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A genomic survey of *Tc1-mariner* transposons in nematodes suggests extensive horizontal transposon transfer events

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ABSTRACT

The number of reports concerning horizontal transposon transfers (HTT) in metazoan species is considerably increased, alongside with the exponential growth of genomic sequence data However, our understanding of the mechanisms of such phenomenon is still at an early stage. Nematodes constitute an animal phylum successfully adapted to almost every ecosystem and for this reason could potentially contribute to spreading the genetic information through horizontal transfer. To date, few studies describe HTT of nematode retrotransposons. This is due to the lack of annotation of transposable elements in the sequenced nematode genomes, especially DNA transposons, which are acknowledged as the best horizontal travelers among mobile sequences. We have therefore started a survey of DNA transposons and their possible involvement in HTT in sequenced nematode genomes. Here, we describe 83 new *Tc1/mariner* elements distributed in 17 nematode species. Among them, nine families were possibly horizontally transferred between nematodes and the most diverse animal species, including ants as preferred partner of HTT.

The results obtained suggest that HTT events involving nematodes *Tc1/mariner* elements are not uncommon, and that nematodes could have a possible role as transposon reservoir that, in turn, can be redistributed among animal genomes. Overall, this could be relevant to understand how the inter-species genetic flows shape the landscape of genetic variation of organisms inhabiting specific environmental communities.

1. Introduction

Transposable elements (TEs) are repeated DNA sequences able to move actively from one locus to another in the host genome. Virtually all extant living organisms, both prokaryotic and eukaryotic, contain TEs in their genome that can heavily affect gene expression (Chuong et al., 2017; Deniz et al., 2019) and genome organization (Caizzi et al., 2016; Moschetti et al., 2020). This observation has led to the conclusion that TEs are not merely selfish DNA, but rather important elements that significantly contribute to genes and genomes evolution and architecture (Feschotte and Pritham, 2007) (Bire and Rouleux-Bonnin, 2012) (Hirsch and Springer, 2017) (Moschetti et al., 2020).

Eukaryotic TEs are classified into two main classes based on their transposition mechanisms (Wicker et al., 2007). Class I elements reverse transcribe a RNA copy to generate new TE copies that are inserted into a new chromosomal location. Class II elements jump directly from a chromosomal locus to another. Three main different Class II transposition mechanisms are currently known (cut/paste (Yuan and Wessler, 2011), rolling circle replication (Kapitonov and Jurka, 2001), and self-synthesis based (Kapitonov and Jurka, 2006)).

Elements of both classes are grouped into subclasses, super-families, families and subfamilies (Arensburger et al., 2016; Piegu et al., 2015; Wicker et al., 2007). Among Class II elements, *Tc1* and *mariner* are two transposon families that move according to the "cut and paste" model and are the founders of the *IS630-Tc1-mariner* superfamily (Plasterk, 1996; Plasterk et al., 1999). These TEs are known to be widespread in many eukaryotic organisms such as fungi, plants, ciliates and worms, arthropods, and vertebrates (including humans), and in prokaryotes as well.

Elements belonging to the *Tc1* and *mariner* families usually transpose

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using the self-encoded transposase as only enzymatic requirement. The presence of terminal inverted repeats (TIRs) at both transposon's end is essential for the transposon-transposase recognition of Tc1-like elements in the early steps of the transposition (Palazzo et al., 2013; Palazzo et al., 2014). Additional hallmarks of the *Tc1/mariner* are the aminoacidic triad in the catalytic core of the transposase, i.e. DDE (*Tc1* family) or DDD (*mariner* family), and the preference for the TA target site of integration (Shao and Tu, 2001). Eight major groups can be defined within the *Tc1/mariner* superfamily based on the spacing between the last two critical residues in the catalytic domain of the encoded transposase protein (Gao et al., 2017).

The *mariner* family can be further subdivided into five major subfamilies based on their sequence similarities and phylogenetic relationships: mauritiana, cecropia, mellifera/capitata, elegans/briggsae and irritans (Bigot et al., 2005; Robertson and MacLeod, 1993).

The distribution and diversity of active and inactive TEs among living organisms can be justified assuming an appropriate model of their evolution. It is currently assumed that *Tc1/mariner* elements could invade the germline of an organism, through horizontal transmission (Hartl et al., 1997). After a new genomic invasion, TEs increase their copy number following reiterated transposition bursts and, afterwards, they spread through the populations by means of sexual reproduction, i.e. vertical transmission (Bast et al., 2019). During the expansion phase TEs accumulate random mutations, a process called "vertical inactivation", that might lead to the long-term extinction of TEs within a genome or a population (Lohe et al., 1995). During the vertical inactivation process autonomous and non-autonomous elements co-exist in the same genome, and the transposition of non-functional copies (unable to express the transposase but with intact TIRs) might be granted by the *trans*-complementation of functional transposase molecules even by a non-autonomous TE (i.e. with a functional CDS and defective TIRs) (Spradling and Rubin, 1982). In some exceptional instances, non-functional TE copies move by the *in trans*-action of unrelated transposition apparatus (Schmid, 2003) (Moschetti et al., 2004). Under this scenario, the functional to non-functional copies ratio rapidly becomes proximal to zero, envisaging the extinction of a TE. Indeed, when the mutational load progressively inactivates the TE copies in the genome, the transposition rate slows down and, finally, only inactive copies of the transposons persist. The lack of transposition activity, rapidly end up with the elimination of the TE from the host genome, in a process known as stochastic loss (Lohe et al., 1995).

However, TEs often escape vertical extinction by means of horizontal transfer (HT) from a host species to a different, reproductively isolated species. In this way, the life cycle of TEs starts over in a new genomic environment (Schaack et al., 2010).

Horizontal transfer (HT) consists in the passage of genetic material between organisms independently from the parent-to-offspring transmission. This phenomenon is primarily associated with prokaryotes, which can easily and directly exchange genetic material both in wild and in laboratory conditions (Koonin et al., 2001). In Eukaryotes this process may require the aid of a vector that could be represented by a viruses or a parasites (Lohe et al., 1995; Miskey et al., 2005; Sinzelle et al., 2006). A eukaryotic HT process, involving TEs is called Horizontal Transposon Transfer (HTT). Several studies contributed to the understanding of this process and to elucidating the importance of HTT in the evolutionarily dynamics of TEs and genomes. On the basis of HTT available data, it has been postulated that insect Class II elements are more prone to jump between species than Class I elements, although the latter are more similar in structure to the retroviruses (Gilbert and Feschotte, 2018; Schaack et al., 2010). However, one of the most obscure aspect of eukaryotic HT and HTT remains the vector-mediated passage of the genetic information, whose snapshot capturing and experimental reproducibility remain prohibitive both in the wild and in laboratory conditions (Schaack et al., 2010). In this view, it could be hypothesized that class of organisms displaying pervasive presence in many ecosystems play a critical role in this hard-to-understand mechanism. In this respect, Nematodes have colonized nearly all ecosystems, and the parasitic lifestyle of many of them could justify their contribution in exchanging genetic material, either directly or indirectly with their hosts.

More than 100 nematode genomes have been sequenced so far (https://www.ncbi.nlm.nih.gov/genome, last accessed September 2020), although their genes and repeats are still poorly or not annotated in many of them. Nematoda is considered one of the largest animal phyla on Earth, comprising more than 1 million species, 25,000 of which have been characterized so far (Zhang, 2013). They are classified in five major clades on the basis of morphological and evolutionary criteria (Blaxter et al., 1998). The relatively compactness of nematodes' genome, ranging from 50 to 600 Mbp (Coghlan, 2005) (Coghlan et al., 2019) allows to easily producing sequence data, although the high AT content (Mitreva et al., 2005) and the amount of satellite DNA (Subirana and Messeguer, 2013) are sometimes challenging in generating the sequencing data in many nematodes species.

Many different methods can be applied to identify new TEs in unexplored genomes. The molecular identification is rather time-consuming, especially if many different elements are to be characterized. De novo TE detection and annotation by bioinformatics approaches can be also challenging (Ou et al., 2019), but fast and cost-effective bioinformatics pipelines based on homology search make this task easier. In the absence of simple tools based on structural comparison, like those available for retrotransposons search (Marsano et al., 2012; McCarthy and McDonald, 2003) sequence similarity-based search strategies remain valid approaches to detect mariner transposons in sequenced genomes.

