



DNA sequence collection at CNR-IPSP: a resource for nematode identification

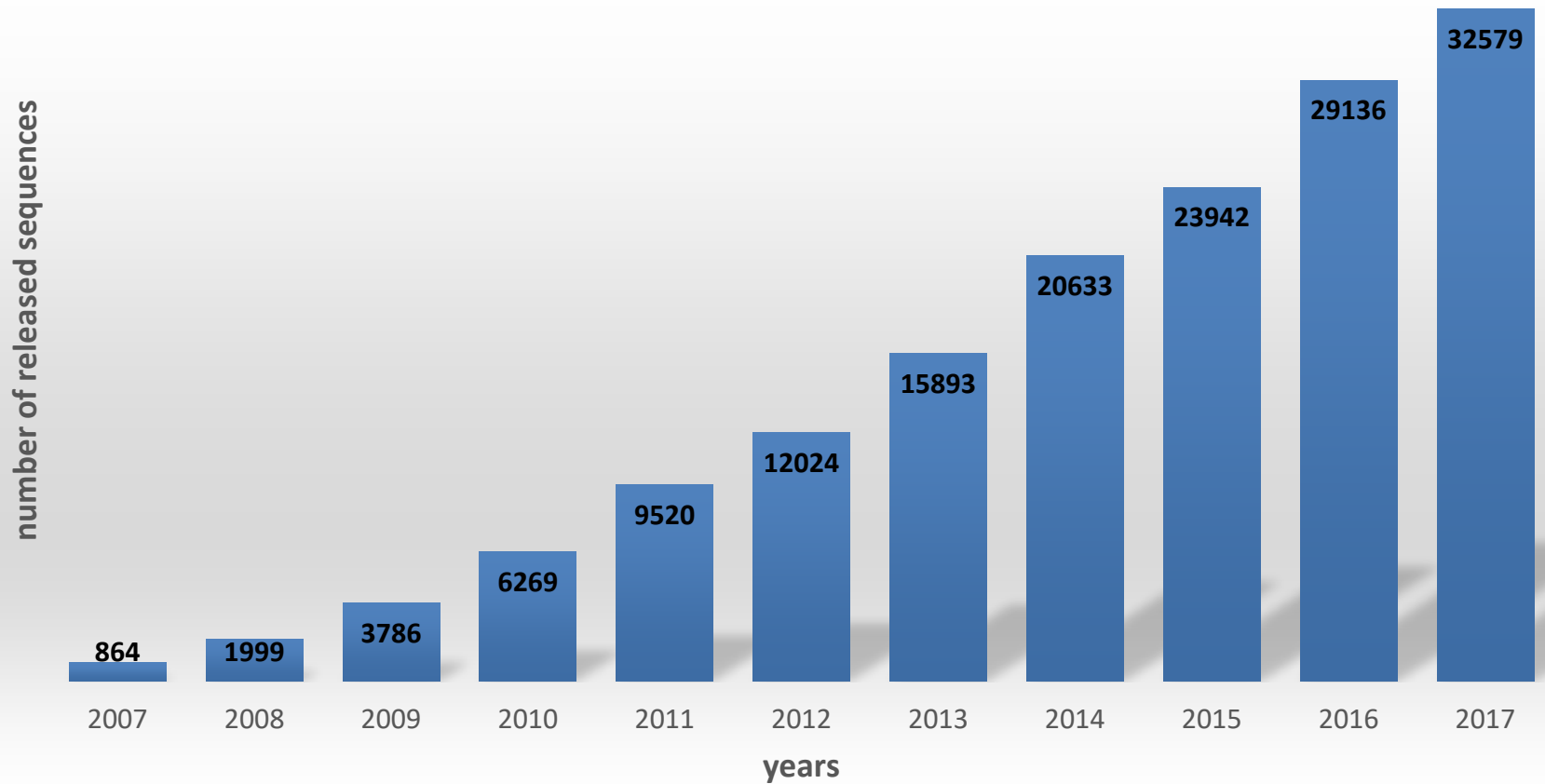
