strong molecular evidence for historical Horizontal Gene Transfer (HGT) events, where an ancestral plant-parasitic nematode likely acquired these catalytic sequences directly from soil bacteria or fungi. The high sequence identity within the respective genera confirms that once these genes were integrated into the nematode genome, they became central evolutionary drivers that enabled these nematodes to transition into highly destructive plant parasites.

Conclusion and Future Prospects

This molecular study successfully utilized an integrative bioinformatics pipeline to characterize the structural configuration and evolutionary relationships of the β-1,4-endoglucanase (*eng*) coding sequences across six economically devastating plant-parasitic nematode isolates. By removing non-coding genomic introns and curating the core aligned columns via Gblocks, this research established a stable dataset of 825 highly informative nucleotide positions.

The pair-wise identity matrix and Maximum Likelihood phylogenetic reconstruction provided absolute statistical confidence (Bootstrap support = 1.00) in resolving the deep evolutionary split between the family Heteroderidae (*Globodera rostochiensis*) and the root-knot nematodes (*Meloidogyne spp.*). The tree topology successfully validated that the endoglucanase gene is strictly conserved within generic boundaries to safeguard vital plant-parasitic functions, yet exhibits significant divergence between genera to accommodate their specialized feeding site strategies (syncytia vs. giant cells).

Agricultural and Therapeutic Significance

Understanding these precise molecular boundaries opens up important avenues for targeted pest management in agricultural systems (Oka *et al.,* 2000). Because the highly conserved consensus regions identified in this study are essential for nematode survival and host infection—yet completely absent in mammalian hosts—they serve as ideal targets for novel, ecofriendly molecular interventions.

Future research can leverage these specific conserved sequences to design highly precise RNA interference (RNAi) silencing vectors or construct transgenic crops expressing gene-specific hairpins (Fire *et al.,* 1998). By knocking down these vital endoglucanase sequences, we can effectively disable the nematode's ability to penetrate root tissues or establish feeding sites, providing a robust, non-chemical line of defines to protect critical vegetable crop agroecosystems.

Table 1. Accession Details of β-1,4-Endoglucanase Gene Sequences Analyzed in This Study

S. No.	GenBank Accession No.	Organism / Species	Common Name	Target Gene	Sequence Length (bp)	Sequence Type
1.	AF056111.1	*Globodera rostochiensis*	Golden Cyst Nematode	*eng-2*	1,176	Coding DNA (Spliced Exons)
2.	AF056110.1	*Globodera rostochiensis*	Golden Cyst Nematode	*eng-1*	1,182	Coding DNA (Spliced Exons)
3.	EU371023.1	*Meloidogyne javanica*	Javanese Root-Knot Nematode	*eng-1*	870	Coding DNA (mRNA derived)
4.	AM231138.1	*Meloidogyne javanica*	Javanese Root-Knot Nematode	*eng-1*	870	Coding DNA (mRNA derived)
5.	AF323088.2	*Meloidogyne incognita*	Southern Root-Knot Nematode	*eng-2*	936	Coding DNA (mRNA derived)

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6.	AF323086.1	*Meloidogyne incognita*	Southern Root-Knot Nematode	*eng-1*	927	Coding DNA (mRNA derived)

Note: Sequence descriptions and accession details were obtained directly from NCBI nucleotide database records used in the present study.