In the present work, we describe the features of 83 new *Tc1/mariner* elements in the genome of 17 nematodes and their relationships with known element of the *Tc1/mariner* superfamily. Furthermore, we present evidence of putative horizontal transfer events that could have occurred between nematodes and the most diverse non-nematode species.

2. Materials and method

2.1. Database search strategy and characterization of TEs

We used a TBLASTX approach to search *Tc1/mariner* elements in nematode genomes. This is a time-saving strategy that allow using the whole DNA queries sequences to search for similar sequences in nematode genomes without extracting coding sequences from the queries. Furthermore, since we used multiple queries retrieved from RepBase, TBLASTX allowed us to use inactive elements (i.e. containing nonsense and frameshift mutations) as queries, avoiding underestimation of the output.

We used a dataset composed of 1718 DNA sequences extracted from RepBase (http://www.girinst.org/repbase/) using "mariner" as search keyword (accessed July 2017). to query 100 nematodes' genome assemblies available on the NCBI genome database (last access to Database January 2017). Since alternative genome assemblies of the same nematode species (if available) were included in the analysis, we analyzed 83 total nematode species (additional details are available in Supplementary Table 1). E value $< 10^{-60}$ and alignment length > 100 bp (roughly equivalent to 50% protein sequence similarity) were taken as criteria to select significant results. The TBLASTX analysis was performed using the remote Blast tool using the following command line: tblastx -remote -db nr -query queryfile -entrez_query "Nematodes[Organism]" -evalue 1e-60 -outfmt 7 -out file.tblastxout. The output was manually inspected, resulting in a set of 8,308 hits in 26 nematode species (obtained from 324 TE queries out of the 1718). Sequences identified in the same genome were clustered on the basis of their sequence identity (>90% identity, over a length > 100 bp) (Flutre et al., 2011; Wicker et al., 2018) using BLASTN. This step allowed the definition of TEs within species in this study and simplified the initial output. Hits sharing > 90%

nucleotide identity with annotated nematode transposon sequences were not considered as new in this study. Our final dataset consists of 101 previously not described nematode TEs, whose features are listed in Supplementary Table 2 Names were assigned according to the Repbase nomenclature. The copy number per genome for each TE was determined using BLASTN against the genome they belong to.

Open Reading Frames (ORFs) were identified with ORFinder (https://www.ncbi.nlm.nih.gov/orffinder/) and verified for the presence of both the canonical transposase domains (DD35E,) and the DNA Binding Domains with the aid of CD-Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Reconstruction of ORFs was performed when multiple copies (>10) were available using DAMBE7 (Xia, 2018).

EINVERTED (http://bioinfo.nhri.org.tw/cgi-bin/emboss/einverted) was used to identify inverted repeat sequences bracketing the transposase gene.

Matches to annotated TEs were identified using the "CENSOR" tool (Kohany et al., 2006) implementing Repeatmasker in RepBase (Jurka et al., 2005) using the default parameters and searching against the whole database.

Orthologous genes in different species were searched using TBLASTX. Protein sequences annotated in the closest species were used as queries to search organism-specific mRNA databases or, alternatively, organism-specific WGS databases. In the latter case, exon–intron structure was inferred from the TBLASTN output with the additional support of GENEWISE (Emboss). DotPlot analyses and sequence manipulation were performed using the DNA Strider editor (version 1.5a8) (Marck, 1988).

2.2. Evolutionary analyses

The 18S RNA genes were used to reconstruct the phylogenetic tree of the nematodes, as previously reported (Coghlan et al., 2019; Szitenberg et al., 2016). The transposase genes or the COI genes were used to compare the evolution of TEs and host species. Alignments were performed using MUSCLE (Edgar, 2004) implemented at the http://www.phylogeny.fr/ website using the default parameters. jModelTest v2.1.10 (Darriba et al., 2012; Posada and Buckley, 2004) was used to select the best evolutionary model that fitted adequately the input sequence data. The Bayesian phylogenetic trees were obtained using MrBayes 3.2 (Ronquist et al.). The Markov Chain Monte Carlo (MCMC) was run for $10x10^6$ generations and sampled every 1000 generation with a burn-in fraction of 25%. Phylogenetic trees were visualized using the FigTree 1.4.4 software (http://treebioedacuk/software/figtree/) and the posterior probability was used as a statistical support for each branch.

The phylogenetic relationships among the nematode TEs identified in this study were inferred using the NJ method at the www.phylogeny.fr webserver (Dereeper et al., 2008). Multiple sequence alignments of the transposase protein sequences were performed with Multalin using the Blosum62 matrix and manually trimmed at both alignment ends to remove poorly aligned transposase regions. Statistical significance of branches was evaluated with the Bootstrap method (100 replicates). Reference sequences of *Tc1/mariner* elements used to aid the classification of nematode elements are listed in Supplementary Table 3. Pairwise distances between animal species included in this study were calculated using MEGA 7 (Kumar et al., 2016) and the pairs of orthologous genes reported in Supplementary table 4. Multiple alignment files used to generate phylogenetic trees in this study are provided in Supplementary Files 1–4.

For the genetic distance comparison analyses multiple sequence alignments of the coding sequences were performed with Multalin using the DNA 5–0 matrix. Codon alignments were built using Codon Alignment v2.1.0 (https://www.hiv.lanl.gov/content/sequence/CodonAlign/codonalign.html). In-frame stop codons were removed to restore the transposase-encoding ORF. The corresponding gaps generated in the multiple alignments were not taken into account (pairwise deletion method).

Kimura-2 Parameters genetics distances were calculated using MEGA (version 7.0.26) (Kumar et al., 2016).

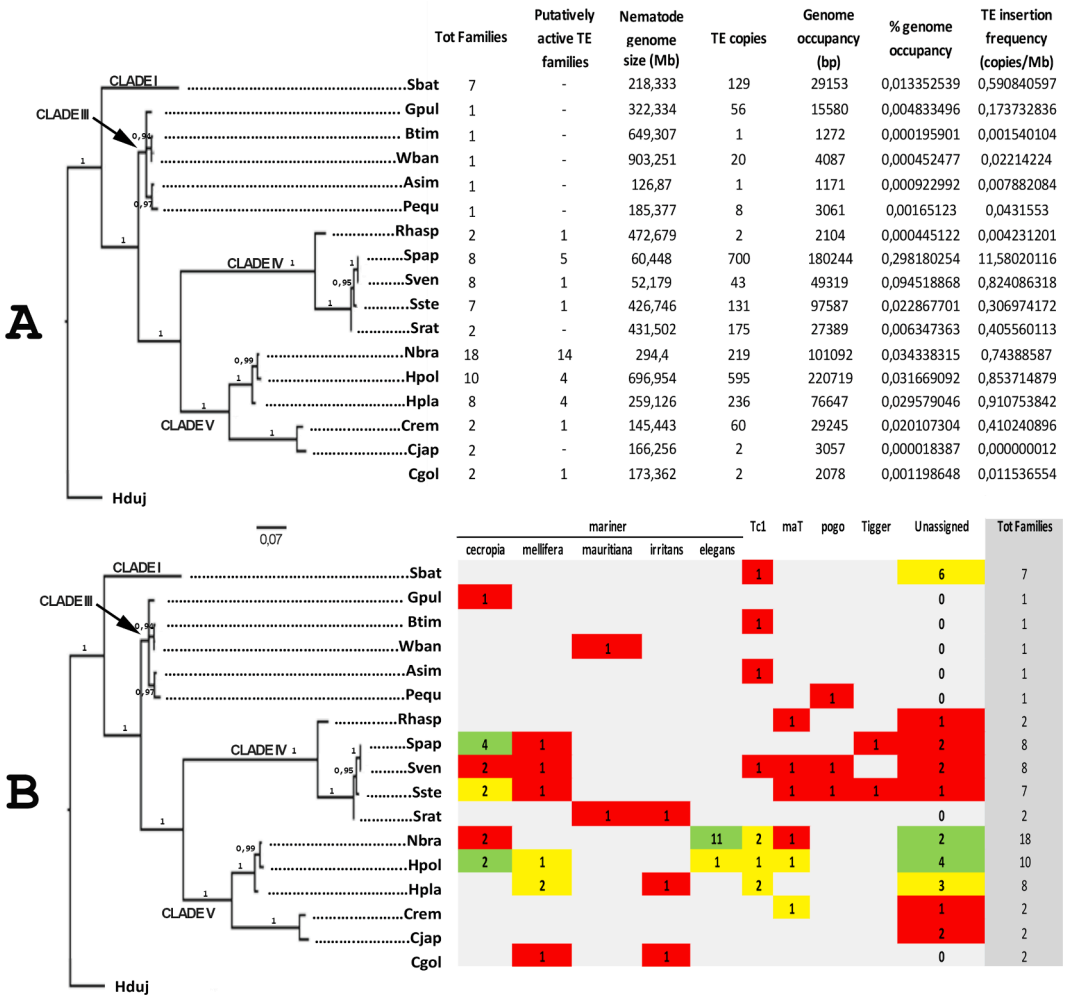
Overall genomic nucleotide identity between species included in this study was calculated using BLASTN. Alignments shorter than 80 nucleotides were filtered out from the output to avoid underestimation of the results.