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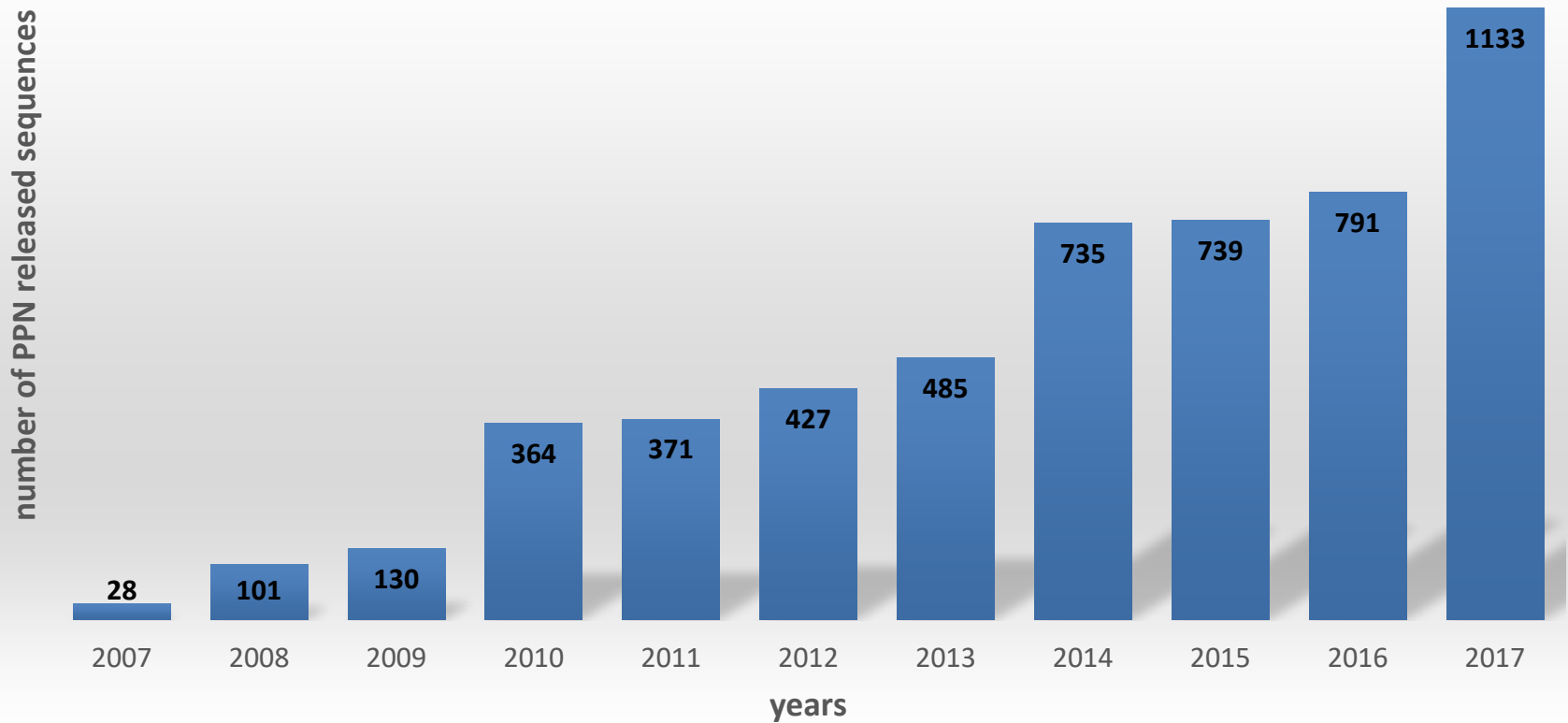