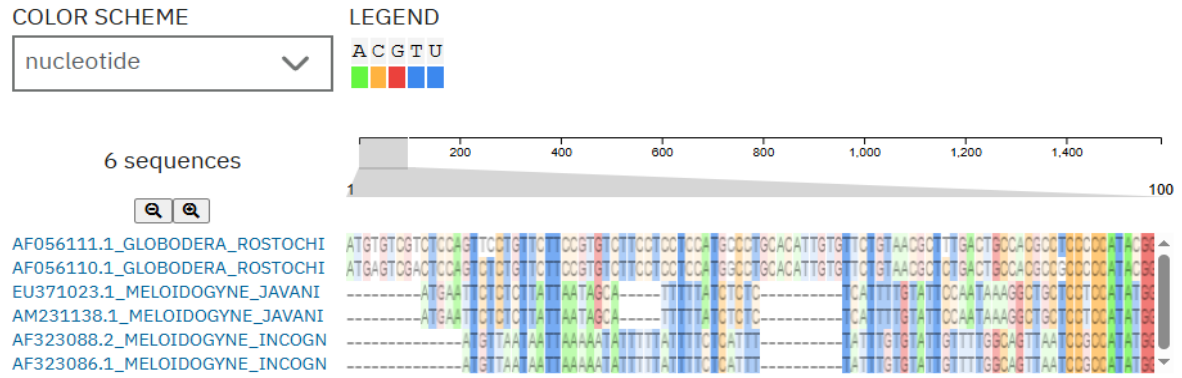

Fig. 2: Magnified view of a highly informative downstream alignment block (nucleotides 481–720) generated on Phylogeny.fr. Solid vertical color blocks highlight absolute sequence identity (100% consensus), showing structural constraints, while systematic nucleotide substitutions mark the defined evolutionary boundaries dividing Globodera and Meloidogyne.

```
AF056111.1      ATGtgTCgTCTCcAgTTccTgtTcTTccGTgTCTTCCTCcTCCATGcCCTGCACATTGTG
AF056110.1      ATGAgTCgaCTCcAgTctcTgtTcTTccGTgTCTTCCTCcTCCATGgCCTGCACATTGTG
EU371023.1      ATGAATtCTCTCTtATTAATAgcATTTT-TATCTctCTCAT-------------------
AM231138.1      ATGAATtCTCTCTtATTAATAgcATTTT-TATCTctCTCAT-------------------
AF323088.2      ATG-----TtaaTAATTAAaAATATTTT-TATtTTCtcatTttAT---------------
AF323086.1      ATG-----TtaaTAATTAAaAATATTTT-TATtTTCtcatTttAT---------------

AF056111.1      TTcTGTAacGCTTTGACtGCcACGCCTCCcCCATAcGGgCAATTgTCcGTctccGGcAcc
AF056110.1      TTcTGTAacGCTcTGACtGCcACGCCgCCcCCATAcGGgCAATTgTCcGTttccGGcAcc
EU371023.1      -TtTGTATTcCaaTaAagGCTg---CTCCtCCATATGGACAATTATCTGTgAAAGGAAGt
AM231138.1      -TtTGTATTcCaaTaAagGCTg---CTCCtCCATATGGACAATTATCTGTgAAAGGAAGt
AF323088.2      TTgTGTATTGtTTTGgCAGtTA---aTCCgCCATATGGAaggcTtTCaGTaAAAaatgGg
AF323086.1      TTgTGTATTGtTTTGgCAGtTA---aTCCgCCATATGGAaggcTtcCTGTaAAAaatgGg

AF056111.1      aAATTgGTcGGCTCcAAcGGACAACCcGTTCAgCTgATcGGAAaTTCgCTgTTCTGGcAc
AF056110.1      aAATTgGTcGaCTCcAgTGGACAACCcGTTCAgCTgATcGGAAaTTCgCTgTTCTGGcAT
EU371023.1      CAATTAGTgGGCagTAATGGACAACCAGTTCAACTtgTtGGAATgTCACTTTTCTGGt--
AM231138.1      CAATTAGTgGGCagTAATGGACAACCAGTTCAACTtgTtGGAATgTCACTTTTCTGGt--
AF323088.2      CAATTAactGGtTCTAATGGACAggCAGTTCAAtTAAggGGAgTTTCtCTTTggTtcAgT