3. Results

3.1. Identification of new Tc1/mariner like elements in nematodes

We used a BLAST-based approach to find new *Tc1/mariner* elements in sequenced nematode genomes. Similar approaches were successfully applied to identify *Tc1/mariner* elements in previous works from several groups (Gao et al., 2017; Palazzo et al., 2016; Wallau et al., 2014) (Bouallègue et al., 2017). A set of *mariner*-like sequences annotated in RepBase (see Materials and Methods) were used in a TBLASTX search against 100 genome assemblies corresponding to 83 nematode species (Supplementary Table 1). 8,308 significant hits (see Methods section) obtained from 324 TE queries in 26 nematode species. This output was subjected to within-species clustering allowing the definition of TEs in this study (i.e. sequences belonging to the same genome that share > 90% nucleotide identity). The final dataset obtained consists of 101 TE sequences (listed in Supplementary Table 2). A comparison with the GenBank and Repbase databases led us to conclude that 83 TEs (Supplementary Table 2 and Supplementary File 5), distributed in 17 nematode species are putatively new TEs. TEs lacking flanking sequences or essential structural features of *Tc1/mariner* elements (such as. the presence of transposase-encoded domains,) were regarded as possible false positives.

In order to infer the functional status (i.e. transposition competency) of the new TEs identified, we first evaluated the presence of ORFs encoding a putative transposase, containing both the DNA binding and the catalytic domains, and the presence of TIRs flanking the transposase-coding gene. The presence of the functional domains of the transposase was detected in many of the analyzed elements (Supplementary File 6). Furthermore, in order to assess the transposition competency of TEs the correct spacing between the catalytic aminoacid triad (DDD or DDE (Shao and Tu, 2001)) of the transposase was verified by visual inspection of the transposase proteins alignment (not shown). Additionally, the presence of TIRs flanking the transposase gene was assumed as a criterion to establish whether an element is potentially active or not, since TIRs are crucial to the transposase-transposon recognition. All the above-described features are summarized in Supplementary Table 2. Considering these criteria, we inferred the presence of thirty-two potentially active nematode TEs distributed in 9 nematode species. The results of the survey are summarized in Fig. 1A. *Tc1/mariner*-like TEs were found in species belonging to four (out of five) nematode clades. From a genomic perspective, the impact of the *Tc1/mariner* elements in the genome of the respective nematode species does not appear to be related to any particular feature of the nematodes, such as parasitism or mating system. As a general trend, nematode genomes contain a small number of DNA transposons if compared with vertebrate genomes (Sotero-Caio et al., 2017), but with a similar genomic coverage (Gao et al., 2017). In addition, while some nematode species host functional and non-functional families (*Strongiloydes stercoralis, Haemonchus placei, Strongyloides papillosus, Strongyloides ratti, Caenorhabditis remanei, Nippostrongylus brasiliensis, Heligmosomoides polygirus, Strongyloides venezualensis, Rhabditophanes* sp. *KR3021* and *Cylicostephanus goldi*), other species contain only non-functional *mariner* TEs (*Soboliphyme baturini, Brugia. timori, Wuchereria bancrofti, Gongylonema pulchrum, Anisakis simplex, Parascaris equorum*). The copy number of the nematode *mariner*-like elements per genome could be also extremely variable, ranging from single (e.g. *Mariner-2_Sste*) to hundreds of copies



	Tot Families	Putatively active TE families	Nematode genome size (Mb)	TE copies	Genome occupancy (bp)	% genome occupancy	TE insertion frequency (copies/Mb)
Sbat	7	-	218,333	129	29153	0,013352539	0,590840597
Gpul	1	-	322,334	56	15580	0,004833496	0,173732836
Btim	1	-	649,307	1	1272	0,000195901	0,001540104
Wban	1	-	903,251	20	4087	0,000452477	0,02214224
Asim	1	-	126,87	1	1171	0,000922992	0,007882084
Pequ	1	-	185,377	8	3061	0,00165123	0,0431553
Rhasp	2	1	472,679	2	2104	0,000445122	0,004231201
Spap	8	5	60,448	700	180244	0,298180254	11,58020116
Sven	8	1	52,179	43	49319	0,094518868	0,824086318
Sste	7	1	426,746	131	97587	0,022867701	0,306974172
Srat	2	-	431,502	175	27389	0,006347363	0,405560113
Nbra	18	14	294,4	219	101092	0,034338315	0,74388587
Hpol	10	4	696,954	595	220719	0,031669092	0,853714879
Hpla	8	4	259,126	236	76647	0,029579046	0,910753842
Crem	2	1	145,443	60	29245	0,020107304	0,410240896
Cjap	2	-	166,256	2	3057	0,000018387	0,000000012
Cgol	2	1	173,362	2	2078	0,001198648	0,011536554

Fig. 1. Overview of the transposable element types, loads, and distribution, in the genomes of nematodes. Bayesian phylogenetic tree of the nematode species into which TEs have been found in this work. The tree was inferred using the 18S RNA sequences under the GTR + G + I evolutionary model. A) The TE loads in each nematode genome is summarized along the nematode tree. B) The distribution of the new TEs described in this work among different *Tc1/mariner* subfamilies is described along the same tree reported in panel A. Note that *C. goldi* was not included in the phylogenetic tree due to the lack of annotated 18S RNA gene. However, we have positioned *C. goldi* near other members of Clade V (Coghlan et al., 2019). The 18S RNA sequence of the tardigrade *Hypsibius dujardini* was used as outgroup (Szitenberg et al., 2016). The posterior probability values > 80% are shown at branches.

(*Mariner-10_Nbra*). As discussed below, the amplification status, together with the intactness of the elements identified could be indicative of a particular stage of the TE life in a given genome.

3.2. Phylogenetic relationships between the nematode elements

We determined the phylogenetic relationships of the new TEs with known elements of the *IS630/Tc1/mariner* superfamily. This analysis allowed the classification into specific subfamilies results. The results are summarized in Fig. 1B and shown in details in Fig. 2. The conceptual translations of the transposase genes of either representative or reconstructed elements were used to build up a multiple alignment. 18 mariner elements belonging to the 5 described *mariner* sub-families, 3 *Tc1-like* elements, 2 *IS630*-like elements of the *IS630* family, the *pogo* element and the *Tigger1* element were used as reference (see Supplementary Table 3) to classify the new nematode elements into one of the known *mariner* sub-families or into the *Tc1-maT* clade. As can be observed in Fig. 2, 14 elements in the tree fall within the *Tc1-maT* clade, 4 elements are included in the *pogo* and *Tigger* clades, whereas the vast majority are *mariner*-like elements. It appears relevant that the genome of *N. brasiliensis* (294.4 Mbp) contains 14 *mariner* families and 4 *Tc1/maT* families, resulting in the highest variability among the nematodes analyzed in this study (Fig. 1, panels A and B). Moreover, *N. brasiliensis* displays the highest ratio of potentially active elements over total families (~79%) among the nematodes analyzed. Notably, the genome of *S. papillosus,* which is one of the smallest in size among the species analyzed in this study, contains 8 different elements (Supplementary Table 2 and Fig. 1B) accounting for over 700 copies in this genome (Fig. 1A), thus resulting in the genome with the highest insertion frequency (~11,6 copies/Mb).

