Journal of Invertebrate Pathology 217 (2026) 108641

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Comparative genomics of parasitoid lifestyle as exemplified by Mermithidae and Nematomorpha

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ARTICLE INFO

Keywords:
Evolution
Behavior
Extended phenotype
Genomes
Host-parasite relationship
Parasitoidism

ABSTRACT

Mermithidae and Nematomorpha are parasitoids united by the commonalities in their lifestyle. Immature stages infect arthropod hosts, can manipulate their host to induce water-seeking behavior, and both have a final free-living non-feeding reproducing adult stage, often killing their host upon emergence. Some species are of great economic importance, being evaluated as biological control agents against mosquito vectors responsible for diseases like malaria, but with scarce genomic resources currently available. This study aimed to investigate the genetic features of the genomes of both Mermithidae and Nematomorpha to identify similarities linked to their ecological lifestyles. We performed a comparative analysis of 12 genomes, comprising parasitoid, parasitic and free-living worms. The investigation revealed enrichment in various protein families known to be linked to host-tissue remodelling, nutrient acquisition, and modulation of host immune responses, chemosensation, host-seeking behaviour, and stage-specific signalling in other parasitic nematodes. Enrichment in domains associated with the cuticle and extracellular matrix could be linked with the intense growth or transcuticular feeding of the parasitic juveniles. The analysis also uncovered a diverse array of conserved transposable element superfamilies across both lineages. The identification of enriched, expanded and contracted gene families and conserved transposable element superfamilies provide the first insights into the potential genomic adaptations associated with parasitoidism in nematode and nematomorph worms.

1. Introduction

Mermithids (a family of the phylum Nematoda) and Nematomorphs (a phylum of parasites known as horsehair worms or hairworms) represent two distinct groups of invertebrates that exhibit intriguing similarities in their parasitoid lifestyles and the alterations in behavior they induce in their definitive hosts. Parasitoids in general are a subgroup of parasites which “develop on or within another organism (=host), derive nourishment from it, and ultimately cause its demise during or at the end of this development” (modified after Eggleton and Gaston (1990) and Maggenti et al. (2005)). The parasitoid lifestyle is better known in insects (Eggleton and Belshaw, 1992) but there are important biological differences between them and parasitoid worms. In both cases, killing the host by the immature stages forces the adult stage to utilize an alternative survival strategy. In insects, the free-living adult feeds on a different source of nutrients and occupies a different ecological niche, compared to its parasitoid larvae. The biology of parasitoid nematodes and nematomorphs is different. Adult mermithids and nematomorphs are free-living and do not feed. The parasitoid juveniles of both mermithids and nematomorphs source their nutrition from a variety of arthropod species in a similar way (de Valdez, 2005; Mishina et al., 2023; Sato et al., 2012). The cuticle of parasitoid juvenile nematodes undergoes a certain degree of degeneration, allowing for transcuticular feeding by the haemocoel-dwelling parasitoid (Rutherford et al., 1977; Skaling and MacKinnon, 1988; Poinar and Hess, 1977), while its digestive system reduces in complexity (Schmidt-Rhaesa, 2005). Accumulated nutrients are stored in a modified intestine (trophosome) in Mermithidae and in parenchyma in Nematomorpha. This energy store allows free-living non-feeding adults to engage in mating and egg-laying for a long period of time, producing huge numbers of progeny, after emerging from the host (Hanelt et al., 2005; Nickle, 1972).

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https://doi.org/10.1016/j.jip.2026.108641
Received 16 September 2025; Received in revised form 5 April 2026; Accepted 27 April 2026

Needing an aquatic environment to complete their development, some Mermithidae and Nematomorpha are known to induce water-seeking behavior in their terrestrial arthropod hosts (Maeyama et al., 1994; Vance, 1996; Poulin, 1998; Thomas et al., 2002; Ponton et al., 2011; Allahverdipour et al., 2019; Herbison et al., 2019b). In nematomorphs, this manipulation may involve alterations in neurotransmitter levels and the regulation of the host's nervous system through parasitoid-produced molecules like serotonin, dopamine, and octopamine (in *Paragordius tricuspidatus*) (Thomas et al., 2003) and Wnt family proteins (e.g. in *Paragordius tricuspidatus*, and *Spinochordodes tellinii*) (Biron et al., 2005,2006). In mermithids, the species *Thaumamermis zealandica* induces an increase in haemolymph osmolality in its semi-terrestrial crustacean host *Bellorchestia quoyana* (formerly *Talorchestia*), providing a potential explanation for the observed water-seeking behavior (Williams et al., 2004). Another mermithid species, *Mermis nigrescens*, induces erratic or hyperactive behavior in the European earwig *Forficula auricularia*, potentially through initial modulation of Mtpα (Mitochondrial trifunctional protein α subunit). Proteomic data reveal shared pathways related to neural transmission and synaptic regulation in mermithids and nematomorphs, which could be involved in manipulating host neural processes. Clathrin appears to be a key protein in this manipulation process (Herbison et al., 2019a).

Substantial biological differences between mermithids and nematomorphs relate to their free-living stages. The infective juveniles of mermithids are actively seeking their host and use digestive enzymes to penetrate the body wall and to reach the haemocoel (Shamseldean and Platzer, 1989), whereas the larvae of nematomorphs are semi-sessile and rely on ingestion of the intermediate paratenic host to reach the definitive host (Hanelt et al., 2005). Mermithid adults are armed with a variety of sensory structures and likely engage them in host-finding (Robinson et al., 1990) and mate-finding (Burr and Babinszki, 1990; Burr et al., 2000). Our knowledge of the receptors present in Nematomorpha is limited – existing ultrastructural studies provide minimal insights into their functionality and role (Schmidt-Rhaesa, 2004; Sokolova et al., 2022). From the phylogenetic point of view, the adoption of a parasitoid lifestyle in all extant species of Nematomorpha suggests that this characteristic likely originated early in the evolutionary history of the group, and is synapomorphic to the phylum. Within the phylum Nematoda, a parasitoid lifestyle is not unique and originated independently in four different lineages: Mermithidae, Marimermithidae, Benthimermithidae and *Neocamacolaimus* (Miljutin, 2013; Tchesunov et al., 2023; Holovachov and Boström, 2014) but only Mermithidae were evolutionary successful, with about one thousand known species infesting a broad variety of hosts.