Ribosomal sequences of nematodes released in the primary database up to July 2017





Ribosomal sequences of plant parasitic nematodes released in the primary database up to July 2017





Resource Review

PPNEMA: A Resource of Plant-Parasitic Nematodes Multialigned Ribosomal Cistrons

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This paper describes the PPNEMA database, grouping and analysing **ribosomal ITS regions** sequenced in plant-parasitic nematodes and present in the primary databases. Thus, PPNEMA is a **preprocessed archive of data** ready to be used from researchers interested in phylogenetic studies on phytoparasitic nematodes, or to recognize a nematode by comparing its rDNA cistrons with the PPNEMA available genus specific multialigned groups.

The innovative aspect of PPNEMA is the availability of the rDNA sequences in groups of multialigned sequences.



General statistics about PPNEMA data in 2007

General	Functional Element	Genus	Redundants
General Statistics			
Total DB Entries			2405
Number of Reference Sequences			1482
Number of Redundant Sequences			923
Number of Reference sequences with redundant sequences			260
Number of Genera			26
Number of Species			405
Total Number of Alignments			208

The ITS sequences were grouped according to genus and species and multialigned in order to be used by researches interested either in phylogenetic studies or to recognize a nematode species by comparing its own sequences to the PPNEMA available genus specific multialigned groups.

