MOLECULAR CHARACTERIZATION OF THE FALSE ROOT-KNOT NEMATODE *Nacobbus aberrans* FROM NORTH AMERICA

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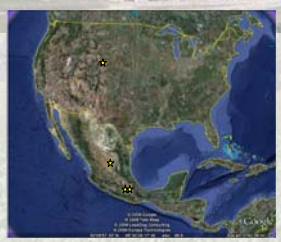
Introduction

The false root-knot nematode *Nacobbus aberrans* (potato pathotype) meets The International Plant Protection Convention (IPPC) definition of a quarantine pest and has been considered a Medium risk priority pest of quarantine importance in the United States. Currently, the taxonomic status and systematic relationships among North America isolates of *N. aberrans*, are unclear. Our objective is to clarify these relationships.

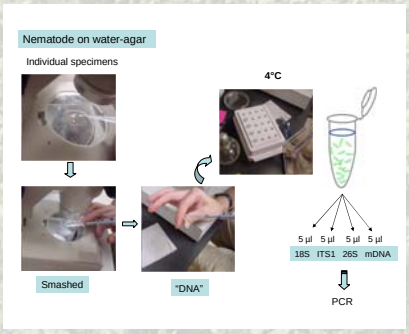
Materials and Methods

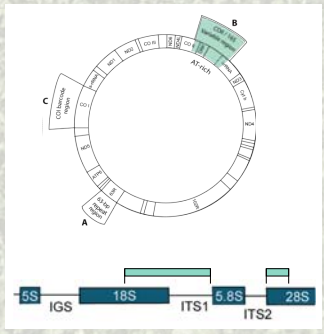
We have initiated morphometric and molecular analysis of single specimens from populations affecting sugarbeet (Western Nebraska, US); tomato and chard (Mexico State, Mexico) and drybean (Zacatecas, Mexico).

		CROP
USA:		
	1. Scottsbluff, Nebraska	Sugar beet
Mexico:		
	2. Xalatlaco, Mexico State	Chard
	3. Experimental Unit Tlapiaxco, UACH	Tomato
	4. Experimental Unit Agroecologia, UACH	Chard
	5. Colegio de Postgraduados, Montecillo	Tomato
	6. Guadalupe, Zacatecas	Dry bean



The nematodes were heat relaxed, mounted on 2% water-agar media and each stage was photographed and analyzed morphometrically. Following morphological analysis each specimen was disrupted and used as a DNA template for PCR reactions to amplify the molecular markers using primer sets for SSU rRNA (18S), Internal Transcribed Region 1 (ITS1), LSU rRNA (28S), and COII/16S variable region of the mitochondrial genome.





Each nematode-DNA template was amplified and sequenced for all four molecular markers. Each marker was considered separately in the construction of the Maximum Likelihood phylogenetic trees using PhyML program.

Results

The ribosomal 18S marker, an evolutionarily conservative marker, displayed no nucleotide sequence variation among isolates; however, both ITS1, 28S and COII/16S markers exhibited differentiation and subdivision by geography (Fig. 1). Three groups were shown: **1.**Drybean (Zacatecas, Mexico), **2.**Chard and Tomato (Mexico State, Mexico) and **3.** Sugarbeet (Western Nebraska, US).