The emergence of parasitoid species in several animal phyla, and their significant diversity, while at the same time representing only a small portion of all animal parasites overall, provides an interesting and manageable study model to study convergence in functionality and phenotype (Poulin 2011). Genomic resources for both Nematomorpha and Mermithidae are still limited. The sizes of few published genomes range from 250–500 Mb, they exhibit a reduced gene content when compared to other metazoa (Cunha et al., 2023; Eleftheriadi et al., 2024; Guiglielmoni et al., 2024; Bhattarai et al., 2024; Kirangwa et al., 2024) and a comprehensive comparative analysis of these genomes within an evolutionary framework is yet to be conducted. Here we introduce a new genome for a mermithid species *Romanomermis iyengari* and provide the first comparative genomic analysis to identify the likely evolutionary drivers and mechanisms behind the parasitoid lifestyle of nematomorphs and mermithids. By conducting inter-phyla comparative analyses of gene families, focusing on expansion vs. contraction and gain vs. loss, we look for possible genetic factors involved in the interaction with the hosts and crucial for parasitoid survival, and try to identify common patterns among genomes of these two distantly related but ecologically convergent lineages. Transposable elements were found to be very abundant in the genome of an insect parasitoid species, *Nasonia* (Wurm and Keller, 2010), although the significance of this remains unclear, and thus we also investigate the contribution of transposable elements (TE) to genome complexity in parasitoid worms, paving the way towards understanding their role in the evolution of the parasitoid lifestyle.

2. Materials and methods

2.1. Data Collection

We conducted a comparative genomics analysis encompassing all published and one new genome of parasitoid worms, including three mermithid species *Romanomermis culicivorax*, *R. iyengari*, and *Mermis nigrescens*, and four nematomorph species *Acutogordius australiensis*, *Gordionus montsenyensis*, *Gordius aquaticus*, and *Nectonema munidae* (Guiglielmoni et al., 2024; Bhattarai et al., 2024; Eleftheriadi et al., 2024; Cunha et al., 2023). Additionally, our analysis includes a selection of nematode parasites, i.e., *Trichinella spiralis*, *Onchocerca volvulus*, and *Brugia malayi*, as well as free-living limno-terrestrial species, *Plectus sambesii* and *Mesodorylaimus* sp., all chosen based on the completeness of their genomes (Suppl. Table 1). Data analyses and interpretations were performed within a phylogenetic context, using published tree topologies as a reference (Ahmed et al., 2022; Cunha et al., 2023).

2.2. HMW DNA extraction of *Romanomermis iyengari*

The *Romanomermis iyengari* nematodes were selected from moss samples provided by Professor Dr. Edward Platzer from the University of California Riverside. High-molecular-weight DNA from 20 individuals of *R. iyengari* was extracted using a salting out protocol as follows. The nematodes were collected and washed in water, then flash-frozen using liquid nitrogen in a salt-based extraction buffer (Tris-HCl 100 mM, ethylenediaminetetraacetic acid 50 mM, NaCl 0.5 M and sodium dodecylsulfate 1 %). Samples were incubated overnight at 55°C after addition of 5 µL of proteinase K. DNA was precipitated using NaCl 5 M, yeast tRNA and isopropanol, and incubated at room temperature for 30 min, then pelleted at 18,000 g for 20 min at 4°C. The DNA was washed twice with 80 % ethanol and spun at 18,000 g for 10 min at 4°C. The DNA pellet was eluted in an elution buffer (D3004-4–10 Zymo Research) and incubated at 50°C for 10 min. RNA was removed by incubating with RNAse (Qiagen, 19101) for 1 h at 37°C. DNA concentration was quantified using a Qubit 4 fluorometer with 1X dsDNA kit. HiFi libraries were prepared with the Express 2.0 Template kit (Pacific Biosciences, Menlo Park, CA, USA) and sequenced on a Sequel II/Sequel IIe instrument with 30 h movie time. HiFi reads were generated using SMRT Link (v10, Pacific Biosciences, Menlo Park, CA, USA) with default parameters.

2.3. Genome assembly and annotation of *Romanomermis iyengari*

PacBio HiFi reads were assembled using NextDenovo v2.5.2 (Hu et al., 2024). Assembly statistics were generated using assembly-stats v1.0.1, and ortholog completeness was assessed using the Benchmarking Universal Single-Copy Orthologs (BUSCO) tool v6.0.0 with parameters −m genome against the *metazoa_odb10/odb12* and *nematoda_odb10/odb12* lineages, both to utilize the most recent BUSCO dataset, and to retain backwards comparability with other studies utilising “odb10” versions (Tegenfeldt et al. 2025). Subsequently, PacBio HiFi reads were aligned to the HiFi assembly using minimap2 v2.24 with parameters-ax map-hifi, and the resulting mapped reads were sorted using SAMtools v1.6. Contigs were aligned against the nt database using BLAST v2.13.0, and the outputs were processed using BlobToolKit v4.1.5 to remove bacterial contaminants (Suppl. Fig. 1). Reads were remapped using minimap2 v2.24, and the output was provided to purge dups v1.2.5 to eliminate uncollapsed haplotypes using duplicated BUSCO genes as a guide and assembly statistics were recalculated using assembly-stats v1.0.1. Protein-coding genes were predicted using the BRAKER3 pipeline (Gabriel et al., 2024), leveraging protein alignments from related species as evidence. To construct a protein database, proteins from *Romanomermis culicivorax* and *Mermis nigrescens* were combined with OrthoDB v11 (Kuznetsov et al., 2023) Metazoa and Eukaryota gene orthologs. The protein database was used as input to BRAKER3 for gene prediction in the soft-masked genome of *Romanomermis iyengari*. Subsequently, BUSCO analyses were conducted (utilizing the *nematoda_odb10/odb12* and *metazoa_odb10/odb12* lineages) on the predicted protein sequences to evaluate completeness.

2.4. Orthology inference and comparative analysis

Orthology analysis of seven parasitoid worm species and five control nematode species was performed using OrthoFinder v2.5.5 (Emms and Kelly, 2019) with default parameters, using the longest isoform for each gene. Orthologous group distributions across species were visualised using the UpSetR package (Conway et al., 2017). Orthology clustering results generated by OrthoFinder were further analysed using KinFin (Laetsch and Blaxter, 2017), with species assigned to two groups: parasitoid worms ($n = 7$) and control worms ($n = 5$) to enable taxon-aware comparisons of shared and group-specific protein clusters between groups. Orthogroup representation was assessed using pairwise representation tests, which evaluate differences in mean protein counts between predefined taxon sets. Significance thresholds of $p \leq 0.05$ and $p \leq 0.01$ were applied, together with a fold-change cutoff of $|\log_2 FC| \geq 1$ (corresponding to the empirical 95th percentile, $|\log_2 FC| \approx 1.03$). Orthogroups that were significantly over- or under-represented in parasitoid taxa were identified in this way.

2.5. Gene family evolution and convergence analysis

For gene family analysis, the rooted species tree was generated by OrthoFinder and converted into an ultrametric chronogram. Branch lengths were transformed from substitutions per site to absolute time using the *chronos* function in the R package *ape* (v5.8). A primary calibration point was applied to the root, representing the divergence between Nematoda and Nematomorpha, with a median age of 550 million years (Ma) based on consensus estimates from the literature and TimeTree.org. A relaxed clock model was employed to account for rate variation across lineages.

