A COI DNA barcoding survey of *Pratylenchus* species in the Great Plains Region of North America

Mehmet Ozbayrak,[1] Tim Todd,[2]
Timothy Harris,[1] Rebecca Higgins,[1]
Kirsten Powers,[1] Peter Mullin,[1]
Lisa Sutton[1] and Thomas Powers[1]*

[1]Department of Plant Pathology,
University of Nebraska-Lincoln,
Lincoln, NE, 68583-0722.

[2]Department of Plant Pathology,
Kansas State University,
Manhattan, KS, 66502.

*E-mail: tpowers1@unl.edu

Abstract

Pratylenchus species are among the most common plant parasitic nematodes in the Great Plains Region of North America. Our goal was to survey *Pratylenchus* species diversity across the Great Plains region using a mitochondrial COI DNA barcode. The objectives were to (i) determine species boundaries of the common *Pratylenchus* species within the region, (ii) assess the host associations of the barcoded *Pratylenchus* specimens, and (iii) determine *Pratylenchus* distribution patterns throughout the region. A total of 860 soil samples, primarily associated with eight major crops, were collected from Colorado, Kansas, Montana, Nebraska, North Dakota, and Wyoming. From this total, 246 soil samples provided the majority of 915 individual nematode specimens that were amplified by PCR and sequenced for a 727 to 739 bp region of COI. Maximum likelihood, neighbor-joining, and Bayesian phylogenetic trees all recognized 19 distinct and well-supported haplotype groups. The most common and widespread haplotype group, representing 53% of all specimens was *P. neglectus*, detected from 178 fields in 100 counties and associated with fields growing wheat, corn, dry beans, barley, alfalfa, sugar beets, potatoes, and a vineyard. The second most prevalent haplotype group was *P. scribneri*, representing 30% of all specimens and recovered from 104 fields in 45 counties, and most frequently associated with corn. Mixed field populations were encountered in approximately 20% of infested fields, with *P. neglectus* and *P. scribneri* most often occurring together in corn-soybean cropping systems. Less frequently encountered from the region were *P. hexincisus*, *P. thornei*, *P. penetrans*, *P. alleni*, and *P. zeae*. Eight additional haplotype groups, potentially new *Pratylenchus* species, were discovered in the survey.

Keywords

COI, DNA barcoding, Species delimitation, ABGD, GMYC, TCS, *Pratylenchus*, Root lesion nematode, Taxonomy.

Global estimates indicate that there are approximately 100 described species in the genus *Pratylenchus* Filipjev, 1936 (Janssen et al., 2017; Singh et al., 2018). In total, 27 of these species have been reported from North America by Castillo and Vovlas (2007). This number has increased to 29 with the descriptions of *P. floridensis* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Moens & Inserra, 2010 and *P. parafloridensis* De Luca, Troccoli, Duncan, Subbotin, Waeyenberge, Moens & Inserra, 2010. Recent studies in the Great Plains Region suggest more species await description (Yan et al., 2017a, 2017b). Although most *Pratylenchus* species descriptions were based solely on morphological features, many species have now been placed in a phylogenetic context using molecular characters (Araya et al., 2016; Fanelli et al., 2018; Flis et al., 2018; Hammas

et al., 2018; Inserra et al., 2007; Palomares-Rius et al., 2014; Singh et al., 2018; Subbotin et al., 2008). These phylogenetic trees provide a framework for species delimitation and establish testable species hypotheses for species discovery (De Luca et al., 2010; De Luca et al., 2012; Janssen et al., 2017; Qing et al., 2019).

Root lesion nematodes in the genus *Pratylenchus* are migratory, intercellular endoparasites that penetrate the root of the host plants and feed and reproduce within the root epidermis and cortex. This feeding behavior results in root lesions that enhance fungal and bacterial infection, secondarily contributing to yield and economic losses in agricultural production (Jones et al., 2013; Smiley, 2015). Yield loss can be underestimated due to the complex nature of root diseases and the biotic interactions that underlie symptomatic nutrient deficiency (May et al., 2016). The severity of losses depends on a multitude of factors (Nicol et al., 2011), not the least of which is species identity and corresponding host associations.