Finally, the genomes of *W. bancrofti, S. ratti, A. simplex, B. timori, C. goldi, P. equorum* bear few families (2 or less) and, overall, no more than 10 copies of *Tc1/mariner* TEs.

In conclusion, the above described analyses show that most of the *mariner*-like elements identified in this study are distributed among five *mariner* families, and many of them are potentially active in nematodes.

3.3. Inference of putative horizontal transposon transfer events

Using the "CENSOR" tool (Kohany et al.) implemented in the Repbase database, we noticed an unexpected high identity in some of the DNA pairwise comparisons between TEs of unrelated species. A striking example is *Mariner-2_Sste*, from *S stercoralis*, which displays 86% identity at the DNA level with the *Mariner-3_AEc* element of the ant

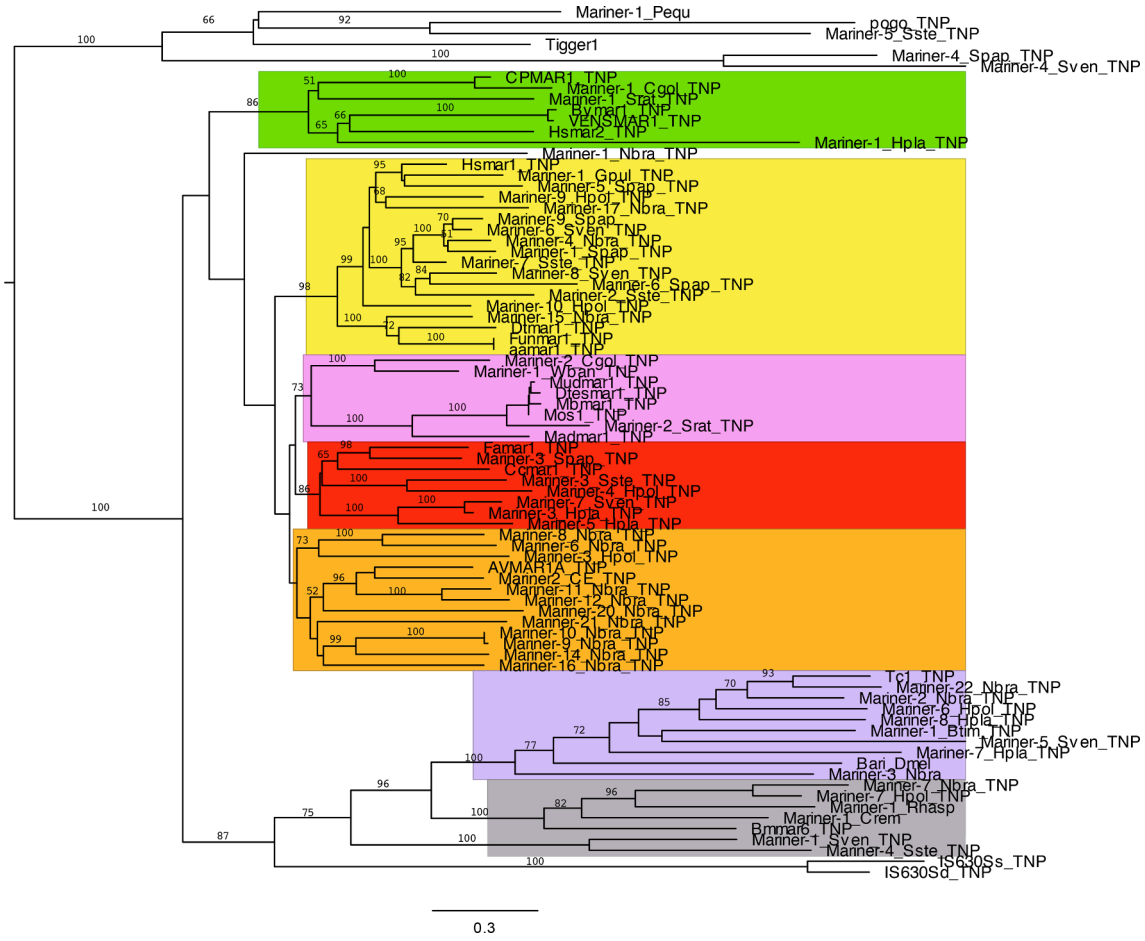


Fig. 2. Phylogenetic relationships of the *Tc1/mariner* elements identified in this study. NJ tree based on the multiple alignment of the transposase protein sequences. 57 new *Tc1/mariner* elements identified in nematodes are marked with *. Color background marks the five major *mariner* groups (red: mellifera; purple: mauritiana; orange: elegans; yellow: cecropia; green: irritans) and *Tc1-maT* (gray: maT; lilac: Tc1). Bootstrap values > 0,5 are displayed at the branches. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A. echinatior (see Table 1 for other matches). These observations prompted us to start a systematic search for horizontal transfer events involving nematode transposons.

We extensively searched for highly similar DNA sequences against the genomic databases at the NCBI in distantly related non-nematode genomes, using the elements listed in Supplementary Table 2 as query sequences. The top-ranking results, i.e. lowest E-value and higher score over longest alignment length, are shown in Table 1. The reported matches detected display at least 80% nucleotide identity, which exceeds the expected identity values when comparing transposable elements in distant species.

Surprisingly, in some instances the extent of sequence identity was observed through the entire transposon sequence, including the TIRs, an observation that strongly supports the hypothesis of horizontal transposon transfer. A representative example is shown in Fig. 3. The TIRs of *Mariner-2_Sste* are nearly identical (26/28 bp and 30/30 bp identical nucleotides at the 5′TIR and 3′TIR respectively) to that of the *Trachymyrmex cornetzi* (an ant species) element identified by BLAST search. Furthermore, there is an extensive identity in the intervening DNA region containing the transposase gene resulting in a global identity>83% (Fig. 3). By contrast, the DNA sequences flanking both elements are completely unrelated, excluding the possibility of sequence contamination related artifactual results.

We have further investigated if the presence of very similar *mariner*-like elements in the genomes of distant species (Table 1) could be the result of horizontal transfer events.

We first compared the transposons' tree (based on the transposase gene) with the species' tree (based on the COI barcode region) to highlight phylogenetic incongruences that result from HTT (Fig. 4). This comparison clearly shows that while the nematode *mariner*-like elements cluster together with their non-nematode homologues (Fig. 4A), in the species tree the nematodes and non-nematodes species cluster apart (Fig. 4B). The uneven distribution of the taxa in the transposon tree is assumed as a strong evidence of HTT (Boto, 2010).

To further assess that the nucleotide identity observed between nematode and non-nematode *mariner*-like elements was due to HTT, we next compared the mean genetic distance of non-mobile orthologous genes (Supplementary Table 4) to the genetic distance of the transposase genes in couples of organisms in which highly similar TEs have been found. Assuming a Gaussian distribution of the pairwise genetic distances, and considering an exclusion range limit of $\mu \pm 3\sigma$ (the expected population fraction below the μ-3σ limit is < 0,13%) with respect of such distribution an HTT event could be hypothesized if the TEs genetic distance falls outside the exclusion range.

As shown in Fig. 5, among the putative HTT cases involving 9 nematode transposons, the genetic distances of *Mariner-1_Pequ* (Fig. 5, panel H) with the respective orthologous transposon, are comprised within the considered limits of μ–3σ in all the comparisons made (namely against *Apteryx australis*, *Crocodylus porosus*, and *Gavialis gangeticus*), suggesting that the observed DNA identity is probably not due to horizontal transfer, at least considering the adopted criteria. Conversely, the Kimura-2 Parameters (K2P) distance of *Mariner-1_H*pla (Fig. 5, panel A), *Mariner-5_Sven* (Fig. 5, panel F), *Mariner-6_Hpla* (Fig. 5, panel G) and *Mariner-4_Sbat* (Fig. 5, panel I) between the most similar

Table 1
Predicted HTT events involving *Tc1/mariner* elements of nematodes.

