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Phylogenetic Analysis of 28S rRNA Gene of Indigenous *Xiphinema pachydermum*

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A B S T R A C T

An attempt has been made to determine the phylogenetic relationship to trace out the evolutionary pattern of the test sequence of *Xiphinema pachydermum* partial 28S rRNA gene and to find out relationship of the same with other selected sequences of NCBI by constructing phylogenetic trees. Nucleotide sequence of 28S ribosomal RNA gene of *Xiphinema pachydermum* from Solan, Himachal Pradesh (AM779749.1) showed maximum homology of 93% with *Xiphinema inaequale* isolate HP 28S large subunit ribosomal RNA gene, partial sequence (HM163210.1) from Baijnath, Himachal Pradesh. The pairwise similarity score of 38 nucleotide sequences with test sequence elucidated 90-93% sequence identity with other sequences from India. Sequences from South Africa showed 91% identity with the test sequence while sequences from Brazil, Czech Republic, Japan, China and Slovakia showed 90% similarity. USA, Italy, Portugal, Spain, and Canada depicted 89% sequence homology with the Solan isolate. Multiple sequence alignment of 38 selected sequences of *Xiphinema* species having similarity score between 89 to 93 percent was performed using Clustal Omega and its output was used in Phylip 3.695 for constructing phylograms. The test sequence, AM779749.1 showed maximum closeness with another Indian isolate from Himachal Pradesh, HM163210.1 (*Xiphinema inaequale*) at significant bootstrap values of 100 both by Neighbor Joining and Maximum Parsimony methods respectively, which are further in close proximity to cluster of sequences containing another isolate from Himachal Pradesh, HM163211.1 (*Xiphinema lambertii* isolate XL 28S large subunit ribosomal RNA gene, partial sequence). While close proximity to the sequences from Czech Republic, Slovakia, and Italy, however having low bootstrap value may depict that insect might have migrated from Central Europe to India or vice versa. The predicted molecular weight of deduced amino acid sequence was 6031.69Da and was found to be rich in glycine (13.6%), serine (13.6%), alanine (11.9%), proline (10.2%) and aspartic acid (8.5%).

Introduction

Plant parasitic nematodes are widely distributed in all types of habitats and pose serious threat to the production of horticultural and agricultural crops. *Xiphinema* species, among the longidorids, are known nematode vectors of several plant viruses and play a vital

role in transmission of diseases, which leads to either total loss of crops or reduction in yield and quality of the product. Strawberry is one of the major fruit crops which is highly infested by a number of plant parasitic nematode species directly or in association with microorganisms. A virus transmitting nematode named *Xiphinema pachydermum* (infecting strawberry in Himachal Pradesh) with was procured and partial 28S rRNA gene (Verma, 2007) was isolated and sequenced (NCBI Accession Number AM779749) followed by *in silico* analysis useful for numerous applications to obtain a better understanding of both patterns and processes of evolution. During the systematic survey conducted for the prevalence of virus transmitting nematodes in association with strawberry at Solan, Sirmaur, Kangra, Una, Bilaspur, Hamirpur, Chamba, Mandi and Kullu districts of Himachal Pradesh revealed the presence of *Xiphinema* species among the longiroids prevalent in the rhizosphere of strawberry plants (Verma and Luqman Khan, 2012).