In general, *Pratylenchus* species are polyphagous, parasitizing a broad variety of plants including cereals, fruits, vegetables, forage crops, industrial crops, cotton, coffee, potatoes, and ornamental plants, as well as weed species (Bélair et al., 2007; Castillo and Vovlas, 2007). Host preferences, however, can differ significantly among species. For example, although *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 has associations with 400 different plant species, the species is most commonly associated with fruit trees such as apple (*Malus* sp.) (Wallace and MacDonald, 1979), cherry (*Prunus avium*), and peach (*Prunus persica*) (Askary et al., 2012), yet potatoes (Morgan et al., 2002) and corn (MacGuidwin and Bender, 2015) are also commonly listed as hosts. *P. scribneri* Steiner in Sherbakoff & Stanley, 1943 is primarily associated with agronomic crops such as potatoes (Brown et al., 1980; Yan et al., 2015), corn, and reportedly soybeans (Reboish and Golden, 1985).

The geographical distribution of *Pratylenchus* extends from cold temperate and sub-alpine ecosystems to tropical, equatorial ecosystems worldwide. The distribution and abundance of individual species may be influenced by temperature optima (Acosta and Malek, 1979; Dickerson, 1979) and soil properties (Thompson et al., 2010). Many *Pratylenchus* species exhibit a preference for sandy soils with a relatively high level of oxygen (Castillo and Vovlas, 2007; Olabiyi et al., 2009).

Postglacial history is a legacy effect that may have shaped *Pratylenchus* distribution in North America prior to the intensive cultivation brought by European settlers. Presently it is not clear which species were introduced to the region, and which might have existed on native grasses prior to European settlement. An early study of nematode community composition of the tallgrass prairie in Kansas recorded 228 species from 80 genera, including 23 genera of plant parasitic nematodes and two *Pratylenchus* species, *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stehoven, 1941 and *P. penetrans* (Orr and Dickerson, 1967). Four *Pratylenchus* species were reported in the Northern Great Plains by Thorne and Malek (1968) including *P. scribneri* and *P. agilis* Thorne & Malek, 1968, which was later synonymized with *P. scribneri* by Subbotin et al. (2008). Both species were reportedly associated with prairie and cultivated potato fields in Nebraska. Two other species were collected in cultivated fields, *P. tenuis* Thorne & Malek, 1968 from an unspecified host, and *P. hexincisus* Taylor & Jenkins, 1957 from corn in South Dakota. *Pratylenchus alleni* Ferris, 1961 was described from soybean fields in southeast Illinois (Ferris, 1961). Smolik and Lewis (1982) recorded *P. tenuis* and *P. scribneri* from the mixed shortgrass prairie ecosystems of western South Dakota. Corn has been frequently recorded as a host for lesion nematodes with *P. scribneri, P. hexincisus,* and *P. tenuis* reported in fields in South Dakota (Smolik, 1977; Smolik and Evenson, 1987) and *P. agilis, P. alleni, P. flakkensis* Seinhorst, 1968, *P. hexincisus, P. neglectus*, and *P. scribneri* in corn fields of Iowa (Norton, 1983). Todd et al. (2014) reported *P. neglectus* and *P. thornei* Sher & Allen, 1953 in wheat fields of Kansas and Colorado. This accounting of *Pratylenchus* species reported from the Great Plains region totals nine different species, not including *P. agilis*.

The objectives of this study were to (i) barcode *Pratylenchus* specimens for species identification across the Great Plains region using the cytochrome oxidase subunit 1 (COI) gene of the mitochondrial DNA (mtDNA) as a gene barcode, (ii) determine the species boundaries among COI barcoded specimens, (iii) assess the host associations of COI barcoded *Pratylenchus* species, and (iv) determine *Pratylenchus* species distribution patterns across the Great Plains region.

Material and methods

Sample collection

Soil and root samples analyzed in this study were obtained from USDA Cooperative Agricultural Pest Survey Program (CAPS), Wheat and Corn Disease Surveys in Kansas and Nebraska, and field samples submitted to the University of Nebraska-Lincoln Disease Diagnostic Clinic, representing different crops and geographic regions primarily within the Northern

Great Plains of North America. Five statewide surveys associated with the CAPS program were conducted by Departments of Agriculture in Kansas, Montana, Nebraska, North Dakota, and Wyoming. A minimal number of samples were acquired from South Dakota and no samples were collected from Iowa. Sample collection sites (county locations), nematode identification (NID) numbers, and host information for each analyzed specimen are presented in Supplementary Table 1 and Figure 1.