Nematode species	Nematode element	Target species element	Target accession	Relevant alignment parameters			
				% identity	Alignment length	E-value	bit score
Strongyloides stercoralis	**Mariner-2_Sste**	**M. ruginodis**	X73313.1	84,657	1297	0.0	1264
		T. cornetzi	LKEY01019312.1	84,562	1302	0.0	1279
		T. septentrionalis	LKEZ01037515.1	84,568	1296	0.0	1273
		T. zeteki	LKFA01010087.1	84,361	1298	0.0	1258
		L. humile	ADOQ01008464.1	84,206	1298	0.0	1251
		A. echinatior	AEVX01005702.1	83,994	1312	0.0	1240
		H. saltator	AEAC01008466.1	83,243	1295	0.0	1182
		C. costatus	LKEX01017719.1	83,231	1300	0.0	1177
		A. colombica	LKEW01019396.1	83,282	1292	0.0	1175
		M. pharaonis	BBSX02013245.1	83,765	1158	0.0	1088
		S. invicta	AEAQ01009973.1	80,335	1312	0.0	974
	Mariner-6_Sste	**D. quadriceps**	JPHR01023093.1	81,420	1803	0.0	1459
		A. rosae	AOFN01004550.1	81,152	1146	0.0	900
		N. vespilloides	LJCH01002934.1	80,332	1266	0.0	905
		E. mexicana	LLKC01012681.1	80,237	2196	0.0	1613
		D. novaeangliae	LGHO01002810.1	79,569	2183	0.0	1522
		S. mimosarum	AZAQ01079607.1	78,830	1965	0.0	1290
		C. lectularius	JHUN01035419.1	80,000	1560	0.0	1144
		S. mediterranea	AUVC01115618.1	78,894	1592	0.0	1062
		L. hesperus	JJRX01348035.1	75,975	2052	0.0	989
		P. tepidariorum	AOMJ01182454.1	79,107	1321	0.0	859
Heligmosomoides polygyrus	**Mariner-1_Hpla**	**A. glabripennis**	AQHT01016807.1	83,359	1304	0.0	1160
		L. decemlineata	AYNB01038757.1	81,544	1295	0.0	1018
	Mariner-6_Hpla	**H. vulgaris**	ABRM01014025.1	89,223	1262	0.0	1570
Soboliphyme baturini	**Mariner-4_Sbat**	**D. novaeangliae**	LGHO01000243.1	84,919	2102	0.0	2076
		M. martensii	AYEL01069060.1	83,881	1551	0.0	1445
		C. exilicauda	AXZI01159886.1	81,968	1392	0.0	1138
	Mariner-7_Sbat	**S. zaharoni**	LDMZ01020256.1	84,692	1104	0.0	1085
		X. maculatus	AGAJ01023144.1	83,619	1166	0.0	1068
		A. citrinellus	CCOE01001708.1	83,779	1122	0.0	1046
Wuchereria bancrofti	**Mariner-1_Wban**	**A. reticulatus**	AB056894.1	86,331	695	0.0	752
		D. suzukii	AWUT01009455.1	85,755	695	0.0	730
		D. ficusphila	AFFG02008209.1	85,755	695	0.0	730
		A. planipennis	JENH01067712.1	84,339	696	0.0	678
		A. tumida	MRBJ01000042.1	83,761	702	0.0	664
Parascaris equorum	**Mariner-1_Pequ**	**A. australis**	LK065141.1	83,565	937	0.0	852
		G. gangeticus	XM_019519190.1	82,997	941	0.0	821
		C. porosus	MDVP01000001.1	80,896	937	0.0	697
Strongyloides venezuelensis	**Mariner-5_Sven**	**L. polyphemus**	AZTN01137836.1	85,386	958	0.0	987

The two species involved in the putative HTTs are designated as Nematode species and target species. Relevant alignment parameters are reported for each pair of elements compared.

elements identified in the compared species, are significantly different from the average values of the vertically inherited coding sequences.

The analysis performed on *Mariner-1_Wban*, *Mariner-6_Sste*, *Mariner-2_Sste*, *Mariner-7_Sbat* (Fig. 5, panels B, C, D, E respectively) reveal that not for all species the K2P distance of transposases fall outside the limit of μ–3σ, suggesting that a more limited range of species was involved in HTT.

To provide stronger evidence of HTT events among the pairs of taxa described in Table 1 and Fig. 5 we further tested the HTT hypothesis using a genome-wide comparison approach. The available genomes assemblies of the species involved in putative HTT events were compared using BLASTN. The identity scores and the length of the alignments were then compared to the same parameters obtained from the BLAST comparison between *mariner* elements. Assuming a vertical transmission, we expect that the alignment quality (i.e. length and score of the alignment) of the aligned *mariner* elements fall within the range of the distribution of the respective genome pairwise comparison. Conversely, we expected that horizontally transferred sequences fall outside the distribution obtained from genomic comparison (i.e. having alignment length score significantly greater than that of genomic comparison).

In this respect, the results obtained, and summarized in Table 2, mirror those obtained from the genetic distance comparison, demonstrating that the *mariner* elements analyzed are "alien" sequences in one or the other genomes, since the observed alignment parameter of the transposase largely exceed the average identity of the respective (masked) genomes.

4. Discussion

In this work we have investigated the abundance, diversity, the distribution, and the structural organization of *Tc1/marine*r transposons in the genomes of nematodes, a large group of animals widely distributed across many ecosystems. Our analyses revealed the presence of new (both inactive and potentially active) elements in nematodes, as well as potential HTT events.

A first observation resulting from our study concerns the detection of *Tc1/mariner* elements in only 26 out of 83 nematode species analyzed. This could be explained considering either 1) the low quality of some genome assemblies, 2) a high degree of sequence divergence of the *Tc1/mariner* elements in those nematode species, or 3) the actual absence of *Tc1/mariner* elements in a given species. In our final dataset, we identified TEs in *C. briggsae* and *C. elegans*, *M. chiwoodi*, *M. javanica, M. incognita, H. bacteriophora, T. colubriformis* annotated in the Repbase and GenBank databases which constitutes a good internal control of our experimental strategy. Since we did not used extremely stringent search criteria (see Methods), and we have detected *Tc1/mariner* elements even in low quality assemblies (i.see N50 and BUSCO values in Supplementary Table 1), the apparent lack of *mariner*-like TEs in 57 out of the 83 species analyzed could be probably explained with the real lack of *mariner*-like elements in some nematode genomes. This situation

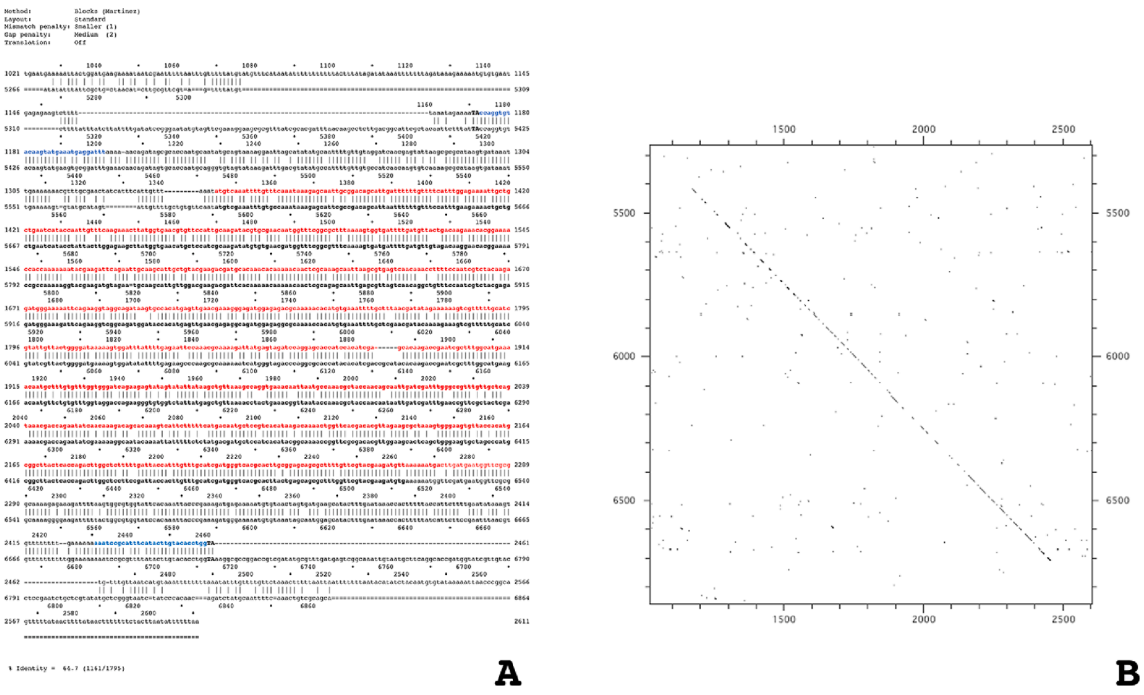


Fig. 3. Striking DNA identity observed between two distantly related mariner transposons. A) Global alignment obtained with DNA Strider (Blocks method) showing the high-level of conservation between the *Mariner-2_Sste* element and the contig LKEY01019312.1 of *Trachymyrmex cornetzi*. 150 bp of sequences flanking the elements upstream and downstream were included in the alignment to highlight the lack of identity in the flanking sequences. The two *mariner* elements, excluding the flanking regions, share > 83% DNA identity (not shown). The TIRs of *Mariner-2_Sste* are shown in blue, its coding region in red and the duplicated target sites (TA) are in uppercases. B) DotPlot alignment (window = 7; stringency = 7) of the same sequences showed in A. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