However, as the sequence number increased, the cost of PPNEMA maintainance became higher and higher; so the database update was stopped.

IPSP sequence collection includes:

- ≈ 1,500 rDNA sequences (ITS, 18S and 28S) coming from all major plant parasitic, entomopatogenic and free-living nematode groups.
- ≈ 100 mitochondrial DNA sequences (COI and COII) coming from most plant parasitic, entomopatogenic and free-living nematodes.
- ≈ 150 nuclear sequences for *hsp90* gene for most plant parasitic, entomopatogenic and free-living nematodes.
- Metagenomic sequences of nematode communities from soil environmental samples obtained by NGS approach.

Our diagnostic protocol for nematodes:

- DNA extraction from individual nematode
 - identical PCR program for all primers
 - easy to standardize
 - specific and sensitive
- Cloning and sequencing

All genera of PPN, EPN and free-living nematodes can be amplified



2 Case Study

1. Case study: *Pratylenchus penetrans*

- Nematodes belonging to the genus *Pratylenchus* are among the 10 species of phytoparasites which cause considerable damage to crop.
- In the United States, economic losses of \$ 51,000,000 have been calculated per year.
- They are characterized by a very conserved morphology.

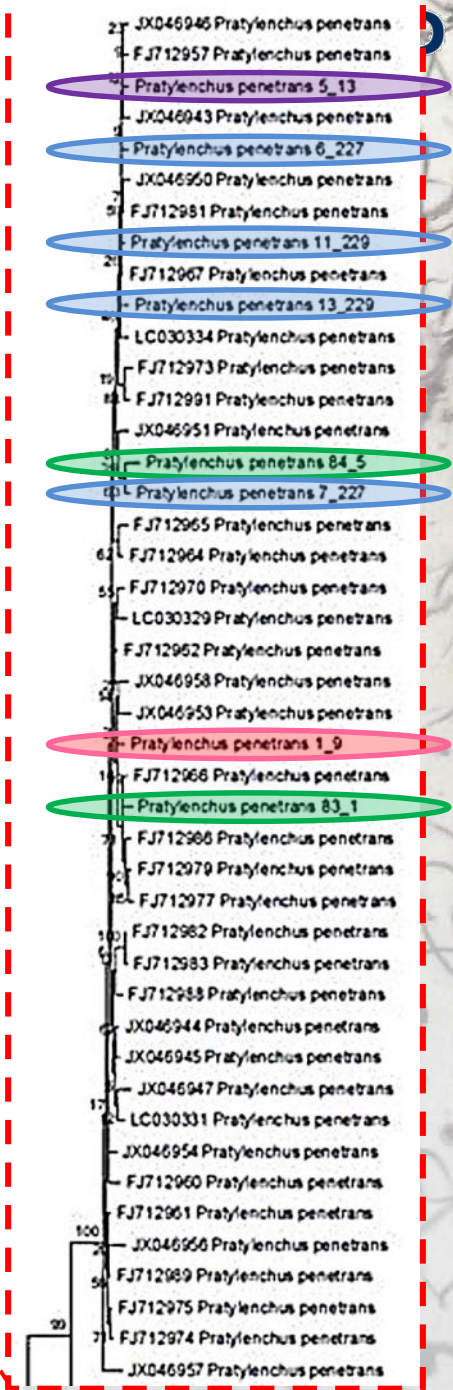
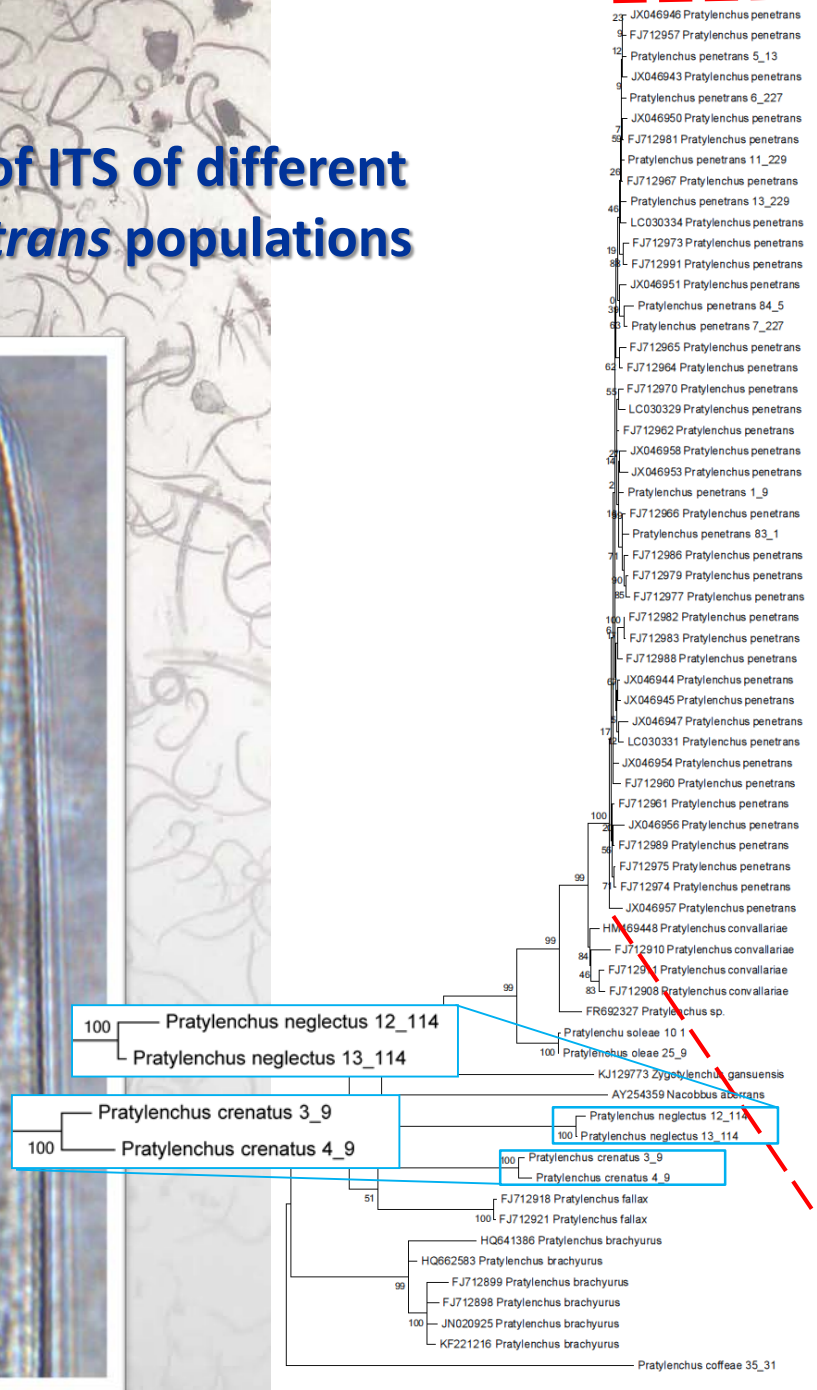
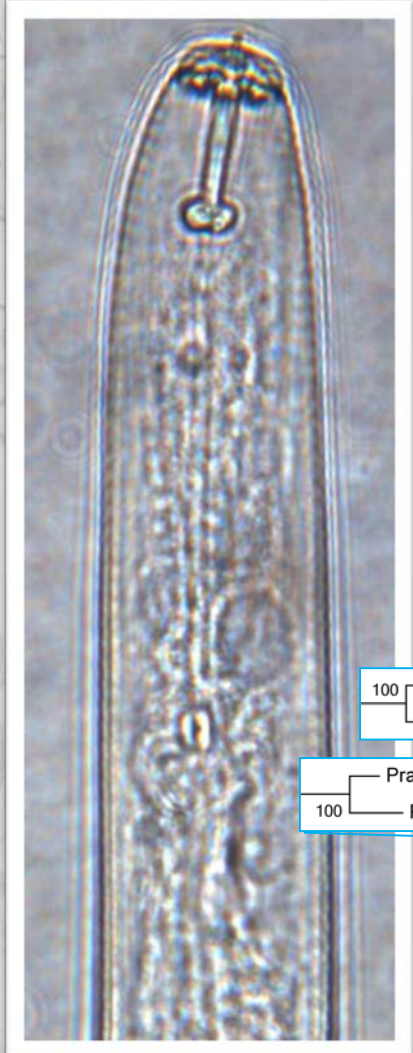