Test sequence

>AM779749.1 *Xiphinema pachydermum* partial 28S rRNA gene, isolate 1

TTTGAACGAAGGCTGCGAGATCTGAGA
CNTTCGAGCTANAAGAAANGGACGGA
ACCGTCTGACGCCCTCG

GGAATCAGTCGAACGACGCGCGAACG
CGTCTCGATCGTCGGAGCGTAAAAGTC
CGGCTCGAGGCGTCTAG

TAGTCGCGTTCGTAAGGCGCATTTCTC
GAGGGTTAGCGCCGCGACCGATGCGAA
CGGCCGACGACGCCGG

CGAGTTGAGGTCGAACGCCCTTCGCGG
GTATTCGTAACCAGCTCGTTCGGTAGA
TATTGTCGGATCTCTA

TTAATTAAGCATCGAGCGAATGACTAT
CAAATACGTCGGCGTAAAACGCTCGAC
GAGCGGGGGAGATTCG

GCCGGTCTTACGGTACGTGCTCGCGGG
TTCTCGTTCGGCGCGGGATCTTTTCCCG
ATGACCCCTTGGACG

CTACCGCGTCTAAGGCGTCCGCGGGGC
CCGAGTCGCCCCCCCGTCCTGATTGGT
GTTGAAACCCGTATGA

TGGAGTCTAAGCGTTAGAGCTAAGATT
TTGCCCTGCCAAAGGCTGTATCCGGGA
AAAAGGGAAAAGGTGG

TGCCCCTGACTGCCGAGGGGCGGTTCC
GTGCGTTTCCCGGCGGGGGGTGGGGTT
TGGCCGTGGACTGTAC

AGGAGGCTGTTGTGGTTTTAATTATTA
AGACCCTTCCGCTTGCGGTGAGGTTAC
TAATGGGTGACGTTTT

CGTGGGCTGGGGGTGGCCCAGTGTAAA
GTATGTGTGGGGTGGGCGCAATCGGTT
CCCGTGGTTCTCGTGC

TGCGGGTTTGGTCTTGGATGAGGTGAG
CTTTATTCAGTTTTGA

Therefore, in the present study, an attempt has been made to determine the phylogenetic relationship (Nei and Kumar, 2000)

To trace out the evolutionary pattern of the test sequence of *Xiphinema pachydermum* partial 28S rRNA gene and to find out relationship of the same with other selected sequences of NCBI.

Materials and Methods

Various physical and chemical parameters (https://web.expasy.org/protparam/) including amino acid content calculation were

performed to know more about the protein test sequence by using the ProtParam package of the ExPASy web server (http://www.expasy.ch/tools/protparam.html) and the secondary structure was predicted by using SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) tool. Phylogenetic trees were constructed using Maximum Parsimony and Neighbor Joining (Saitou and Nei, 1987; Tamura *et al.*, 2004) methods using Phylip 3.695 (the *PHYL*ogeny*I*nference *P*ackage, http://evolution.genetics.washington.edu/phylip.html). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. Nucleotide sequence of 28S ribosomal rna gene of *Xiphinema pachydermum* from Solan, Himachal Pradesh (AM779749.1) was compared with other sequences of *Xiphinema* species retrieved from NCBI database using BLAST (Altschul *et al.*, 1990). Table 1 shows the percent homology of *Xiphinema pachydermum* 28SrRNA gene with other *Xiphinema* species.

Results and Discussion

Translation of *Xiphinema pachydermum*28S rRNA gene

The nucleotide test sequence AM779749.1 of 813bp was translated using Expasy Translate tool and a protein sequence of 59AA with largest ORF was obtained for further analysis, the FASTA format of which is as given below:
> VIRT-62476:5'3' Frame 3, start_pos=99
MTIKYVGVKRSTSGGDSAGLTVRARGFS
FGAGSFPDDPLDATASKASAGPESPPRPD
WC

Physical and chemical parameters of deduced amino acid sequence of *Xiphinema pachydermum*28S rRNA gene

Various physical and chemical parameters viz., molecular weight (6031.69), theoretical pI (7.92), amino acid composition, atomic composition, instability index (41.04), aliphatic index (46.44) and grand average of hydropathicity (-0.420) were obtained for the deduced protein sequence of *Xiphinema pachydermum*28S rRNA gene. The instability index indicated that the protein is unstable. The deduced protein sequence was found to be rich in glycine (13.6%), serine (13.6%), alanine (11.9%), proline (10.2%) and aspartic acid (8.5%). The percentage of arginine and threonine was 6.8% and while that of lysine, phenylalanine and valine was 5.1%. It was found to be deficient in leucine (3.4%), and cysteine, glutamic acid, isoleucine, methionine, tryptophan and tyrosine with one count only (Table 2). Rest of the amino acids were absent. Total number of negatively (Asp + Glu) and positively (Arg + Lys) charged residues were found to be 6 and 7 respectively. Total number of atoms were found to be 831 and the chemical formula was obtained as $C_{262}H_{407}N_{75}O_{85}S_2$. The atomic composition showed 262, 407, 75, 85 and 2 atoms of Carbon, Hydrogen, Nitrogen, oxygen and sulphur respectively. The exact mass of the protein sequence was deduced using the Isotopident package of the ExPASy web server (http://education.expasy.org/student projects/isotopident/htdocs/). The mono-isotopic mass and the exact mass calculated were found to be 6027.927and 6029.934Da. The probability of combination was 74.51% of predicted mass of 6027 amu.