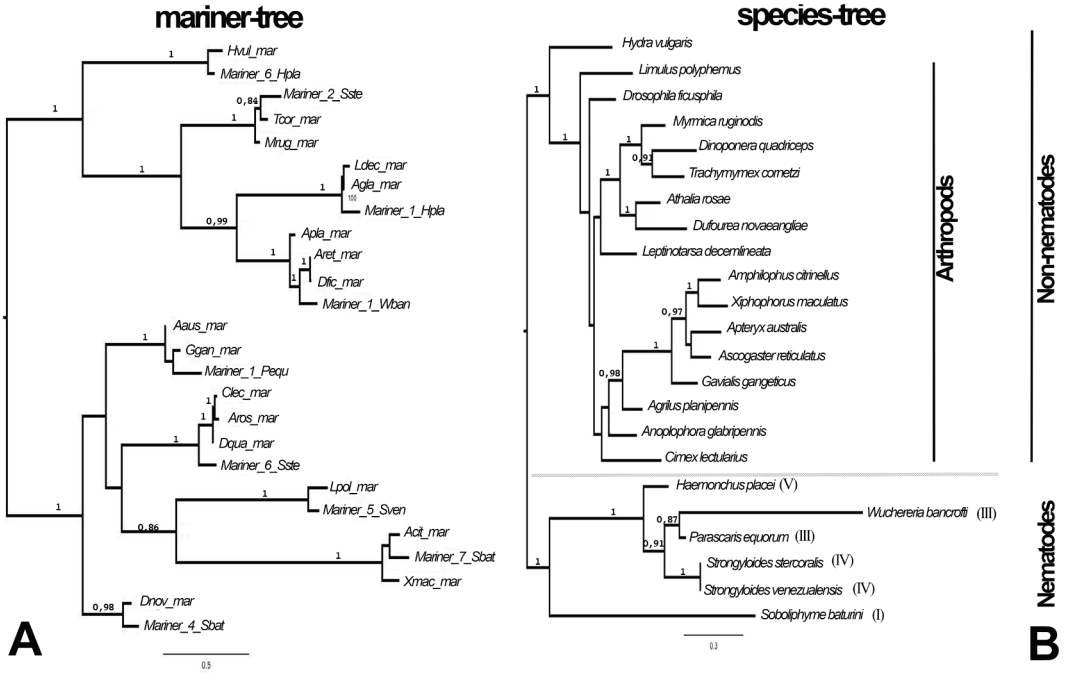


Fig. 4. Comparison of the transposase tree and the species trees The phylogenetic trees based on multiple alignment of nucleotide sequences are compared. A) Bayesian tree of the transposase genes of the TEs putatively involved in HTT events (TIM3 + G evolutionary model). B) Bayesian tree of the mitochondrial COI genes of the species involved in putative HTT events (GTR + I + G evolutionary model). The posterior probability values > 80% are shown at branches. Numbers in brackets indicate the respective nematode clade (see also Supplementary table 1).

appears possible when looking at pair of closely related species with opposite quality of the genome assemblies (eg. B*rugia timori* and *B. pahangi*), where *Tc1/mariner*-like elements have been identified only in the species associated with low quality assembly (*B. timori*). However, we cannot exclude that the quality of genome assembly could affect the identification of new TEs. Indeed, at the time of our search, in 2016, we

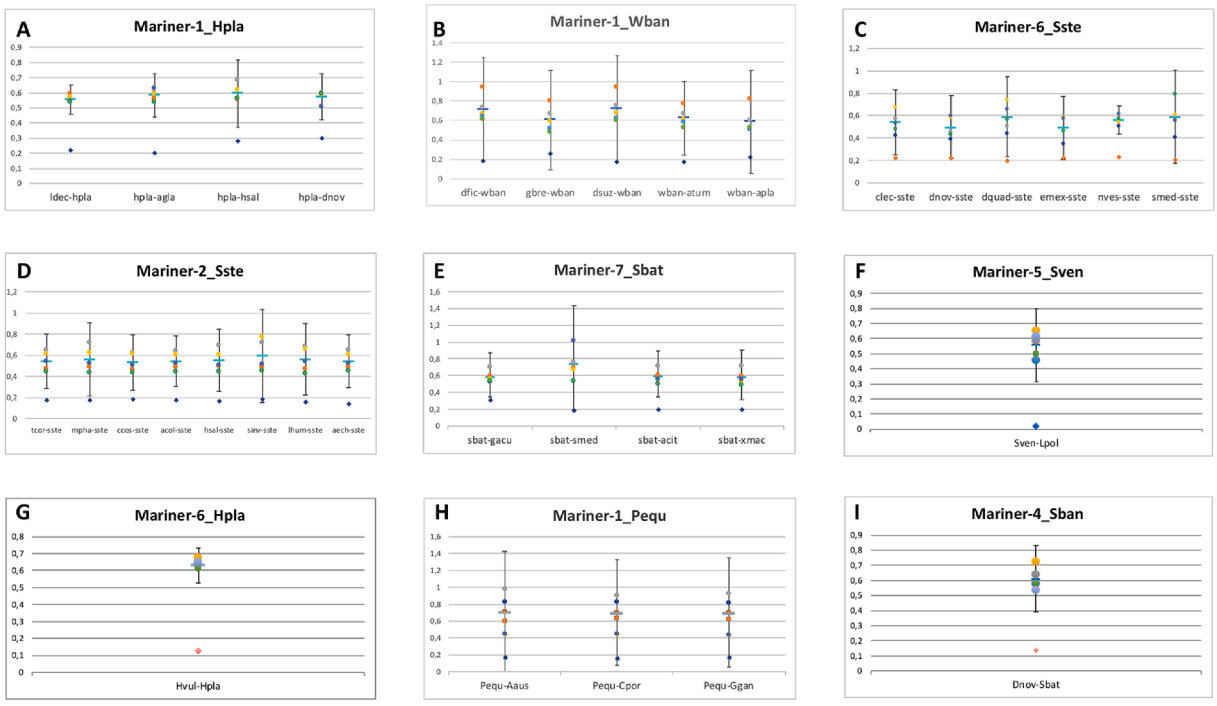


Fig. 5. HTT inference through K2P distance analysis. Comparison of the Kimura-2 Parameters (X axes) distances of the CDS encoding vertically inherited genes (colored circles) and putative horizontally transferred transposase genes (colored diamonds). The species compared in each panel are reported in Table 1. Bars represent the K2P distance mean values of vertically transmitted genes. Error bars represent 3X the Standard Deviation.

Table 2
Evaluation of the genomic nucleotide identity between pairs of taxa (here designated as Nematode and Partner) potentially involved in HTT and the relative pairs of mariner elements. The relevant alignment parameters (score highlighted in pink and length highlighted in blue) are shown. The values compared (3XSD are in red types). N: number of alignments; mean: mean score or length; SD: standard deviation; max: maximum score or length value; min: minimum score or length value.