AF323086.1      CAATTAactGGtTCTAATGGACAggCAGTTCAAtTAAgaGGAATTTCtCTTTggTtcAgT
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Fig. 3: Detailed MUSCLE alignment view focusing on the 5' terminal initiation region of the endoglucanase gene. Blue-shaded highlights visualize uniform consensus sites, while targeted gaps (dashes) accommodate evolutionary insertions or deletions (indels) separating the distinct nematode genera immediately following the conserved start codon.

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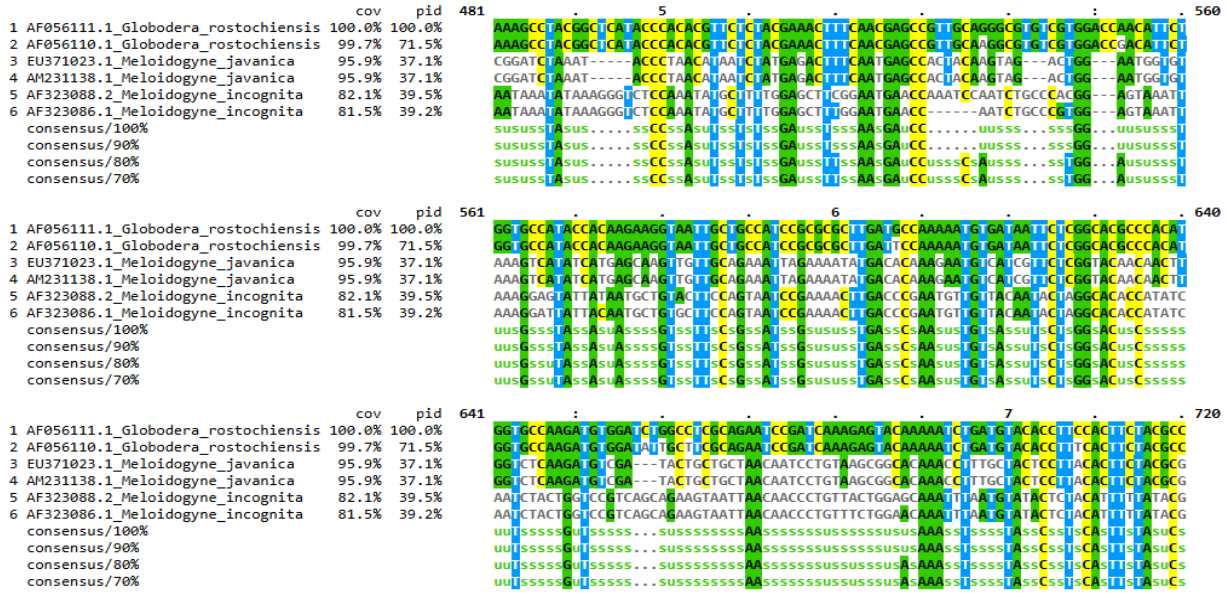

Fig. 4: Multiple sequence alignment block (nucleotides 481–720) of the Beta-1,4-Endoglucanase (eng) coding sequence across six plant-parasitic nematode isolates generated via Clustal Omega. Colored columns represent degrees of nucleotide conservation, while the consensus lines at the base quantify conservation thresholds 70%to 100). Note the distinct generic clustering where sequences exhibit tight intra-generic conservation but clear inter-generic variation between *Globodera* and *Meloidogyne*.

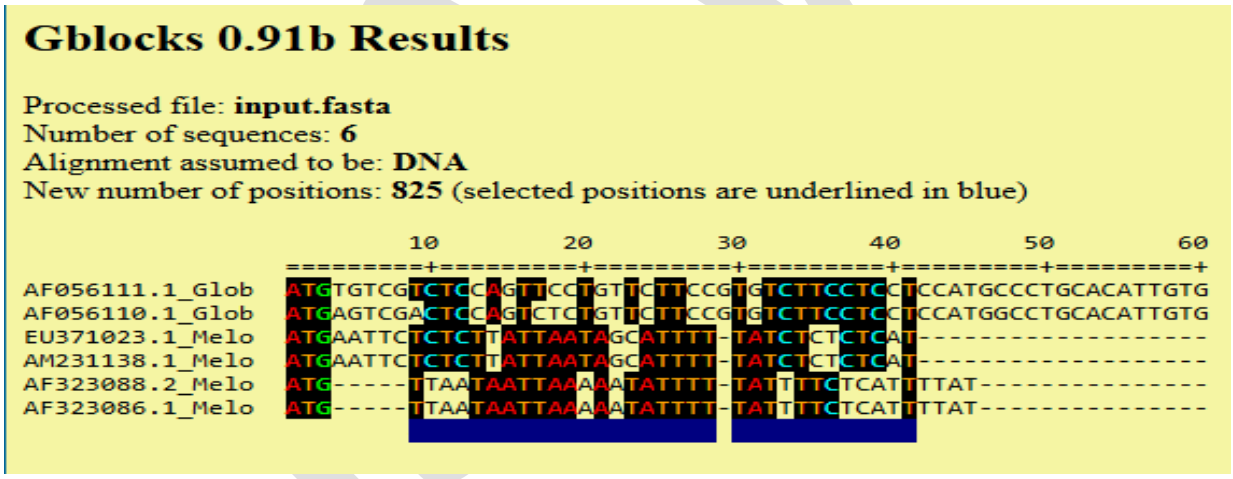

Fig. 5: Automated alignment curation using Gblocks 0.91b on the endoglucanase dataset. Blue underline blocks indicate highly reliable, statistically informative alignment positions selected for downstream phylogenetic reconstruction, effectively eliminating ambiguous, hyper-variable regions to ensure tree stability.

Table 2: Percent Identity Matrix of Beta-1,4-Endoglucanase Coding Sequences (CDS)

Sequence ID / Organism	1	2	3	4	5	6
1. AF056111.1_Globodera_rostochiensis	**100.00**	87.81	51.24	51.24	41.87	41.81