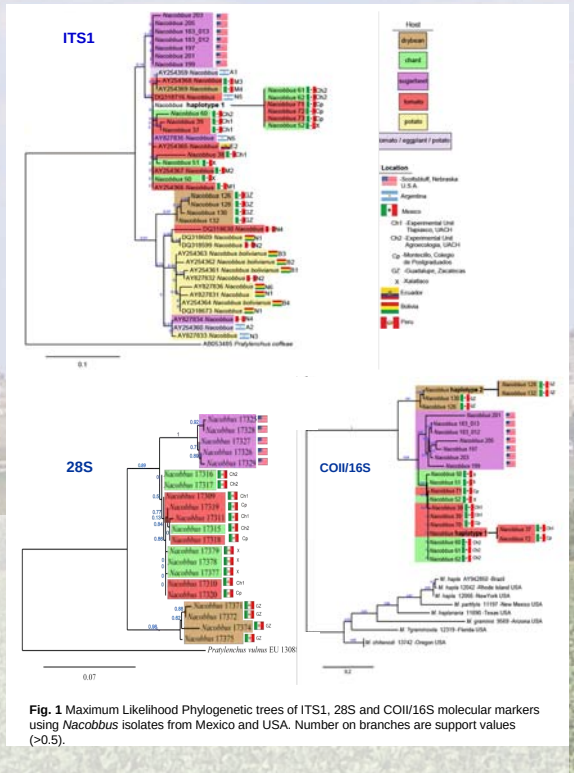


Fig. 1 Maximum Likelihood Phylogenetic trees of ITS1, 28S and COII/16S molecular markers using *Nacobbus* isolates from Mexico and USA. Number on branches are support values (>0.5).

The DNA sequences provided sufficient information to identify "pure" diagnostic characters (DeSalle *et at.,* 2005) which were specific for each of the populations (Table 1)

Table 1. Multiple region ITS1, 28s and COII/16S barcode of *N. aberrans* isolates from Mexico and USA.

Isolate	Host	Mol. Marker	Diagnostic Characters
Agronco, Mexico	Chard	ITS1	G[G] T[G]G[A] T[G] G[T] A[C] A[T] T[A] G[
		28S	T[T] C[ ]G[G] C[
		COII/16S	C[G] G[T] T[A] A[A]C[T] T[A] A[C] T[
CP, Mexico	Tomato	ITS1	G[G] T[G] G[A] T[G] G[T] A[C] A[T] T[G[
		28S	T[C] A[C] G[G]C[
		COII/16S	C[G] G[T] T[A] A[A]C[T] T[A] A[C] T[
Zac., Mex	Drybean	ITS1	T[T] C[G] A[G] A[A] T[T] G[T] G[C] C[T] A[A] T[
		28S	C[CTTATTGCTTTCAAAGTGTCTCGCTAAACTTAAATTTA] GCGCGAAAATTATTTAACAAT]
		COII/16S	TAAAATTAATTATTTTGAAAAGCTGTTATTTTTCTAAA] AATTGAAAAATTTCTGTTTAAATTTTTTAACTTACTG] AAACGCGAT]

Isolate	Host	Mol. Marker	Diagnostic Characters
Scottsbluff, NE, USA	Sugarbeet	ITS1	A[A]T[A]C[G]A[T]A[G]T[T] A[C] G[A]A[A] C[
		28S	A[A] A[C] C[T] A[C] A[T] T[A] A[G] A[A] C[A]
		COII/16S	C[A]T[C]G[A]G[G]A[A]A[A]A[G]A[A]G[A]A[T]A[A]A]
Xalatlaco, Mexico	Chard	ITS1	T[C] G[G] T[G] G[G] G[A] C[T] G[A[
		28S	T[C] A[C] G[G] C[C] C[
		COII/16S	C[G] G[T] T[A] A[A] C[T] T[A] A[C] T[
Tlapiaxco, Mexico	Tomato	ITS1	T[C] G[G] G[G] G[A] C[A] T[G] A[T] G[A]
		28S	T[C] A[C] G[G] C[C]
		COII/16S	C[G] G[T]T[A] A[A] C[T] T[A] A[C] T[

Discussion and Conclusions

These results indicate that drybean from Mexico and sugarbeet US populations can be considered as separate groups which are subdivided by geography. The taxonomic nature of these groups requires additional comparison with South American isolates.

References: 1. DeSalle, R. Egan, M. G. and Siddall M. 2005. The Unholy Trinity: Taxonomy, Species Delimitation and DNA Barcoding Philosophical Transactions: Biological Sciences, 360 (1462): 1905-1916.