		Genomic alignment scores						Genomic alignment lengths					mariner alignment			
NEMATODE	PARTNER IN HTT	n	mean	SD	mean+3XSD	max	min	mean	SD	mean+3XSD	max	min	score	length	NEMATODE TE	PARTNER TE
S. stercoralis	T. cornetzi	393	99,04	53,01	258,06	452	50,1	159,30	103,13	468,68	785	81	807,00	1231,00	Mariner-2_Sste	LKEY01019312.1
S. stercoralis	L. humile	249	84,54	35,39	190,71	357	50,1	157,03	98,83	453,51	690	81	823,00	1057,00	Mariner-2_Sste	ADOQ01008464.1
S. stercoralis	A. echinatior	471	115,70	86,51	375,23	490	46,1	188,35	142,15	614,82	696	81	781,00	1069,00	Mariner-2_Sste	AEVX01005702.1
S. stercoralis	H. saltator	284	121,68	130,81	514,12	846	48,1	185,30	152,37	642,40	878	81	815,00	1079,00	Mariner-2_Sste	AEAC01008466.1
S. stercoralis	C. costatus	356	89,40	45,93	227,20	391	50,1	156,46	102,15	462,90	785	81	789,00	1058,00	Mariner-2_Sste	LKEX01017719.1
S. stercoralis	A. colombica	512	86,43	32,02	182,50	321	50,1	150,09	93,30	429,99	785	81	751,00	1041,00	Mariner-2_Sste	LKEW01019396.1
S. stercoralis	M. pharaonis	279	84,70	41,37	208,80	343	44,1	155,08	109,33	483,06	792	81	368,50	474,00	Mariner-2_Sste	BBSX02013245.1
S. stercoralis	S. invicta	281	88,02	40,67	210,04	280	46,1	155,26	105,65	472,22	701	81	632,00	1013,00	Mariner-2_Sste	AEAQ01009973.1
S. stercoralis	C. lectularius	436	87,95	37,71	201,08	311	52	142,35	78,54	377,96	872	81	311,40	575,50	Mariner-6_Sste	JHUN01035419.1
S. stercoralis	E. mexicana	914	92,16	49,37	240,26	494	42,1	156,13	105,40	472,35	984	81	304,33	596,67	Mariner-6_Sste	LLKC01012681.1
S. stercoralis	D. novaeangliae	518	100,59	85,17	356,10	842	48,1	166,71	143,41	596,94	1188	81	279,33	615,67	Mariner-6_Sste	LGHO01002810.1
S. stercoralis	S. mediterranea	3229	120,45	56,83	290,94	575	46,1	155,08	130,22	545,74	1303	81	410,00	1112,00	Mariner-6_Sste	AUVC01115618.1
S. stercoralis	N. vespilloides	540	98,75	45,54	235,37	432	50,1	169,19	119,06	526,36	789	81	267,00	465,50	Mariner-6_Sste	LJCH01002934.1
S. stercoralis	A. rosae	398	90,29	50,18	240,83	440	50,1	158,06	107,85	481,62	1146	81	575,50	624,00	Mariner-6_Sste	AOFN01004550.1
S. stercoralis	P. tepidariorum	427	88,62	29,87	178,23	202	46,1	134,18	59,94	313,99	629	81	225,35	462,00	Mariner-6_Sste	AOMJ01182454.1
S. stercoralis	D. quadriceps	235	104,45	63,11	293,78	349	46,1	160,15	107,12	481,51	585	81	351,33	589,67	Mariner-6_Sste	JPHR01023093.1
H. placei	A. glabripennis	32174	112,99	87,99	376,94	1150	46,1	157,85	116,97	508,76	1297	81	437,50	517,50	Mariner-1_Hpla	AQHT01016807.1
H. placei	L. decemlineata	361774	155,66	77,97	389,56	944	44,1	227,14	145,85	664,69	1301	81	287,67	320,67	Mariner-1_Hpla	AYNB01038757.1
H. placei	H. vulgaris	40913	228,56	146,30	667,47	1336	46,1	259,24	162,12	745,61	1306	81	1292,00	1233,00	Mariner-6_Hpla	ABRM01014025.1
S. baturini	D. novaeangliae	703	144,87	166,33	643,85	1447	50,1	227,33	279,23	1065,02	2102	81	1057,00	1496,00	Mariner-4_Sbat	LGHO01000243.1
S. baturini	M. martensii	56639	132,64	63,82	324,11	799	42,1	194,33	106,63	514,21	1514	81	797,00	1473,00	Mariner-4_Sbat	AYEL01069060.1
S. baturini	C. exilicauda	24566	94,54	41,54	219,14	650	46,1	177,77	118,79	534,14	1260	81	650,00	1260,00	Mariner-4_Sbat	AXZI01159886.1
S. baturini	X. maculatus	3109	91,67	42,52	219,24	833	50,1	130,21	60,87	312,81	1166	81	728,00	1106,00	Mariner-7_Sbat	AGAJ01023144.1
S. baturini	A. citrinellus	3643	77,09	34,82	181,56	801	46,1	107,66	48,85	254,20	1161	81	680,00	960,00	Mariner-7_Sbat	CCOE01001708.1
W. bancrofti	D. suzukii	304	148,75	106,92	469,51	591	50,1	179,77	120,38	540,92	744	81	507,00	501,00	Mariner-1_Wban	AWUT01009455.1
W. bancrofti	D. ficusphila	410	164,59	104,67	478,61	571	50,1	182,23	104,96	497,12	588	81	393,50	381,00	Mariner-1_Wban	AFFG02008209.1
W. bancrofti	A. planipennis	4897	165,31	91,37	439,43	607	46,1	239,13	131,71	634,25	699	81	318,00	407,00	Mariner-1_Wban	JENH01067712.1
W. bancrofti	A. tumida	937	130,26	77,58	363,01	607	48,1	152,39	96,05	440,55	1362	81	400,50	432,00	Mariner-1_Wban	MRBJ01000042.1
P. equorum	A. australis	6814	147,01	104,64	460,93	928	42,1	226,19	181,94	772,01	1216	81	545,00	933,00	Mariner-1_Pequ	LK065141.1
P. equorum	G. gangeticus	21193	145,90	91,83	421,39	1512	52,7	195,16	119,09	552,43	4037	81	496,00	939,00	Mariner-1_Pequ	XM_019519190.1
P. equorum	C. porosus	19955	136,48	71,12	349,85	1487	53,6	175,89	96,18	464,43	2730	81	339,00	915,00	Mariner-1_Pequ	MDVP01000001.1
S. venezualensis	L. polyphemus	1270	95,37	68,87	301,98	763	44,1	150,21	107,32	472,17	956	81	714,00	957,00	Mariner-5_Sven	AZTN01137836.1
NEMATODE	PARTNER IN HTT	n	mean	sd	mean+3XSD	max	min	mean	sd	mean+3XSD	max	min	score	length	NEMATODE TE	PARTNER TE
		Genomic alignment score						Genomicalignment length					mariner alignment			

did not found TEs in the genome of *Haemonchus contortus,* while since the latest release of the genome assembly (haemonchus_contortus_MHCO3ISE_4.0) we are able to find TEs sharing high identity (>90%) with the TEs identified in *H. placei* in this study (Supplementary File 7). Since *H. placei* and *H contortus* are sister species, this finding further supports the goodness of our results and calls for near-to-chromosome assembly level of the sequences genomes, especially in genomes with a poor TE content.

A second observation raising from our TE survey in nematodes concerns the low abundance of *Tc1-mariner* elements in many nematode genomes, which is somehow expected since *Tc1-mariner* are in general less abundant than retroelements, owing to their mode of transposition. The observation that the genomes of some species of the Strongyloides genus, *W. bancrofti*, and *B. malayi* have low abundance of DNA transposons is also in accordance with previous papers (Szitenberg et al., 2016).

4.1. Active TE families and their dynamics in nematode genomes

In absence of specific transposition tests, the evaluation of the functional status of a TE could be inferred from the structure of TEs compared to a functional reference element. This would enable the estimation of the relative amount of autonomous versus non-autonomous elements within a certain family. We have carefully inspected each family detected and found that 33 are potentially active due to the presence of TIRs bracketing a coding sequence encoding a putative transposase protein, which in turn contains the transposase-associated protein domains (i.e. the DNA binding domain and the catalytic domain, see Supplementary File 6).

In this study we highlighted that the TE copy number varied considerably, ranging from single to multiple copies. Here, we speculate that both the amplification and the functional status of the TEs identified are symptomatic of the success of the TE in a given genome.

While low-copy or single-copy TE families could be in their early stage of the genome colonization or they could result from abortive genomic invasions, high-copy number TE families could be at the top of their genomic expansion.