Multi-alignment of ITS of *Pratylenchus penetrans* populations

FJ712918	<i>Pratylenchus fallax</i>	ACCAAAAAACGCA	TTACATTTGCGTA	ATAGAGTGAAGAGTATATT	-----	TTTTAATAACTCG	TTGTGAAA	AT	G	-----	TACAC					
FJ712921	<i>Pratylenchus fallax</i>	ACCAAAAAACGCA	TTACATTTGCGTA	ATAGAGTGAAG	TATATT	-----	TTTTAATAACTCG	TTGTGAAA	AT	G	-----	TACAC				
Pratylenchus soleae 10	1	TCAAAGAAAAAAC	CTAATTTGTTT	TGGAATATA	--	TAA	TC	ATTA	-	TGTGTTTATATA	AACATCAA	TATCCC	ATTTTGA			
Pratylenchus oleae 25	9	TCAAAGAAAAAAC	CTAATTTGTTT	TGGAATATA	--	TAA	TC	ATTA	-	TGTGTTTATATA	AACATCAA	TATCCC	ATTTTGA			
FR692327	<i>Pratylenchus sp.</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTGGGAATATATA	TAA	TC	AAATACACT	GT	TTTATATAT	TGGAGAA	-	TATCCC	ATTTTGA			
HM469448	<i>Pratylenchus convallariae</i>	TCAAACAAAAAAC	CAAAAGTGTG	TGTTGGAATAT	--	GTAATC	AATTC	-	TGTTTATATAT	TAGAA	-	TATCCC	ATTTTGA			
FJ712911	<i>Pratylenchus convallariae</i>	TCAAACAAAAAAC	CAAAAGTGTG	TGTTGGAATAT	--	GTAATC	AATTC	-	TGTTTATATAT	TAGAA	-	TATCCC	ATTTTGA			
FJ712908	<i>Pratylenchus convallariae</i>	TCAAACAAAAAAC	CAAAAGTGTG	TGTTGGAATAT	--	GTAATC	AATTC	-	TGTTTATATAT	TAGAA	-	TATCCC	ATTTTGA			
FJ712910	<i>Pratylenchus convallariae</i>	TCAAACAAAAAAC	CAAAAGTGTG	TGTTGGAATAT	--	GTAATC	AA	TTCT	GT	TTTACATAT	CGA	GAA	TATCCC	ATTTTGA		
JX046957	<i>Pratylenchus penetrans</i>	TCAAACAAATAA	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATAACATG	TTTATATAT	TCGA	GAA	TACCC	ATTTTGA		
JX046956	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATCTCATG	TTTATATAT	CTA	GAA	TATCCC	ATTTTGA		
JX046958	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATCTAATG	TTTATATAT	TCGA	CAA	TATCCC	ATTTTGA		
FJ712975	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATCTAATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA		
FJ712974	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA		
FJ712989	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTG	--	TGAAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA		
FJ712961	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATGTTG	TGTTA	--	TC	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
JX046953	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACATG	TTTATATAT	TCGA	GAA	TACCC	ATTTTGA	
JX046944	<i>Pratylenchus penetrans</i>	TCAAATAA	CAAAATG	TGTTG	--	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
JX046945	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CA	AGT	--	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
FJ712966	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
FJ712986	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
FJ712979	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
FJ712977	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
FJ712982	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
FJ712983	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
Pratylenchus penetrans 1	9	TCAAACAAAAAAC	CAAAATG	TATGTTG	TTG	TG	AAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
FJ712965	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TCGA	GAA	TTTT	CCCAATTTTGA	
JX046954	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
FJ712964	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
Pratylenchus penetrans 84	5	TCAAAGAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATCTCATG	TTTATATAT	TAGA	GAC	TATCCC	ATTTTGA	
JX046947	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TACCC	ATTTTGA	
FJ712960	<i>Pratylenchus penetrans</i>	TCAGACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	CTA	GAA	TATCCC	ATTTTGA	
FJ712988	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TAGA	GAA	TATCCC	ATTTTGA	
FJ712962	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TTT	CCCAATTTTGA	
Pratylenchus penetrans 83	1	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
JX046951	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TATTTG	TTG	TG	AAATA	TATAATC	-	AATAGCATG	TTTATATAT	CTA	GAA	TATCCC	ATTTTGA	
LC030331	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	ATTACCATG	TTTATATAT	TCGA	GAAAA	TCCC	ATTTTGA	
FJ712970	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATGCCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
LC030329	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	CAAAAT	ATATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TATCCC	ATTTTGA	
Pratylenchus penetrans 7	227	TCAAAGAAAAAAC	CA	AATGTTG	TTG	TG	AAATAT	ATAATC	-	AGTCTCATG	TTTGTATAT	TAGA	GAA	TATCCC	ATTTT GA	
Pratylenchus penetrans 13	229	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATAT	TAATC	-	AATCCATG	TTTATATAT	TCG	GACT	TATCCC	ATTTT GA	
FJ712973	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	CT	-	TATGTTTATATAT	TCGA	GAA	TATCCC	ATTTT GA
FJ712991	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATATATA	TAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	TAT	CCCAATTTT GA	
JX046946	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	T	CCCAATTTT GA	
JX046950	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
Pratylenchus penetrans 6	227	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
JX046943	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
LC030334	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	CAAAAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
Pratylenchus penetrans 11	229	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
FJ712957	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
Pratylenchus penetrans 5	13	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
FJ712981	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAA	TAT	CCCAATTTT GA	
FJ712967	<i>Pratylenchus penetrans</i>	TCAAACAAAAAAC	CAAAATG	TGTTG	TTG	TG	AAATAT	ATAATC	-	AATACCATG	TTTATATAT	TCGA	GAAAA	T	CCCAATTTT GA	