Nematode extraction

Nematodes were extracted from $100\,cm^3$ field soil using a modified flotation-sieving and sugar centrifugation method (Jenkins, 1964) and from the host-root material using root incubation (Russell, 1987; Todd and Oakley, 1996). A majority of the samples recovered from Kansas was obtained from root extracts.

Morphological analysis and vouchers

Nematodes extracted from soil and roots were first evaluated under a stereo dissecting microscope and select specimens belonging to the genus *Pratylenchus* were handpicked for light microscopy examination and DNA extraction. Following immobilization of live specimens by heating, individual nematode specimens were mounted on temporary glass slides, measured, and photographed with a Leica DMLB light microscope with Differential Interference Contrast and a Leica DC300 video camera. Each nematode was assigned a unique Nematode Identification number (NID). The NID number links images, measurements and DNA of each individual nematode specimen. Usually five individual specimens from each sample were mounted, measured, and photographed in this manner. Images were archived in the database system of the Nematology Laboratory at the University of Nebraska-Lincoln. For PCR amplification, image-vouchered specimens were removed from temporary slides and smashed in an $18\,\mu l$ drop of sterile deionized distilled water using a sterile, transparent micropipette tip. Smashed specimens were transferred to PCR reaction microfuge tubes and stored at $-20°C$ until PCR amplification.
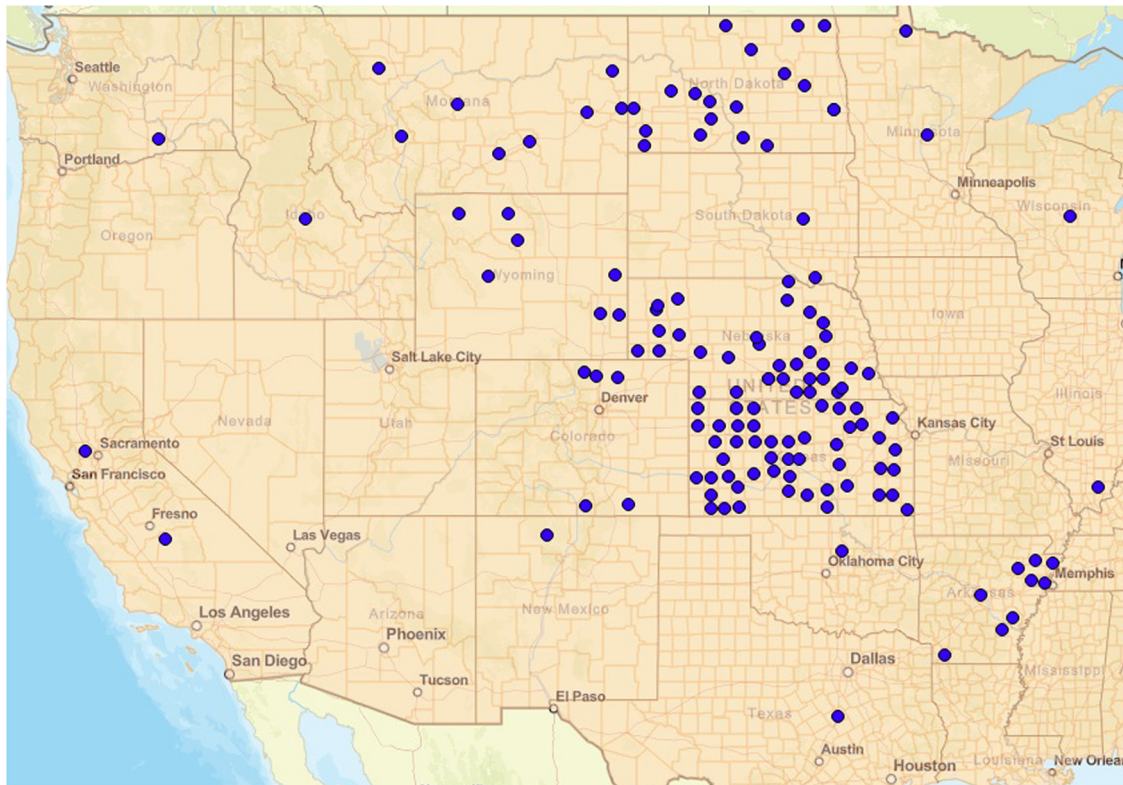

Figure 1: Distribution of sampling sites by county for the *Pratylenchus* survey.