Gene family expansion and contraction dynamics were modelled using CAFE5 v1.1 (Mendes et al., 2020). To account for rate heterogeneity across gene families, a gamma model was implemented. A range of gamma categories ($k = 1$–10) was tested, and the optimal model ($k = 3$) was selected based on the maximum likelihood (lowest $-\ln L = 122880.85$). The global lambda (λ) parameter, representing the rate of gene gain and loss, was estimated under a birth–death model ($\lambda = 0.00189597$). Functional annotation of significant ($p < 0.05$) lineage-specific expansions and contractions was performed using InterProScan v5.77–108.0 (Jones et al., 2014).

2.6. Transposable elements and repeat sequence analysis

To assess the content of transposable elements (TE) within the parasitoid Mermithidae and Nematomorpha, first, a TE and repeat library was built using EDTA v2.1.3 (Ou et al., 2019) with parameters *–sensitive 1 –anno 1*. Proportions of classified and unclassified transposable elements were calculated from the final curated annotations following the FasTE pipeline by Bell et al. (2021), after removal of repetitive elements not classed as TEs, duplicated isoforms, low-confidence and overlapping annotations and elements shorter than 80 bp (Suppl. Table 2). Kimura divergence estimates were obtained from RepeatMasker outputs based on alignments of individual transposable element copies to their family consensus sequences derived from the EDTA-generated species-specific repeat library. Divergence was calculated as the Kimura 2-parameter (K2P) distance between individual transposable element copies and their corresponding consensus sequences, providing differential rates of transitions and transversions, and estimating the sequence divergence since insertion and relative age of TE insertions. Output files were parsed with the R script “RMTrips”, contained in FasTE. A custom R script “RMTrips output to divsum-file format” was used, renaming the TE superfamilies according to Wicker-classification (Wicker et al., 2007), and converting the data format to further plot the TE divergence landscapes with the “Plot Kimura Distance” R script. Stacked barplots were plotted in R.

3. Results

3.1. The genome sequence of Romanomermis iyengari

The assembly size of *Romanomermis iyengari*’s genome is 302.9 Mb, featuring a 37 % GC content and scaffold N50 of 322 kb (Suppl. Fig. 1). The longest scaffold stretched over 3.9 Mb. Gene annotation prediction yielded 16,503 protein coding genes. Overall, BUSCO scores indicate 64.4 % completeness for *metazoa_odb10* and 59.5 for *metazoa_odb12*, with duplicated orthologs at 11.7 % and 8.8 %, respectively, and fragmented orthologs at 1.9 % and 6.8 % respectively. In terms of Nematoda (*nematoda_odb10* and *nematoda_odb12*), BUSCO assessment shows 46.4 % and 70.0 % completeness, with duplicated orthologs at 9.2 % and 14.6 % and fragmented orthologs at 2.2 % and 5.5 %, respectively. 54.28 % of the *R. iyengari* genome has been identified as repetitive elements.

3.2. Identification of orthologous clusters in Mermithidae and Nematomorpha

Orthology inference using OrthoFinder across 12 species identified a total of 146,582 genes, of which 132,194 (90.2 %) were assigned to 12,722 orthogroups, while 14,388 genes (9.8 %) remained unassigned. Among the orthogroups, 2,210 were species-specific, comprising 8,700 genes (5.9 % of total genes). The mean and median orthogroup sizes were 10.4 and 5.0 genes, respectively, indicating a predominance of small gene families. To identify orthogroups specifically representative of parasitoid worms included in our study, we retained those present in at least six of the seven species, allowing for potential incomplete annotations in a single genome. This filtering yielded 18 orthogroups, including three conserved across all seven species (Fig. 1). Identified in this way, a set of protein domains have regulatory, enzymatic, and extracellular functions (Table 1). These orthogroups comprise core molecular components associated with transcriptional control, signalling, metabolism, and cell-environment interactions. Several orthogroups encoded transcription- and chromatin-associated proteins, including a homeodomain-containing orthogroup, the COMPASS component Shg1, DSS1/SEM1 family proteins, and WD repeat- and CID domain-containing proteins. Extracellular and membrane-associated functions were represented by orthogroups containing EGF-like domains, low-density lipoprotein receptor class A domains, a claudin-domain-containing transmembrane protein, and a Noggin-like domain. Proteolytic and extracellular matrix-associated functions were represented by orthogroups containing trypsin and ADAMTS cysteine-rich domains, while enzymatic functions included glycosyl hydrolases, acyltransferases, and queuine tRNA-ribosyltransferase (Table 1).

3.3. Taxon-aware analysis of orthogroup enrichment in parasitoid worms

To investigate gene family differences associated with the parasitoid lifestyle, we performed a taxon-aware analysis using KinFin, comparing seven species of parasitoid worms to five non-parasitoid (control) species. The distribution of $\log_2$ fold-change ($\log_2 FC$) values was centered around zero, indicating that most orthogroups exhibited similar representation across parasitoid and control taxa (Fig. 2A). The rarefaction curves (Fig. 2B) show a rapid initial increase in orthogroup counts for both groups. However, while the control group approaches a plateau

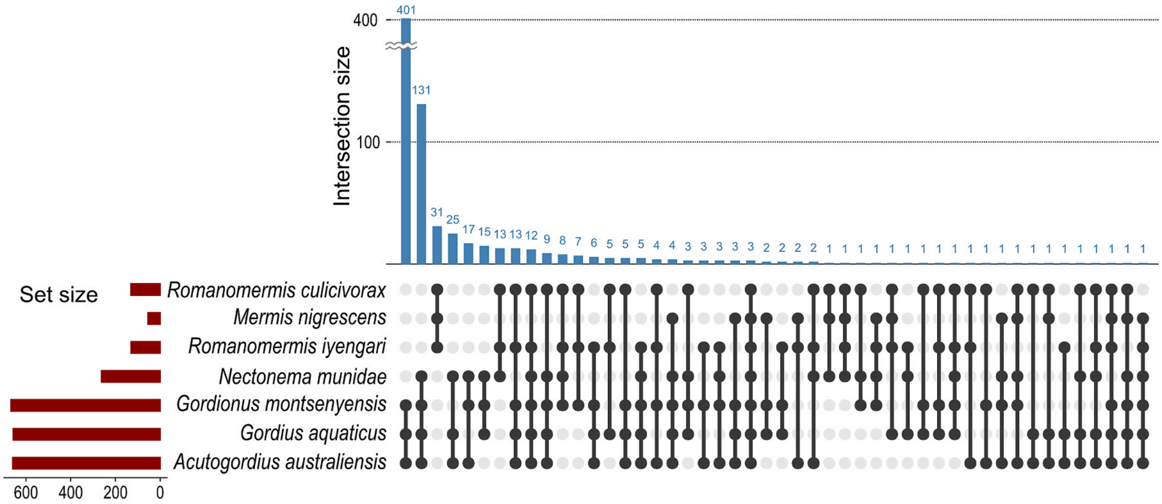

Fig. 1. UpSet plot showing the distribution of putative parasitoid-specific ortholog families across seven studied species. Horizontal bars (left) indicate the total number of ortholog families (set size) in each species. Vertical bars (top) represent the number of ortholog families shared among specific combinations of species (intersection size). Dots below each bar identify the species included in each intersection. Connected dots indicate shared ortholog families across multiple parasitoid worm species.