Phylogenetic analysis

BLASTn showed identity of query sequence with other isolates of *Xiphinema* species. The test sequence showed maximum homology of 93% with *Xiphinema inaequale* isolate HP 28S large subunit ribosomal RNA gene, partial sequence (HM163210.1) from Baijnath, Himachal Pradesh. The pairwise similarity score of 38 nucleotide sequences with test sequence elucidates 90-93% sequence identity with other sequences from

India. Sequences from South Africa showed 91% identity with the test sequence while sequences from Brazil, Belgium, Czech Republic, Japan, China and Slovakia showed 90% similarity. USA, Italy, Portugal, Spain, and Canada depicted 89% sequence homology with the Solan isolate.

Multiple sequence alignment of 38 selected sequences of *Xiphinema* species having similarity score between 89 to 93 percent was performed using Clustal Omega and its output was used in Phylip3.695 for constructing phylograms. Table 1 shows the list of 28S rRNA gene sequences of *Xiphinema* species retrieved from NCBI. All the nucleotide gene sequences of *Xiphinema* species when plotted into an evolutionary tree through Neighbor Joining and Maximum Parsimony trees constructed using PHYLIP 3.695 software as shown in Figure 1 and 2 respectively.

Comparative genomic and proteomic phylogenetic analysis of Indian Isolate of partial coat protein gene sequence of Zucchini Yellow Mosaic Virus (ZYMV) has been done with Maximum Likelihood, Neighbor Joining, Maximum Parsimony, and Unweighted pair Group Method of Arithmetic Averages methods using PHYLIP 3.68 by Sharma N *et al.,* (2012). For the validation of phylogenetic trees, "bootstrap" is be used to place confidence intervals on phylogenies (Felsenstein, 1985). It involves resampling points from one's own data, with replacement, to create a series of bootstrap samples of the same size as the original data. Each of these is analyzed, and the variation among the resulting estimates taken to indicate the size of the error involved in making estimates from the original data. In the case of phylogenies, it is argued that the proper method of resampling is to keep all of the original species while sampling characters with replacement, under the assumption that the characters have been independently drawn by the systematist and have evolved independently. Majority-rule consensus trees can be used to construct a phylogeny showing all of the inferred monophyletic groups that occurred in a majority of the bootstrap samples. The Neighbor Joining tree (Fig. 1) constructed using PHYLIP software showed two distinct clades in the phylogenetic tree. The sequence of *Xiphinema georgianum* from USA branched off early. Most early diverging clade consisted of 11 sequences viz., HM163209.1 (Czech Republic), HM163211.1 (India), AY601603.1 (Italy), AY601602.1 (Slovakia), AY601604.1 (China), AY601601.1 (South Africa), AY601600.1 (South Africa), AY601605.1 (Brazil), JN091972.1 (Japan), AM779749.1 (India) and HM163210.1 (India) including the test sequence with a bootstrap support of 87. Second big clade consisted of 26 sequences wherein first sub-clade consisted of 5 sequences of *Xiphinema floridae* with bootstrap value of 100, second sub-clade included 12 sequences with bootstap value of 43 and the third sub-clade was identified with 6 sequences of *Xiphinema citricolum* from USA and 2 *Xiphinema rivesi* sequences from Spain, and a *Xiphinema santos* sequence from Portugal with a bootstrap value of 69.

The test sequence, AM779749.1 showed maximum closeness with another Indian isolate from Himachal Pradesh, HM163210.1 (*Xiphinema inaequale*) at significant bootstrap value of 100 by Neighbor Joining method, which are further in close proximity to an isolate from Himachal Pradesh, HM163211.1 (*Xiphinema lambertii* isolate XL 28S large subunit ribosomal RNA gene, partial sequence) with a bootstrap value of 87.

Fig.1 Phylogenetic relationship of *Xiphinema pachydermum* 28S rRNA gene sequence with other species of *Xiphinema* by Neighbour Joining method