PCR primers and amplification conditions

The mitochondrial COI gene was amplified by PCR using primer sets of COI-F7b-Prat (F7bP) (5′-GGDTGRACWTTHTAYCCNCC-3′) developed by the UNL nematology laboratory, and COI-JB5 (5′-AGCACCTAAACTTAAAACATAATGAAAATG-3′) (Derycke et al., 2005), resulting in a fragment length of 727 to 739 bp for genetic analysis after trimming the primers from the amplified product. On occasion, forward primer JB3 (5′-TTTTTTGGGCA TCCTGAGGTTTAT-3′) (Derycke et al., 2005) was used in combination with the JB5 primer. The D2-D3 domains of 28S rDNA were amplified using the primer set D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (De Ley et al. 1999). PCR was conducted in a total volume of 30 μl reaction mix consisting of 1.2 μl molecular biology grade water (Sigma-Aldrich), 2.4 μl of each primer, and 15 μl of 2× JumpStart REDTaq ReadyMix Reaction Mix (Sigma-Aldrich), and 9 μl DNA template. Amplification conditions were as follows: initial denaturation (modified hot start) at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 5 min. Annealing temperature was at 48°C for the amplification of D2–D3 domains. Amplification success was evaluated in 1% agarose gels using 0.5X TBE and ethidium bromide.

DNA cleaning and sequencing

High-quality PCR products in a 0.7% TAE agarose gel were extracted by x-tracta Tool (USA Scientific), and cleaned using Gel/PCR DNA Fragments Extraction Kit (IBI Scientific). Cleaned PCR products were shipped to the University of California-Davis DNA Sequencing Facility for sequencing in both directions.

Phylogenetic analysis

DNA sequences were edited using CodonCode Aligner version 8.0.1 (www.codoncode.com) and used in a BLAST search. Sequences were submitted to GenBank, with accession numbers for COI and 28S sequences provided in Supplementary Table 1. Multiple sequence alignment was conducted using MUSCLE (Edgar, 2004) with a gap opening penalty −400 and a gap extension penalty −200, in MEGA version 7 (Kumar et al., 2016). The best DNA Model tool in MEGA 7 was used to determine a best-fit substitution model: general time reversible (GTR) with a gamma distribution (G) and proportion of invariable sites (I). The substitution model (GTR + G + I) was used for maximum-likelihood analysis with 200 bootstrap replications using software MEGA 7 and for Bayesian inference analysis (BI) using the software MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The COI haplotype data set was reduced by removal of redundant sequences using software Jalview.2.10 (Waterhouse et al., 2009).

Species delimitation/delineation

Molecular species delimitation was assessed using Automatic Barcoding Gap Discovery (ABGD) (Puillandre et al., 2012), the Generalized Mixed Yule Coalescent (GMYC) method (Pons et al., 2006), and by statistical parsimony networks (TCS) (Clement et al., 2002), all applied to a non-redundant, 143 specimen COI data set.

ABGD analyses were performed at the online webserver (wwwabi.snv.jussieu.fr/public/abgd/abgdweb. html) using the Kimura (K80) distance model with default parameters.