Table 1
Orthogroups representative of parasitoid worms included in this study (Nematoda and Nematomorpha) and their functional annotation.

Orthogroup	Pfam	Description
OG0001084	PF00008	EGF-like domain
OG0002652	PF00665	Integrase core domain
OG0003973	PF00046	Homeodomain
OG0004098	PF01301	Glycosyl hydrolases family 35
OG0004194	PF04937	Domain of unknown function (DUF659)
OG0004612	PF26158	Transmembrane protein 179/179B Claudin domain
OG0004647	PF17771	ADAMTS cysteine-rich domain 2
OG0004790	PF01702	Queuine tRNA-ribosyltransferase
OG0004881	PF00057	Low-density lipoprotein receptor domain class A
OG0005043	PF00089	Trypsin
OG0005345	PF04818	CID domain
OG0005385	PF00400	WD domain, G-beta repeat
OG0005430	PF01553	Acyltransferase
OG0005461	PF10252	Casein kinase substrate phosphoprotein PP28
OG0005672	PF05160	DSS1/SEM1 family
OG0005752	PF10644	Misato Segment II tubulin-like domain
OG0006020	PF05806	Noggin
OG0006072	PF05205	COMPASS (Complex proteins associated with Set1p) component shg1

with increasing sampling depth, the parasitoid group continues to rise without clear saturation. The absence of saturation in the parasitoid rarefaction curve suggests that gene family diversity within this group remains incompletely sampled, with additional genomes likely to reveal further orthogroups.

We found a subset of orthogroup clusters that were significantly overrepresented in the studied parasitoid worms compared with the control set (Table 2). The enriched orthogroup clusters were associated with signalling, proteolysis, and structural components. Enriched signalling-related domains included G protein-coupled receptors (7 transmembrane receptors), protein kinases, protein-tyrosine phosphatases, myotubularin-like phosphatases, RhoGAP domains, and Sec7 domain-containing proteins, together with a homeodomain-containing orthogroup. Proteolytic activity and its regulation were represented by enrichment of serpins, metalloproteases (M12 family), and ADAMTS cysteine-rich domains. Structural and extracellular-associated domains were also enriched, including collagen triple helix repeats, cuticlin domains, and zona pellucida (ZP-C) domains, alongside protein interaction modules such as leucine-rich repeats, armadillo repeats, and zinc finger domains (Table 2).

3.4. *Gene family evolution in parasitoid worms*

We next examined gene family evolution at the species level. Substantial variation in expansion and contraction patterns was observed across taxa (Fig. 3). Among three studied nematode parasitoid species, *Mermis nigrescens* showed the strongest expansion signal, whereas *Romanomermis* species exhibited more moderate or balanced patterns. Among four studied nematomorph parasitoid species, expansion-biased and contraction-biased profiles were both observed across species. Within the expanded gene families of the parasitoid worms, we consistently observed a subset of protein domains including signal transduction (G protein-coupled receptors, protein kinases and phosphatases), protein turnover (ubiquitin-associated proteins), membrane trafficking (Sec7/Mon2- and VPS-related proteins), extracellular interaction domains (fibronectin type I), and nucleic acid processing and transcriptional regulation machinery. A full list of lineage-specific expanded and contracted domains is provided in Suppl. Tables 3–16.

3.5. *Transposable elements*

Both nematomorph and mermithid genomes exhibit a high proportion of transposable elements compared to outgroup species. Using the Kimura substitution level as a measure of divergence from the TE consensus sequence, and plotting it as transposable elements and repeat divergence landscapes (Fig. 4, Suppl. Fig. 3), the following observations can be made: (a) the genome of *Nectonema munidae* contains TEs at various divergence levels with prominent TE superfamilies including *DTA_hAT* and *DTA_hAT_MITE*; (b) the *Gordius aquaticus* landscape shows a broad distribution of TEs with peaks at low and intermediate divergence levels; (c) the profiles of *Gordionus montsenyensis* and *Acutogordius australiensis* display a high percentage of TEs at multiple divergence levels; (d) all three studied mermithid species, *Mermis nigrescens, Romanomermis culicivorax* and *R. iyengari* show significant TE presence with multiple peaks at various divergence levels; (e) *Mesodorylaimus* sp. shows low overall TE content with a few peaks; (f) divergence landscapes for the remaining outgroup species, *Brugia malayi, Onchocerca volvulus*, *Plectus sambesii* and *Trichinella spiralis,* show smaller proportions of TE elements in their genomes compared to parasitoid nematomorphs and mermithids, and do not reveal any common patterns in TE divergence among them.

All studied species of Nematomorpha have a comparable genome size between 200 and 300 Mb, while mermithid genomes are larger and