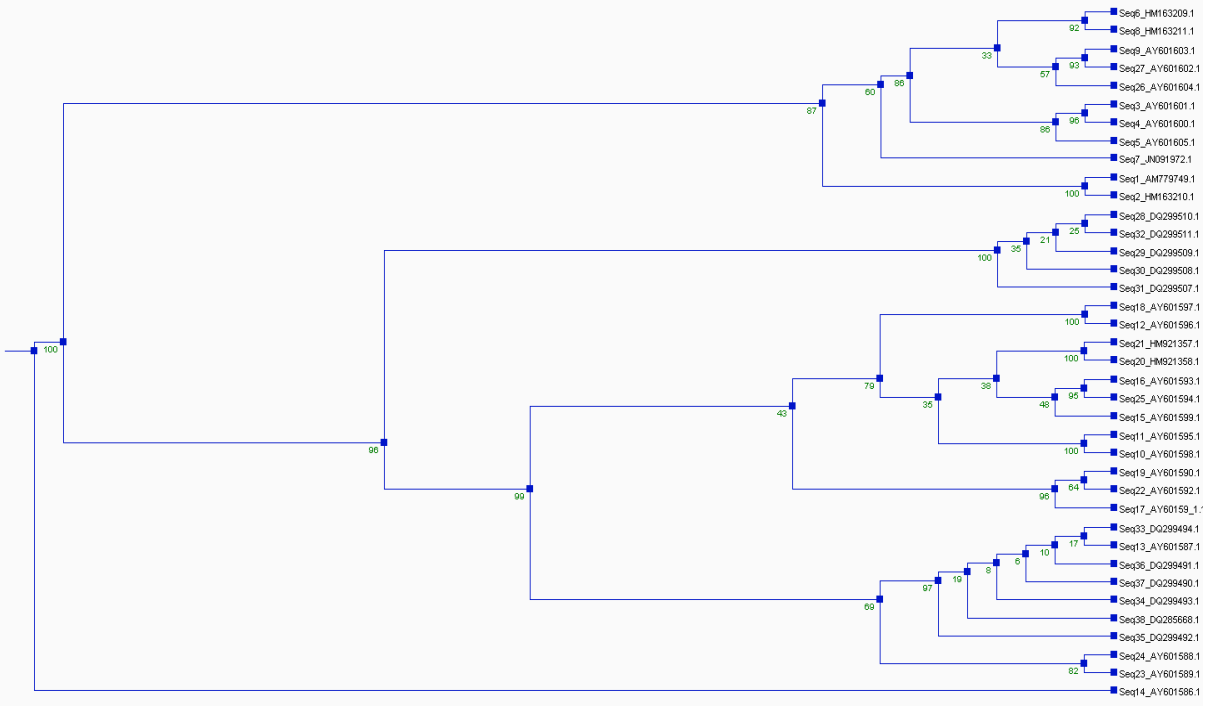

Fig.2 Phylogenetic relationship of *Xiphinema pachydermum* 28S rRNA gene sequence with other species of *Xiphinema* by Maximum Parsimony method