Phylogenetic tree of ITS of different *Pratylenchus penetrans* populations



2. Case study: molecular identification of nematodes associated to aquatic imported plants

- Many ornamental plants can support advanced larval injury before external symptoms are observed.
- A good cooperation between the regulatory agencies, crop consultants, farmers and growers is essential for success in all kinds of phytosanitary programmes.
- The increasingly declining skills in classical identification and diagnosis in nematology is evident.
- In order to fill this gap molecular tools have been developed to assist the morphological identification.



Sample of 20 nematode specimens in ethanol 96%

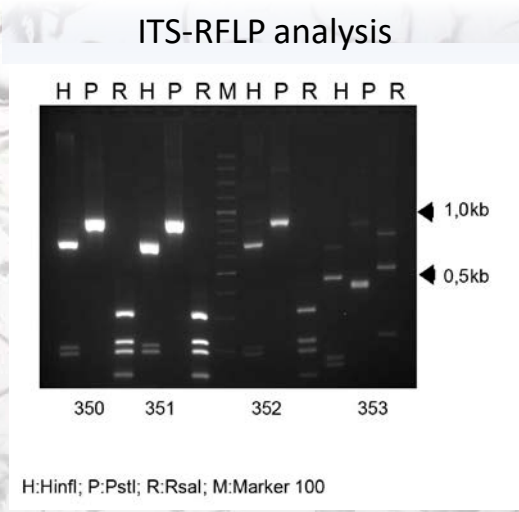


<http://www.aquaestetica.ro/en/plante-de-acvariu/>

Molecular analysis

Total DNA amplification (PCR)
markers: 18S rDNA, 28S rDNA and ITS region

Sequencing results



ITS

two species belonging to genera *Hirschmanniella* and *Plectus*

18S

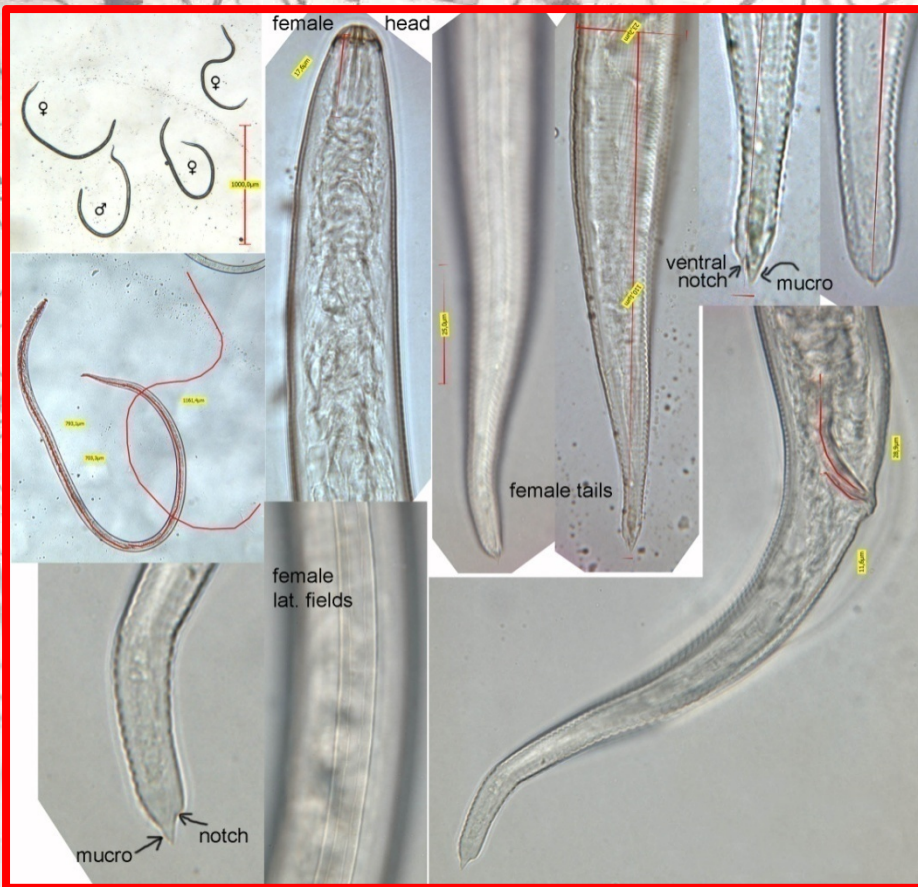
two sequences: *Chronogaster typica* (99%) and *Aphelenchoides fragariae* (96%)

26S

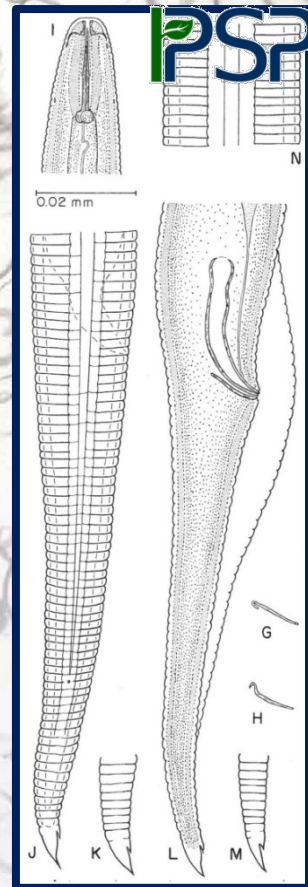
two sequences: *A. fragariae* (92%) and a Rhabditidae