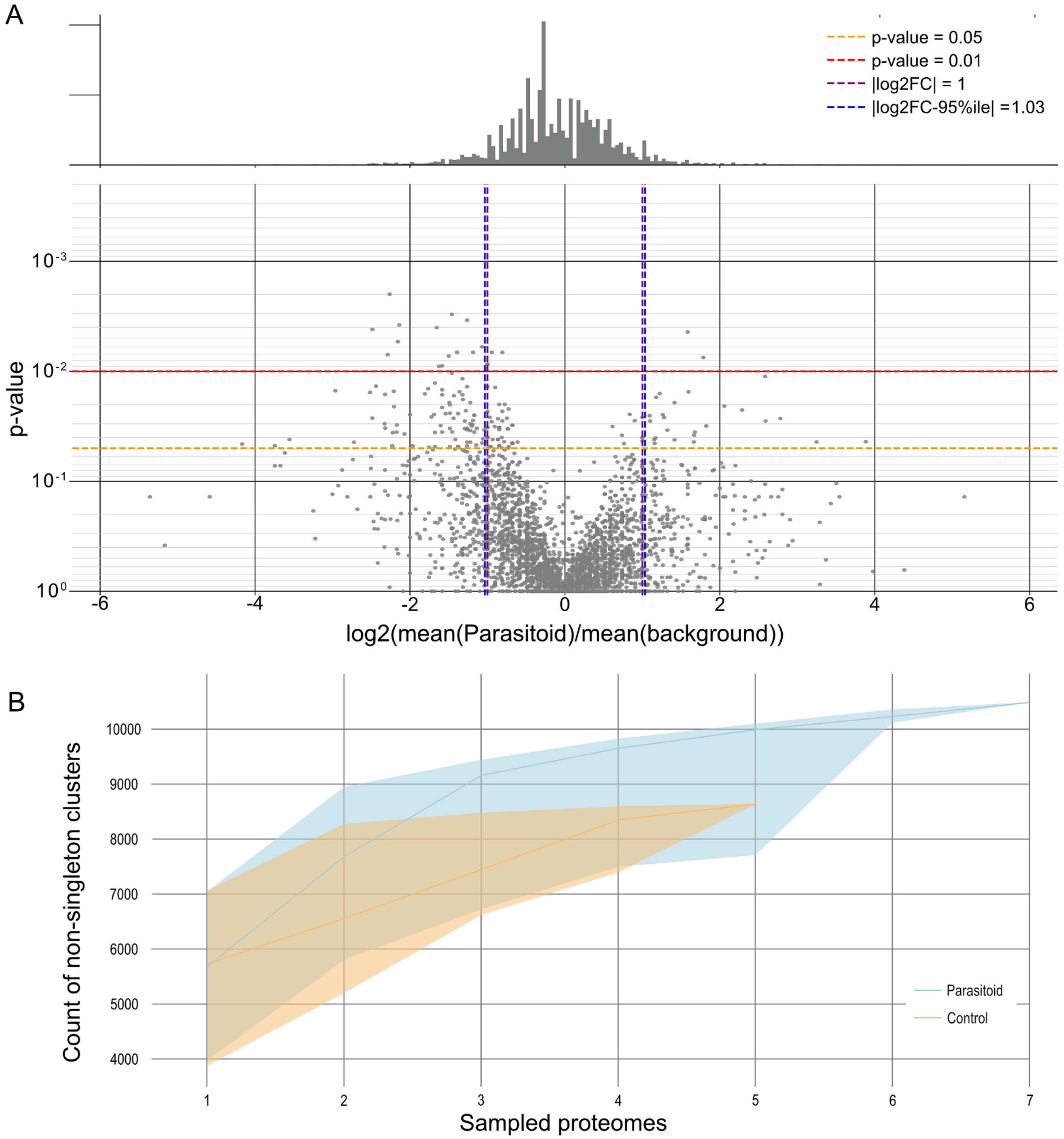

Fig. 2. **A.** Pairwise representation (volcano) plot of ortholog clusters comparing parasitoid and control sets of species. Each point represents an ortholog family, plotted by log2 fold change in abundance (x-axis) versus statistical significance (p-value, y-axis). Vertical dashed blue lines indicate fold-change thresholds (|log2FC| = 1.03), while horizontal dashed lines denote significance thresholds (p = 0.05 and p = 0.01). Clusters falling above these thresholds represent gene families significantly enriched or depleted in the parasitoid group. **B.** Rarefaction curves showing the accumulation of non-singleton ortholog clusters with increasing numbers of sampled proteomes in parasitoid and control set of species. The x-axis represents the number of randomly sampled proteomes, and the y-axis indicates the count of shared (non-singleton) ortholog clusters. Solid lines show the mean number of clusters recovered, while shaded areas represent variability across sampling replicates. The curves illustrate how cluster diversity increases with additional genomes and approaches saturation as sampling depth increases.

vary in size (Suppl. Table 1). Nevertheless, all studied Nematomorpha and Mermithidae species exhibit a high combined length of TEs within their genomes, indicating extensive TE content (Fig. 5A, B), compared to other nematodes included in this study. Of them, *Mermis nigrescens* displays the highest combined TE length among the Mermithidae. On the other hand, *Brugia malayi* and *Onchocerca volvulus* show relatively low TE content, while *Plectus sambesii*, *Trichinella spiralis* and *Mesodorylaimus* sp. show intermediate values, with contributions from multiple superfamilies (Fig. 5A, B). Comparing the composition of TEs between all species included in this study (Fig. 5C), it is evident that DNA elements of the superfamilies *hAT*, *CACTA*, *Mutator*, *Transib* and *Mariner* are conserved among the species, while also containing a large portion of DNA elements that could not be further discriminated. Furthermore, all genomes contain Helitron elements and the composition of retroelements is dominated by the superfamily *Gypsy* (Fig. 5C).

Table 2
Orthogroup clusters significantly overrepresented in parasitoid worms included in this study (Nematoda and Nematomorpha) and their functional annotation.

Orthogroup	Pfam	Description	Score
OG0001125	PF10177	Uncharacterised conserved protein (DUF2371)	0.004390259712
OG0000424	PF25057	Cuticlin *N*-terminal domain	0.007489104269
OG0000342	PF07884	Vitamin K epoxide reductase family	0.01115317518
OG0000395	PF00903	Glyoxalase/Bleomycin resistance protein/Dioxygenase superfamily	0.01536943574
OG0004056	PF06602	Myotubularin-like phosphatase domain	0.01595880467
OG0000825	PF01369	Sec7 domain	0.01723344726
OG0001166	PF00046	Homeodomain	0.01929607018
OG0000809	PF00042	Globin	0.02071681966
OG0000240	PF00079	Serpin (serine protease inhibitor)	0.02249427122
OG0002175	PF00233	3′5′-cyclic nucleotide phosphodiesterase	0.02480842455
OG0001315	PF00102	Protein-tyrosine phosphatase	0.02534731868
OG0001173	PF00069	Protein kinase domain	0.02628545207
OG0000162	PF00001	7 transmembrane receptor (rhodopsin family)	0.02688306854
OG0000129	PF01391	Collagen triple helix repeat (20 copies)	0.02828929242
OG0001581	PF00105	Double treble clef zinc finger, C4 type	0.02835967896
OG0000867	PF17771	ADAMTS cysteine-rich domain 2	0.03139516531
OG0000976	PF01182	Glucosamine-6-phosphate isomerases/6-phosphogluconolactonase	0.03426400773
OG0002318	PF13855	Leucine rich repeat	0.03569831062
OG0000231	PF00122	P-type ATPase actuator domain	0.03808200715
OG0003232	PF13688	Metallo-peptidase family M12	0.04042397934
OG0001266	PF00514	Armadillo/beta-catenin-like repeat	0.04172920643
OG0001415	PF00303	Thymidylate synthase	0.04196889079
OG0000143	PF00100	ZP-C domain	0.04359664489
OG0000144	PF10256	Golgin subfamily A member 7/ERF4 family	0.04390199203
OG0000969	PF00620	RhoGAP domain	0.04390199203
OG0000500	PF00282	Pyridoxal-dependent decarboxylase conserved domain	0.04528449914

4. Discussion

4.1. *Orthogroup enrichment analysis of parasitoid worms reveals signalling, proteolysis, and structural components functional domains*

Proteolytic enzymes and their inhibitors have been widely implicated in host–parasite interactions in nematodes, including tissue penetration and modulation of host immune responses. The enrichment of proteases and protease inhibitors in parasitoid worm species included in this study is consistent with roles described for these molecules in other parasitic nematodes, including host-tissue remodelling, nutrient acquisition, and modulation of host immune responses (Zang and Maizels 2001; Hewitson et al., 2009). In insect-parasitic and entomopathogenic nematodes, secreted serine proteases, metalloproteases, and serpins have been shown to contribute to cuticle degradation, penetration of host barriers, and suppression of melanisation and other protease-amplified immune reactions. The presence of orthogroups encoding trypsin-like proteases, M12 metalloproteases, ADAMTS-related domains, and serpins in seven analysed parasitoid worms therefore aligns with conserved mechanisms of host interaction reported in parasitic nematodes, without implying a unique or exclusive association with parasitoidism (Cantacessi et al., 2009).