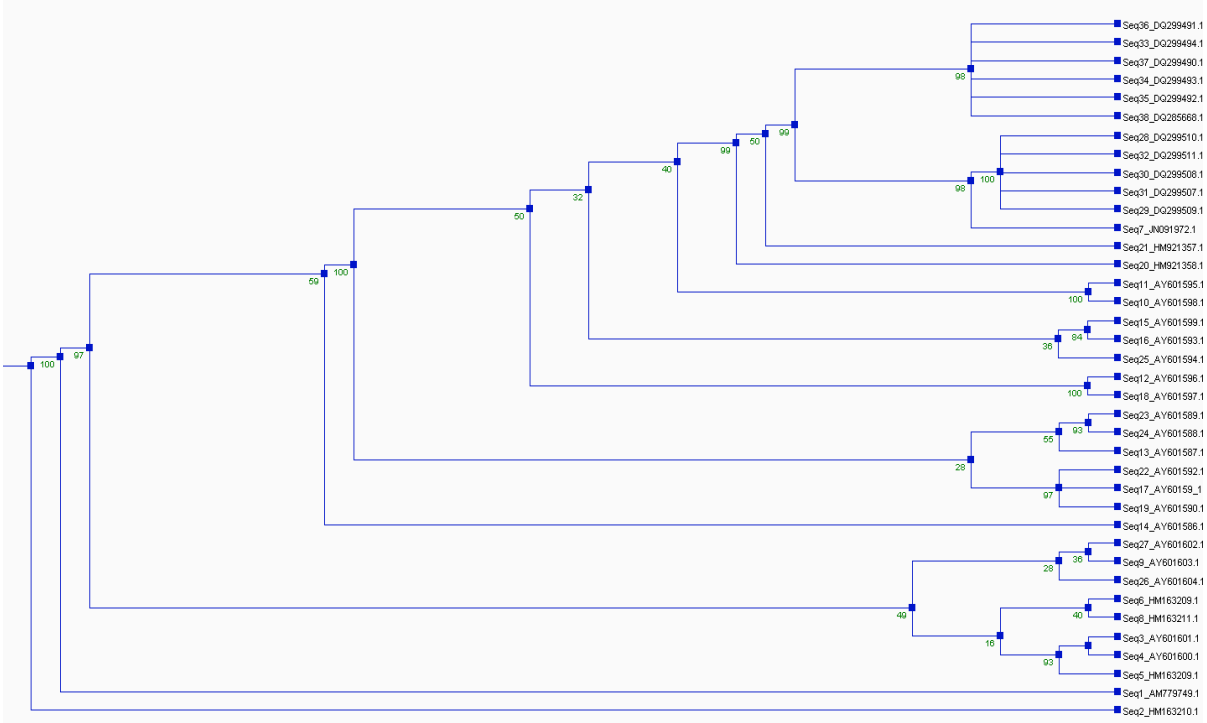

Table.1 List of 28S rRNA gene partial sequences of *Xiphinema* species retrieved from NCBI

S. No.	Accession Number	DNA Sequence Length (bp)	Organism	Country	Maximum identity
1	AM779749.1	813	*Xiphinema pachydermum*	Solan, HP, India	100%
2	HM163210.1	787	*Xiphinema inaequale*	Baijnath,HP, India	93%
3	AY601601.1	770	*Xiphinema brevicollum*	South Africa	91%
4	AY601600.1	770	*Xiphinema diffusum*	South Africa	91%
5	AY601605.1	770	*Xiphinema brevicollum*	Brazil	90%
6	HM163209.1	771	*Xiphinema brevicollum*	Czech Republic	90%
7	JN091972.1	831	*Xiphinema americanum group sp. LZ-2011*	Japan	90%
8	HM163211.1	773	*Xiphinema lambertii*	Baijnath,HP, India	90%
9	AY601603.1	775	*Xiphinema taylori*	Italy	89%
10	AY601598.1	769	*Xiphinema utahense*	USA	89%
11	AY601595.1	769	*Xiphinema thornei*	USA	89%
12	AY601596.1	774	*Xiphinema bricolensis*	USA	89%
13	AY601587.1	773	*Xiphinema santos*	Portugal	89%
14	AY601586.1	779	*Xiphinema georgianum*	USA	89%
15	AY601599.1	771	*Xiphinema americanum*	USA	89%
16	AY601593.1	771	*Xiphinema thornei*	USA	89%
17	AY60159 1.1	772	*Xiphinema americanum*	USA	89%
18	AY601597.1	774	*Xiphinema incognitum*	USA	89%
19	AY601590.1	773	*Xiphinema pacificum*	USA	89%
20	HM921358.1	805	*Xiphinema rivesi*	Spain	89%
21	HM921357.1	815	*Xiphinema rivesi*	Spain	89%
22	AY601592.1	772	*Xiphinema californicum*	USA	89%
23	AY601589.1	768	*Xiphinema rivesi*	USA	89%
24	AY601588.1	768	*Xiphinema rivesi*	USA	89%
25	AY601594.1	776	*Xiphinema bricolensis*	Canada	89%
26	AY601604.1	768	*Xiphinema brevicollum*	China	90%
27	AY601602.1	773	*Xiphinema taylori*	Slovakia	90%
28	DQ299510.1	832	*Xiphinema floridae*	USA	89%
29	DQ299509.1	832	*Xiphinema floridae*	USA	89%
30	DQ299508.1	832	*Xiphinema floridae*	USA	89%
31	DQ299507.1	832	*Xiphinema floridae*	USA	89%
32	DQ299511.1	832	*Xiphinema tarjanense*	USA	89%
33	DQ299494.1	831	*Xiphinema citricolum*	USA	89%
34	DQ299493.1	831	*Xiphinema citricolum*	USA	89%
35	DQ299492.1	831	*Xiphinema citricolum*	USA	89%
36	DQ299491.1	831	*Xiphinema citricolum*	USA	89%
37	DQ299490.1	831	*Xiphinema citricolum*	USA	89%
38	DQ285668.1	831	*Xiphinema citricolum*	USA	89%

Table.2 Amino acid composition of deduced amino acid sequence of 28S rRNA gene of indigenous *Xiphinema pachydermum*