Mariner-1_Btim could be representative of a recent entrance in this genome, since it is single copy and potentially active element (Table 1 and Supplementary Table 2), whereas *Mariner-2_Sste* could be the result of an abortive invasion, since it is a single-copy element and carries inactivating mutations that disrupt the transposase gene (Table 1 and Supplementary Table 2). Other hypotheses explaining the presence of single copy elements, e.g. selection of single insertion followed by loss of all other genomic copies, should be considered as less parsimonious. Other examples of TEs that are found in single copy per genome have been described so far in other organisms (Gladyshev and Arkhipova, 2009; Palazzo et al., 2016). These observations suggest that TEs genomic invasions are not ever successful events. A failure invasions can be the result of the inhibition of transposition upon the entrance in the genome. One possibility could concern the poor ability internal TE promoter to transcribe the transposase gene in the new genome. Even if the promoter of some *Tc1/mariner* elements are often able to break down the barriers existing between the transcriptional apparatuses of different species (Palazzo et al., 2017) (Palazzo et al., 2019). This feature could not be common among all *mariner* elements, thus resulting in an abortive HTT event. Position effect, induced either by the repressive chromatin environment near the integration site or by heterochromatin (Marsano et al., 2019), could also prevent the transcription of the transposase gene, leaving a single genomic copy of the element. Other layers of TEs repression rely on DNA methylation (Smith et al., 2012; Zhou et al., 2020) and chromatin changes induced by the TE insertion (Moschetti et al., 2020).

Contrary to the observation reported above, the *Mariner-21_Nbra* family consists of autonomous and non-autonomous copies that co-exist together, suggesting the current expansion of the family, whereas the *Mariner-6_Sbat* family could be considered as in its terminal stage of the lifecycle since non-autonomous copies are exclusively found.

4.2. Potential horizontal transfer events and their possible relevance in the evolution of species and ecosystems

Despite the huge number of sequenced genomes, HTs is still a complex and poorly understood phenomenon occurring frequently in metazoan, as demonstrated by many reported cases (Dotto et al., 2015). Undoubtedly, geographical proximity and host/parasite relationships help the exchange of genetic material between unrelated species (Gilbert and Feschotte, 2018; Schaack et al., 2010). In this respect, nematodes being ubiquitous organisms have a great chance to intercept DNA fragments from virtually every organism, thus participating either as a donor or as a recipient in HT events over time.

While many studies have recently focused on horizontal gene transfer in nematodes, very few have dealt with horizontal transposon transfer. Indeed, horizontal transfer is a well-documented process in plant-parasitic nematodes (Haegeman et al., 2011) which have acquired a large set of genes from bacteria. Furthermore, specific studies highlighted the importance of horizontal gene transfer from different sources including insects, in nematodes (Rodelsperger and Sommer, 2011). The central role of endosymbiont bacteria as a vector in horizontal transfer has been demonstrated in many eukaryotic species (Lopez-Madrigal and Gil, 2017). Several DNA transfer events from *Wolbachia* to the genome of animal parasitic nematodes have been reported (Dunning Hotopp et al., 2007; McNulty et al., 2012). Bacteria of the *Wolbachia* genus are closely associated with *Onchocercids* nematode germline cells at the female stage supporting the hypothesis of horizontal transfer from endoparasites. Moreover, the presence of a functional and essential gene acquired from non-*Wolbachia* bacteria has been reported in the human parasitic nematode *Brugia malayi* (Wu et al., 2013).

Methodologically, the inference of HTT events is not an easy task. Several methods based on the rate of synonymous to non-synonymous substitutions comparison, codon usage analyses and the powerful VHICA method which (Wallau et al., 2016) are routinely used to infer HTT. Unfortunately, if the divergence time between the species in which HTT is inferred increases these methods perform poorly due to substitution saturation which causes loss of the phylogenetic signal (Strimmer et al., 2009). In the latter case, the methods based on phylogeny and genetic distance more suitable to infer HTT (Dunemann and Wasmuth, 2019; Suh et al., 2016).

Very recently, horizontal transfer events involving TEs have been also reported to occur between nematodes and unrelated organisms. In details, two studies demonstrate that LINE-like retrotransposons can be exchanged in HTT events involving nematodes (Dunemann and Wasmuth, 2019; Suh et al., 2016). These findings support the notion that host-parasite interactions make nematodes prone to the episodic exchange of genetic material, including “selfish” mobile elements. However, since DNA transposons have been reported to be more prone to HTT than retrotransposons (Schaack et al., 2010) it appears peculiar that HTT events involving nematode DNA transposons are limited to a single case reported in the scientific literature (Laha et al., 2007). This evidence strongly suggests that the breadth of investigations in this field should be expanded given the availability of many sequenced genomes. Here, we have described HTT events involving nine DNA transposons in nematodes. Our results suggest that HTT between nematodes and distantly related metazoan might have occurred several times. Besides, the involvement of the most diverse non-nematode partners in HTT is consistent with the widespread diffusion of nematodes in almost all ecological niches. However, given the heterogeneous scenario observed from our results, it is difficult to infer both the direction and the vector (s) of the HTT events described here.

At least two relevant observations rise from these findings. The first observation deals with the ecological implications of nematodes as a potential source of variability through horizontal transfer in eco-niches

and ecosystems. In natural ecosystems, the relationships among different inhabiting species are the result of co-evolution. Environmental perturbations act as genetic stressors on the community members. The acquisition of novel genetic traits is one of the most effective reactions to environmental perturbations that allow either persistence in the same community or colonization of new environments. Horizontal gene transfer plays a critical role in the determination of gene flows in ecological niches (Andam et al., 2015). Similarly, TE flows can be determining factors in shaping the genetic profiles of eco-niches' resident species (Venner et al., 2017). TEs are powerful modulators of stress response both at short-term (adaptive response) and long-term (evolutionary changes) timescale, and their exchange through HTT could heavily contribute to the remodeling of the genetic robustness of ecological communities. In this context, the potential role of nematodes in disseminating TEs in virtually all ecosystems could be relevant. Environmentally stressed species could indeed augment the power of their stress-response (intrinsically determined by the endogenous TE complement) aided by horizontally transferred TEs. The new genetic features acquired could be then vertically transmitted through the population.

Interestingly insect species are involved in 25 putative cases of HTT detected in this study (Table 2). In this respect, *S. stercoralis* and ants seem to be preferred companion species in exchanging TEs since 11 different ant species belonging to 9 different genera are putatively involved in HTT events of two nematode *Tc1/mariner* elements (namely *Mariner-2_Sste* and *Mariner-6_Sste*). It is well known that insects can act as an environmental source of nematodes and that can disseminate them (Giblin-Davis et al., 2013; White et al., 2019) even in the absence of specific insect-nematode interactions. However, it has been reported that insect species within the order of Blattodea and Diptera act as vectors of Strongyloides species (Adenusi et al., 2018; Fetene and Worku, 2009). Therefore, even in the absence of host-parasitic interactions, there could be intimate connections between insects and nematodes, mainly dictated by ecological constraints, which could justify HTT. Paradoxically, occasional interactions could be the best conditions for HTT to occur, since no defense mechanisms have been developed from any of the interactor species. Here, we highlight that ants could be part of this complex network even if they have been never reported as nematode carriers.

The second observation that can be drawn from our work, is that Nematodes could be possibly regarded as a reservoir species (Gilbert et al., 2016), which could host TEs transiently in their genomes and grant them the opportunity to spread horizontally. As recently reviewed by Blumenstiel (Blumenstiel, 2019), reservoir species play a critical role in the re-invasion and in the resurrection of TEs in species that have lost them. Usually, a good reservoir species is thought to be associated with a high rate of TE acquisition and proliferation in its genome (Gilbert et al., 2016). This is especially true for large genomes, like mammalian genomes, and also some nematodes, whose genomes are smaller in size, display these features.

5. Conclusions

In conclusion, the role of nematodes is recently emerging in the context of HTT. The vast amount of genetic and genomic information available could enable us in shedding light on this obscure evolutionary process that entangles the reconstruction of the phylogenetic history of organisms. Furthermore, the study of the non-mendelian genetic connections between organisms sharing the same ecological niche could be relevant to fully understand how ecological communities evolve.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2021.107090.

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