The enrichment of 7TM (G protein-coupled) receptors in seven studied species, together with intracellular regulators such as kinases, phosphatases, RhoGAP, and Sec7-domain-containing proteins point to potential roles for GPCR-mediated pathways in environmental sensing and developmental regulation in parasitoid worms (McVeigh et al., 2006; Hallem et al., 2011; Crook, 2014). In many parasitic helminths, GPCRs and associated kinases and phosphatases have been implicated in chemosensation, host-seeking behaviour, and stage-specific signalling, while RhoGAP and Sec7-domain proteins are known to regulate small-GTPase-dependent processes such as cytoskeletal remodelling, vesicular trafficking, and cell-surface dynamics. The co-enrichment of 7TM receptors with these regulatory components therefore aligns with broadly conserved mechanisms through which nematodes coordinate sensory input, intracellular signal transduction, and developmental transitions.

We found structural domains associated with the cuticle and extracellular matrix enriched in studied parasitoid worms, consistent with conserved roles in cuticle formation, growth within the host, and structural remodelling during development and emergence (Page & Johnstone, 2007). The nematode cuticle is a collagen-rich, dynamic structure that undergoes repeated remodelling during moulting and growth, processes in which collagens and cuticlin-like proteins contribute to mechanical integrity and barrier function (Page & Johnstone, 2007). ZP-domain-containing proteins are implicated in extracellular matrix assembly and structural organisation, while ADAMTS-related domains have been associated with proteolytic processing of ECM components, suggesting a role in controlled matrix remodelling rather than passive degradation (Hutter et al., 2000). The enrichment of these domains therefore might align with the mechanistic demands of intense growth that the parasitic juvenile undergoes within the body of its host (Gordon et al. 1974) or transcuticular feeding (Rutherford et al., 1977; Skaling and MacKinnon, 1988; Poinar and Hess, 1977).

The rarefaction curves revealed the absence of saturation in orthogroup counts in seven studied parasitoid species, compared to a non-parasitoid control set of species. This pattern suggests that orthogroup diversity is not fully captured by the current dataset limited to only seven species, and that additional high quality genomes from both mermithids and nematomorphs are likely to reveal further gene family diversity, enforcing the need for broader taxon sampling of parasitoid worms. In contrast, the near-plateau observed in control taxa indicates more complete representation of shared gene families in this group, despite it being even more limited taxonomically.

The functional relevance of these genomic signatures to the parasitoid life style remains unclear based on sequence-level analyses alone. Further experimental validation will be required to establish their roles, including approaches such as stage-specific or host-associated RNA-seq, spatial expression profiling, and functional assays (including gene knockdown). In addition, investigation of regulatory mechanisms, including chromatin modification and other epigenetic processes, may help to explain how gene expression is controlled during the development of parasitoid worms and their interaction with the host.

4.2. *High proportion of transposable elements in studied parasitoid worms remains unexplained*

Transposable element content displays a wide variation among the studied species, and the underlying reasons for this diversity remain unclear. There are differences in the copy number of TE families between the different genomes analysed here, as shown in Fig. 5. Since the detection and annotation of TEs strongly depend on the quality and contiguity of analysed genomes and contig sizes, the results presented in this section must be interpreted accordingly. The mermithid genomes included here have varying completeness with *Romanomermis culicivorax* (359 Mbp genome and scaffold N50 of 1 Mb) and *M. nigrescens* (524 Mbp genome and scaffold N50 of 2 Mb) possessing much better contiguity than the genome of *R. iyengari* (303 Mbp genome and scaffold N50 of 322 Kb), which still needs improvement in terms of contiguity and could be misrepresenting TE amounts to some extent. Nevertheless, the determination of TE superfamily composition is not impeded by these limitations.

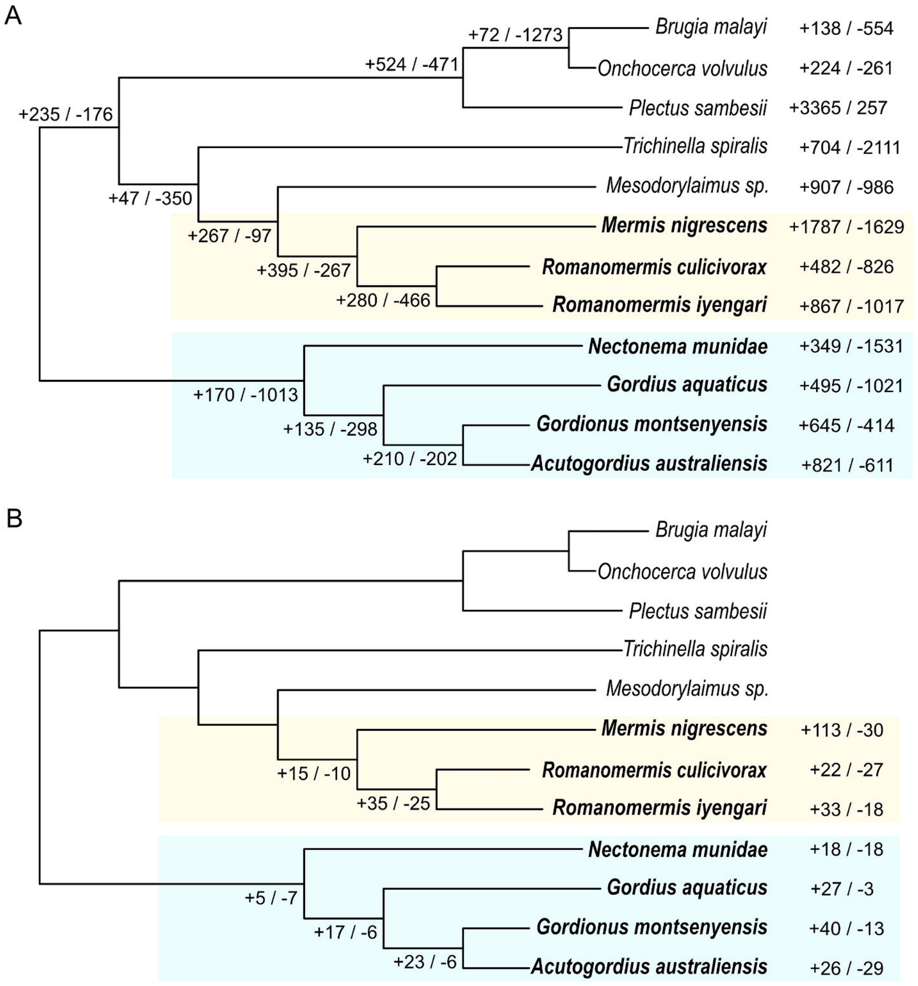

Fig. 3. The number of expanded (+) and contracted (−) gene families. A. Total identified. B. Significantly expanded and contracted gene families. The Cladogram on the left shows the evolutionary history of gene families and differences between species included in the analysis. Bold font highlights parasitoid nematodes, light yellow background highlights parasitoid Nematoda, while light cyan background highlights parasitoid Nematomorpha.