A



B

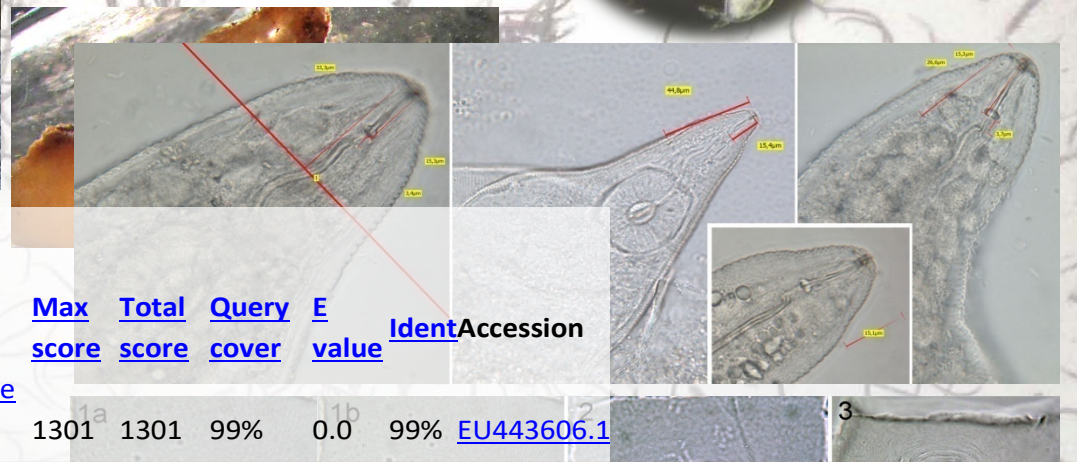
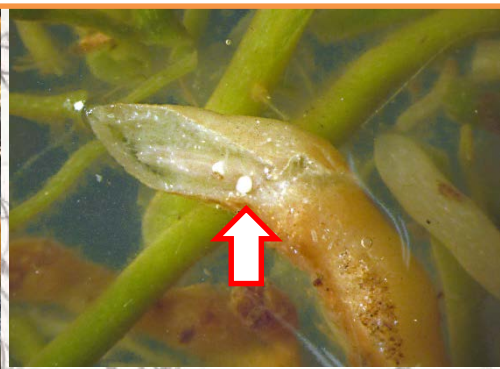
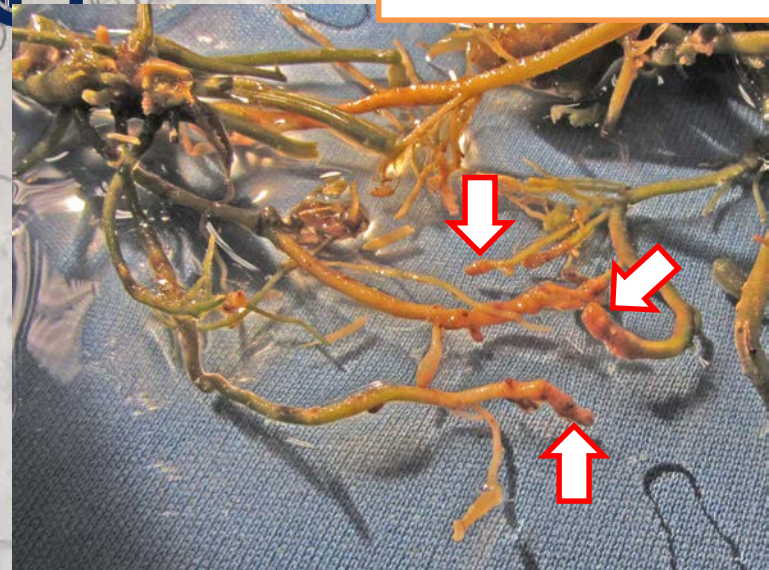


C

- ✓ Pictures above show the morphological analysis of the *Hirschmanniella* species (B= original; C = after Sher, 1968) found on aquatic plants (A);
- ✓ Sequence analysis of the 3 markers showed the specimens to belong to the genus *Hirschmanniella*;
- ✓ Sequences of *Hirschmanniella* from *Vallisneria g.* = to those of the new specimens from *Hygrophila difformis*;
- ✓ no molecular markers have been determined for this species in the database.



Occurrence of *Meloidogyne* sp. on *Anubias barteri*



Sequences producing significant alignments
 Select for downloading or viewing reports

- Select seq gb|EU443606.1|
- Select seq gb|KX130766.1|
- Select seq gb|KF482369.1|
- Select seq gb|KF501128.1|

Description

[Meloidogyne hispanica strain Seville 28S ribosomal RNA gene, partial sequence](#)
[Meloidogyne lucicola ribosomal RNA gene, partial sequence](#)
[Meloidogyne sp. Brazil 28S large subunit RNA gene, partial sequence](#)
[Meloidogyne hispanica 28S ribosomal RNA gene, partial sequence](#)

[Max score](#) [Total score](#) [Query cover](#) [E value](#) [Ident](#) [Accession](#)

Max score	Total score	Query cover	E value	Ident	Accession
1301	1301	99%	0.0	99%	EU443606.1

Table: comparison of main diagnostic characteris of *Meloidogyne* J2 associated to aquatic plants

Species	Body lenght (µm)	Tail length (µm)	Stylet (µm)	DGO(µm)	Index "a"	Tail shape
sp.from <i>Anubias b.</i>	443-491	48-61	11.3 (10.7-12)	2.4-3.6	25.6-32	rounded - clavate
<i>hispanica</i>	356-441	41-53	11.1 (10.4-11.9)	2.2-3.4	24.6-30.9	rounded - clavate
<i>sewelli</i>	460-600	68-82	11-13	7.0-8.0	32-42	sub-acute
<i>graminis</i>	420-510	66-88	12-13	2.2-2.8	29-34	sub-acute
<i>graminicola</i>	415-484	67-76	11-12	2.8-3.4	22-27	rounded - clavate
<i>oryzae</i>	500-615	70-90	14-15	2.0-3.0	30-45	rounded - clavate
<i>naasi</i>	418-465	52-78	13-15	2.0-3.0	25-32	sub-acute

Legend: = matching = little matching = not matching



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