Name	Symbol	Count	Percentage
Alanine	Ala(A)	7	11.90
Arginine	Arg (R)	4	6.80
Aspartic Acid	Asp (D)	5	8.50
Cysteine	Cys (C)	1	1.70
Glutamic Acid	Glu (E)	1	1.70
Glycine	Gly (G)	8	13.6
Isoleucine	Ile (I)	1	1.70
Leucine	Leu (L)	2	3.40
Lysine	Lys (K)	3	5.10
Methionine	Met (M)	1	1.70
Phenylalanine	Phe (F)	3	5.10
Proline	Pro (P)	6	10.20
Serine	Ser (S)	8	13.60
Threonine	Thr (T)	4	6.80
Tryptophan	Trp (W)	1	1.70
Tyrosine	Tyr (Y)	1	1.70
Valine	Val (V)	3	5.10

Sequences from South Africa showed close proximity with a sequence from Brazil as well as with the test sequence with high bootstrap value of 86. Phylogenetic analysis at nucleotide level further revealed that HM163209.1 (Czech Republic), AY601603.1 (Italy), AY601602.1 (Slovakia), and AY601604.1 (China) with low bootstrap (33), showed close proximity with the test sequence. The sequence of *Xiphinema georgianum* from USA segregated at a very early stage was found to be most divergent followed by the sequences of *Xiphinema citricolum* from USA, *Xiphinema rivesi* from Spain and *Xiphinema santos* from Portugal.

The Maximum Parsimony (Fig. 2) tree depicted the similar results, as above, showing *Xiphinema inaequale* sequence to be evolutionarily most similar to the test sequence with a bootstrap value of 100 followed by a group of 8 sequences viz., AY601601.1 and AY601600.1 (South Africa), AY601605.1 (Brazil), HM163209.1 (Czech Republic), HM163211.1 (India), AY601603.1 (Italy), AY601604.1 (China) and AY601602.1 (Slovakia), however with low bootstrap value (49) distantly related with sequences of *Xiphinema citricolum* from USA.

Al-Banna *et al.*, (1997) used nucleotide sequences of the large subunit ribosomal genes (26S rDNA) to examine evolutionary relationships among species of the genus Pratylenchus (Order: Tylenchida, Family: Pratylenchidae), commonly known as root-lesion nematodes. Based on parsimony analyses of approximately 307 aligned nucleotides of the D3 expansion region of the 26S rDNA, it is clear that species of Pratylenchus are a paraphyletic assemblage. The outgroup taxon H. belli shares a common ancestor with the clade that includes *P. vulnus*

and *P. crenatus* while *N. aberrans* and *R. similis* share a common ancestor with 5 other species included in this study.

Morris *et al.,* (2013) sequenced three genes (mtCO1, LSU, and ITS) from nematodes extracted from parasitized *Sirex* spp. collected inside and outside of the range of *S. noctilio* and assessed phylogenetic relationships among native *Deladenus* spp. in the north-eastern United States and the Kamona strain of *D. siricidicola* which suggested cospeciation between four North American *Sirex* spp. and their associated nematode parasites. They discussed nematode-host fidelity in the system and the potential for non-target impacts of a biological control program using *D. siricidicola* against *S. noctilio*.

Using ribosomal (18S, ITS1, ITS2, D2-D3 expansion segments of 28S rDNA) and mitochondrial (partial cox1 and nad4) DNA markers in a study of several populations of -group from Europe and Morocco, two cryptic species *Xiphinema browni* sp. n. (formerly reported as *Xiphinema pachtaicum*) and *Xiphinema penevi* sp. n. were revealed (Lazarova S *et al.,* 2016). The phylogenetic reconstructions inferred from three molecular markers (18S, D2-D3 28S rDNA and cox1) showed that *Xiphinema penevi* sp. n. is part of *Xiphinema pachtaicum*-subgroup and is closely related to *Xiphinema incertum*, *Xiphinema pachtaicum*, *Xiphinema parapachydermum*, *Xiphinema plesiopachtaicum*, *Xiphinema astaregiense* and *Xiphinema pachydermum*. However, phylogenetic relationships of the *X. americanum*-group species reconstructed by Bayesian inference for D2-D3 of 28S rRNA gene sequences did not provide clear species delimitation of the samples studied, although the mtDNA presented interspecific variations useful for demarcation among species (Subbotin S *et al.,* 2016).

From the results obtained it appears that *Xiphinema pachydermum* sequence of Solan, H.P., India has maximum similarity with *Xiphinema inaequale* sequence of Baijnath, H.P., India and shares a common ancestor with high bootstrap value. While close proximity to the sequences from Czech Republic, Slovakia, and Italy, however having low bootstrap value may depict that insect might have migrated from Central Europe to India or vice versa.

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