Understanding which TE superfamilies are conserved within a lineage can shed light on potentially active TEs within the respective group. Our analysis suggests that the superfamilies of *hAT*, *CACTA*, *Mutator*, *Transib*, *Mariner*, *Helitron* and *Gypsy* have persisted successfully among all groups while in *Onchocerca volvulus* and *Brugia malayi*, whole superfamilies like *PiggyBac*, *ERV*, *Jockey* and *SINES* have been eliminated and overall TE activity has been downregulated. Apart from *Nectonema munidae*, it seems that the amounts and divergences of TEs show similar trends in the remaining three nematomorph species. Divergence patterns in all three mermithid species show similarities to each other, while the remaining outgroup species exhibit both much lower TE content and no obvious commonality in TE divergence landscapes.

The biological meaning of these observed patterns remains unknown, and requires further investigation. Mobile TEs often induce deleterious effects upon insertion through inactivation of genes but infrequently also contribute to genome dynamics and adaptability by reorganization and introduction of novelties (Jiang et al. 2017). A high number of TE elements and strong divergence patterns in both parasitoid lineages may lead one to unjustifiably suggest their strong role in the parasitoid lifestyle, especially considering that the *Nasonia* parasitoid wasp also exhibits notably high TE content, yet the factors driving this phenomenon in this insect species remain elusive as well (Wurm & Keller, 2010). In general, the variability of TE landscapes within and across insect orders suggests a complex interplay influenced by factors beyond phylogenetic relatedness, possibly including horizontal transfer (Gilbert et al., 2021). While the impact of insect traits on TE landscapes remains uncertain, recent discoveries suggest TEs play crucial roles in insect adaptations, aging, and antiviral immunity. Thus, TEs are increasingly recognized as essential symbiotic elements in insects, with potentially both harmful and beneficial effects depending on the context (Gilbert et al., 2021). Since our analysis was limited by the availability of high-quality genomes of parasitoid nematodes and nematomorphs, we could only determine the TE content and divergence patterns in very few species, aiming to establish foundational data for future investigations encompassing a broader spectrum of species and chromosome-level genome assemblies.

5. Conclusions

While comparative analysis of parasitoid genome sequences alone cannot fully explain parasitoid biology and interactions with hosts, our findings offer foundational insights into the genomic factors driving parasitoid worm adaptation, laying the groundwork and allowing future research to focus on particular gene families. For example, current analysis identified enrichment in gene families in parasitoid worms that are already known to be involved in host-parasite interactions in other groups of nematodes. Interestingly, we observed enrichment in structural domains associated with the cuticle and extracellular matrix, which could potentially be involved with either transcuticular feeding or with intense growth of parasitic stages, but we did not identify any genomic signatures that could be involved in host manipulation mechanisms – both topics requiring further investigations. Further, the

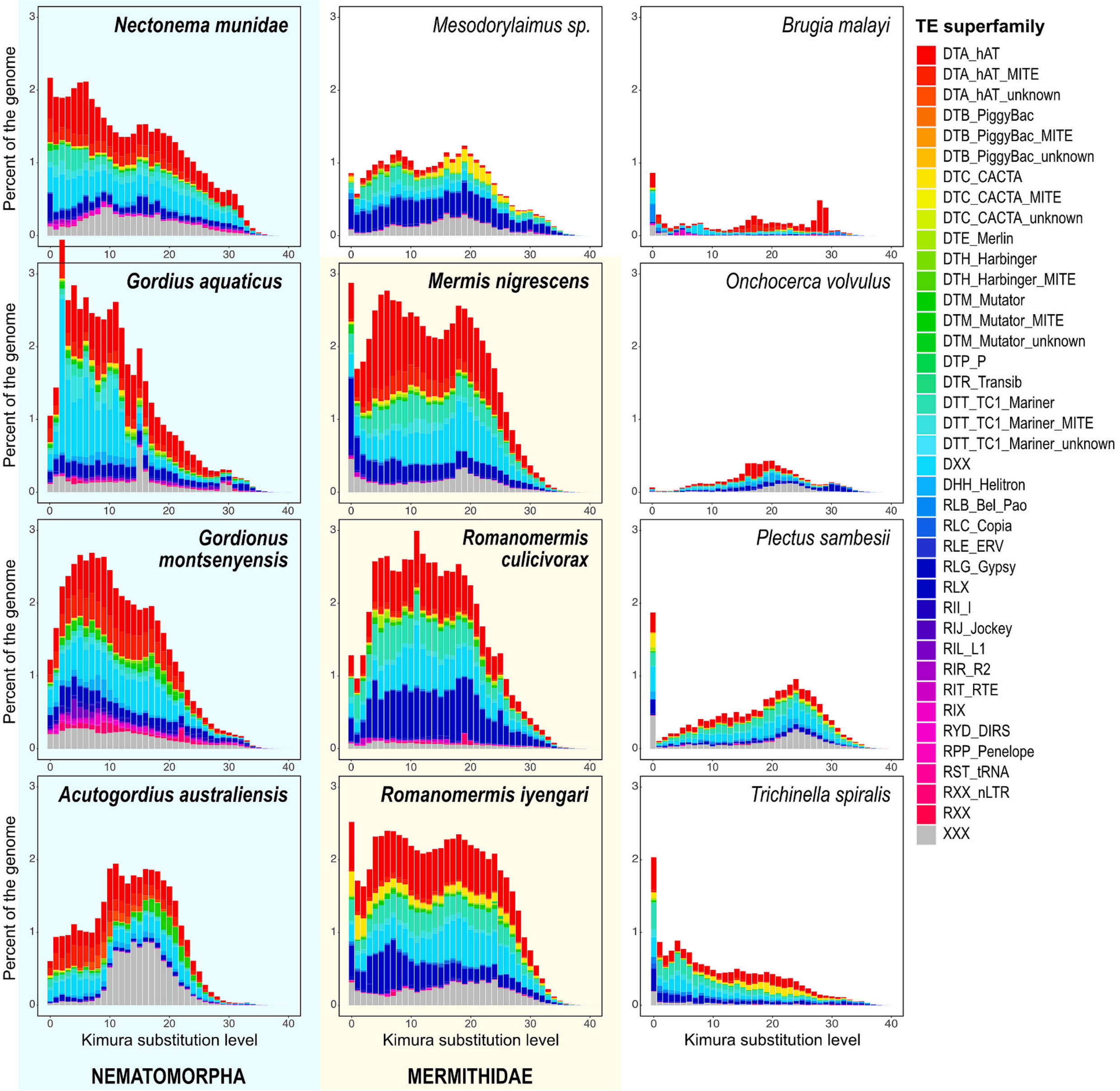

Fig. 4. Transposable elements (TE) and repeat divergence landscapes showing TE portions as percent of the genome (y-axis) over Kimura substitution levels (x-axis) obtained via the "FasTE" pipeline. Legend for TE superfamily (colors) is shown to the right where XXX corresponds to unclassified repeats. Bold font highlights parasitoid nematodes, light yellow background highlights parasitoid Nematoda, while light cyan background highlights parasitoid Nematomorpha.

identification and characterisation of transposable element superfamilies within nematomorph and mermithid genomes provide a valuable starting point for a more in-depth exploration of their genomic impact.