2. AF056110.1_Globodera_rostochiensis	87.81	**100.00**	52.27	52.27	43.21	43.27
3. EU371023.1_Meloidogyne_javanica	51.24	52.27	**100.00**	100.00	51.93	51.62

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4. AM231138.1_Meloidogyne_javanica	51.24	52.27	100.00	**100.00**	51.93	51.62
5. AF323088.2_Meloidogyne_incognita	41.87	43.21	51.93	51.93	**100.00**	96.20
6. AF323086.1_Meloidogyne_incognita	41.81	43.27	51.62	51.62	96.20	**100.00**

Note: Matrix computed via Clustal2.1 using the isolated coding sequences (CDS). Values represent the percentage of identical nucleotides shared between pairs of sequences. Bold values highlighted diagonally indicate absolute identity (100.00%).

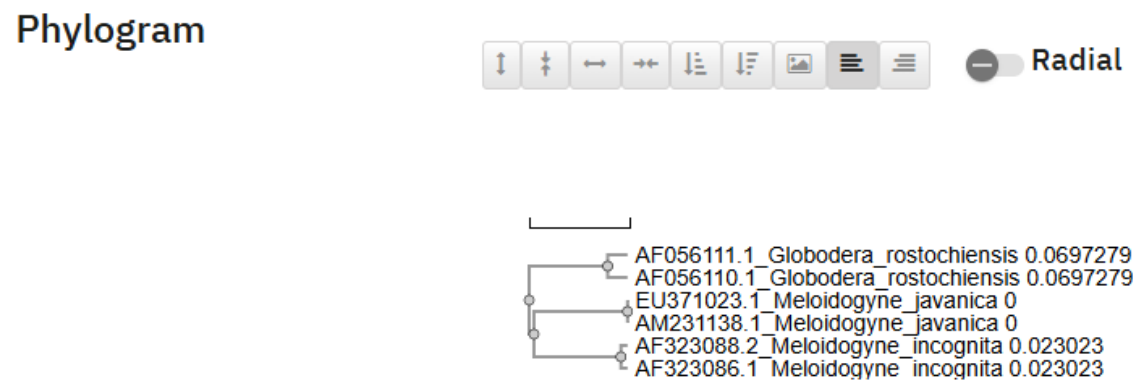

Fig. 6. Neighbor-Joining phylogram of the β-1,4-endoglucanase coding sequence across six plant-parasitic nematode isolates generated via Clustal Omega. The numbers accompanying the species labels represent the computed branch lengths (evolutionary distance), indicating the estimated number of nucleotide substitutions per site. The tree demonstrates a distinct topological bifurcation separating the family Heteroderidae from Meloidogyne isolates.

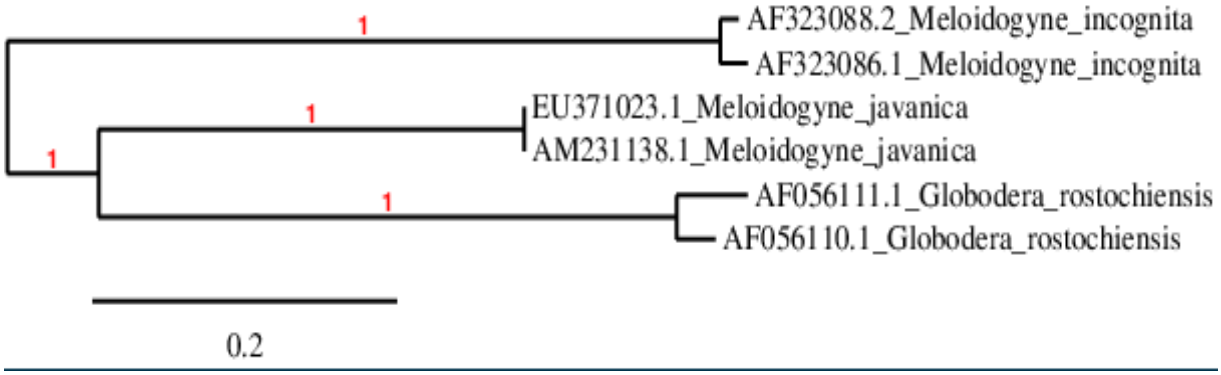

Fig. 7. Maximum Likelihood phylogenetic tree based on the coding sequences (CDS) of the β-1,4-endoglucanase (eng) gene across selected plant-parasitic nematodes. Numbers at nodes indicate branch support values. The tree cleanly resolves the family Heteroderidae (*Globodera rostochiensis*) from the root-knot nematodes (*Meloidogyne spp.*), highlighting the high sequence conservation within respective genera.

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