The significance of studies of gene family evolution and identification of particular gene families associated with specific functions extends beyond mere theoretical insights. They may hold practical value for addressing real-world challenges, particularly in the realm of vector control strategies. Both mermithids and nematomorphs have strong negative effects on the population density of their hosts, in addition to the manipulation of their host's dispersal behavior discussed above. Not covered in this particular study, but no less important, is the ability of mermithids to affect host reproduction success by sterilization, inducing changes in secondary sex characters (intersexes) and mating behavior (Vance, 1996; Ya'cob et al., 2021; Muñoz-Muñoz et al., 2016; Rowell, 2000). Specifically, uncovering the mechanisms of parasitoid-induced changes in dispersal behavior and reproductive development is a promising avenue for applying the knowledge obtained toward developing targeted and environmentally friendly methods for controlling insect pests. By leveraging the understanding of molecular factors involved in parasitoid-host interactions, we may uncover innovative approaches to, for example, curb the transmission of malaria, offering a sustainable and effective means of combating a global health threat.

6. Data availability

In addition to the information presented in the supplementary files, all data used and generated in this study are available at https://doi.org/10.6084/m9.figshare.31927005. The PacBio HiFi reads of *Romanomermis iyengari* were submitted to NCBI database with accession numbers: Bioproject accession PRJNA1126031 and Biosample accession SAMN41918894.

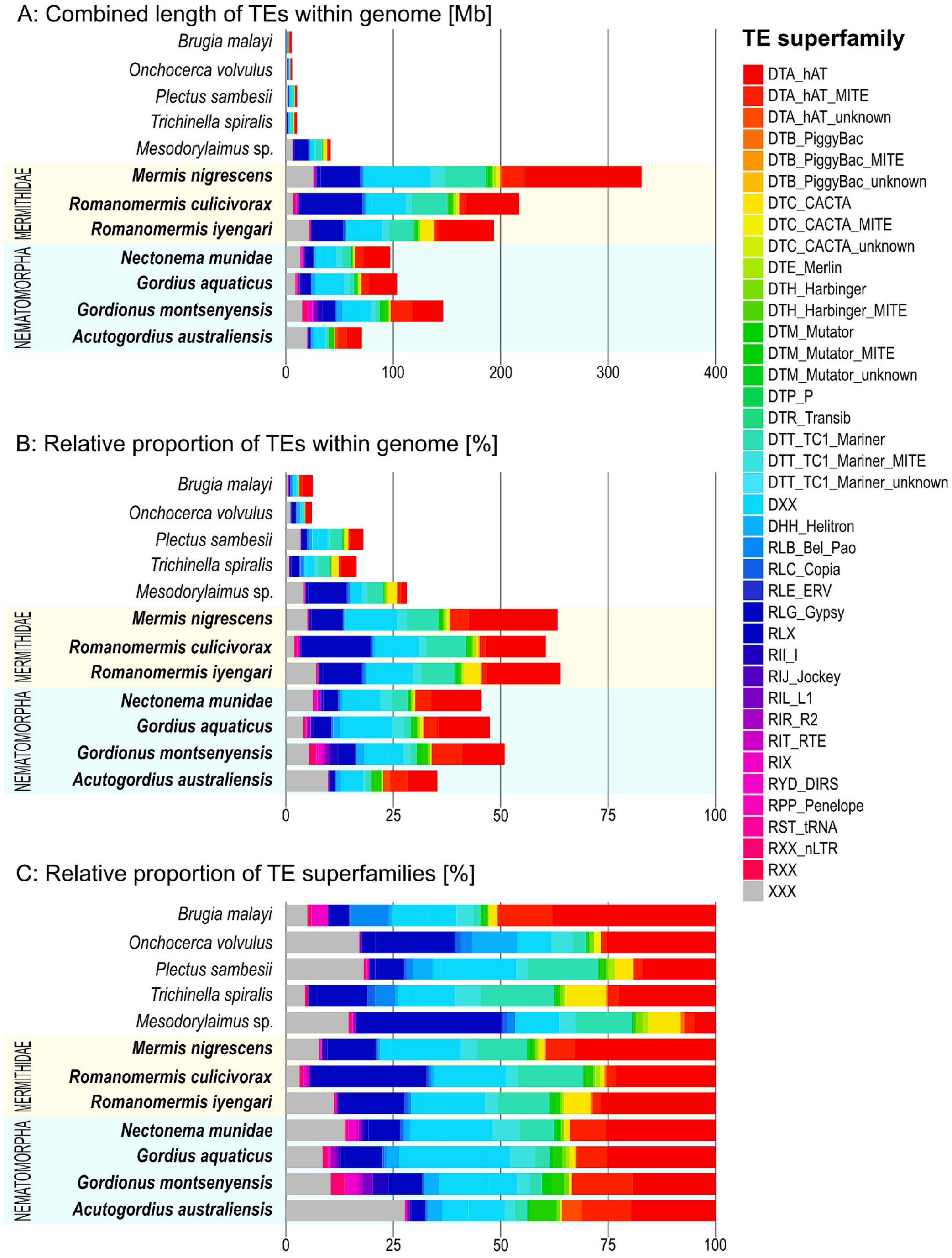

Fig. 5. Stacked barplots show the amounts of transposable elements (TEs) in Mb (A) and as percentage of the genome (B), as well as the relative proportion of TEs (C) within the genomes of species included in the analysis. Legend for TE superfamily (colors) is shown to the right where XXX corresponds to unclassified repeats. Bold font highlights parasitoid nematodes, light yellow background highlights parasitoid Nematoda, while light cyan background highlights parasitoid Nematomorpha.

CRediT authorship contribution statement

Joseph Kirangwa: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Viktoria Bednarski:** Investigation, Formal analysis, Data curation. **Nadège Guiglielmoni:** Investigation, Data curation. **Robert Poulin:** Writing – original draft, Investigation. **Eddy Dowle:** Writing – original draft, Investigation. **Philipp Schiffer:** Project administration, Investigation. **Oleksandr Holovachov:** Writing – review & editing, Writing – original draft, Supervision, Investigation.

Funding

This work was funded by a DFG ENP 434028868 grant awarded to Philipp Schiffer. Nadège Guiglielmoni was supported by the European Union’s Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101110569.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Philipp H. Schiffer reports financial support was provided by Deutsche

Forschungsgemeinschaft. Nadège Guiglielmoni reports financial support was provided by the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant.

Acknowledgments

We would like to thank Professor Dr. Edward Platzer from the University of California Riverside for providing us the culture of *Romanomermis iyengari*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2026.108641.

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