GMYC is a model-based maximum likelihood approach that uses an ultrametric tree to delimit species and determine diversification and coalescence events based on branching patterns. An ultrametric tree was constructed using Beast 2.5.2 (Bouckaert et al., 2014) with the GTR model with Gamma distribution (G) and four gamma categories for nucleotide evolution. The tree was estimated with a strict clock model or a lognormal relaxed clock model assigned to a yule or a coalescence branching model. All other parameters were set as defaults and the tree was not dated for GMYC analysis. Markov Chain Monte Carlo (MCMC) chain was run at 30 to 250 M generations and sampled every 3,000th to 5,000th generation based on model selection. Tracer V1.7 (Rambaut et al., 2018) was used to visualize convergence and evaluate effective sample size (ESS > 200) of traces. A maximum clade credibility tree was produced by TreeAnnotator V2.5.2 (Rambaut et al., 2018) with common ancestor node heights, after discarding 25% of the trees as burn-in. The tree was visualized using FigTree V.1.4 (tree.bio.ed.ac.uk), and Beast analyses were run on the XSEDE server of the CIPRES Science Gateway (Miller et al., 2011). The GMYC analysis was conducted using the single threshold option (Fujisawa and Barraclough, 2013) using the Species' limits by threshold statistics (SPLITS) R Package Version 1.0–19 (http://R-Forge.Rproject.org/projects/splits/) and APE package (Paradis et al., 2004) available for R v3.5.2 (www.R-project.org). After analysis all outputs (estimated threshold time and the list of ML clusters and entities) were exported from R.

TCS is a clustering method that calculates a distance matrix for pairwise comparison of haplotypes to recognize species boundaries, while calculating mutational differences at an assigned cutoff probability. Parsimony criterion applies before the mutational differences reach the cutoff percentage (Templeton et al., 1992). In this study, we evaluated two connection limits (90 and 95%) for species delimitation.

Haplotype network analysis

A haplotype network analysis was used to visualize the relationships among COI haplotypes of *Pratylenchus* species based on their geographic and host information. Haplotype networks were calculated using TCS plug-in (Templeton et al., 1992; Clement et al., 2002) in the software PopART 1.7 (Leigh and Bryant, 2015) using a 95% connection limit.

Divergence time estimation analysis

Molecular divergence time was estimated with a molecular clock analysis using the non-redundant COI data set in BEAST v2.5.2 (Bayesian evolutionary analysis by sampling trees) (Bouckaert et al., 2014). Because no fossil or geological calibration points are available for dating the *Pratylenchus* phylogeny, we used two mutation/clock rates from the literature. The first was a widely used substitutions/site/my/lineage clock rate of 0.0115 corresponding to a common invertebrate COI mitochondrial pairwise sequence divergence rate of 2.3% per site per million years (Brower, 1994). The second was the mitochondrial substitution genome rate of 7.2×10^{-8} per site per generation experimentally calculated for the nematode *Caenorhabditis briggsae* (Dougherty & Nigon, 1949) Dougherty, 1953 (Howe et al., 2010). For *Pratylenchus,* an assumption of two generations per year was applied to the analyses. The BEAST analysis was carried out under a fixed strict clock model with a yule speciation model as tree prior, all other parameters were set as defaults. Markov Chain Monte Carlo (MCMC) chain was run at 30 M generations and sampled every 3,000th generation. Tracer V1.7 (Rambaut et al., 2018) was used to visualize convergence and evaluate effective sample size (ESS > 200) of traces. A maximum clade credibility tree was produced by TreeAnnotator V2.5.2 (Rambaut et al., 2018) with common ancestor node heights, after discarding 25% of the trees as burn-in. The MCMC tree and node ages were visualized using FigTree V.1.4 (tree.bio.ed.ac.uk). Beast analysis was conducted on the XSEDE server of the CIPRES Science Gateway (Miller et al., 2011).

Results

Survey results

A total of 860 soil samples were assayed during the growing seasons of 2017 and 2018. These samples represented statewide surveys of seven major agronomic crops (wheat, corn, dry beans, barley, alfalfa, sugar beets, and potatoes) in Colorado, Kansas, Montana, Nebraska, North Dakota, and Wyoming, as well as four additional crops (soybean, cotton, apple, and vineyard) opportunistically sampled across the Great Plains Region. In total, *Pratylenchus* species were recovered from approximately 71% of all samples. Prevalence of *Pratylenchus* spp. were 89.30% for corn fields (86.40% in Nebraska, 63.60% in Montana, 94.90% in Kansas, and 87.50% in Colorado), 53.15% of wheat fields (100% in Nebraska, 20% in Montana, 37% in North Dakota, 80% in Wyoming, and 80.30% in Kansas), and 41.25% of potato fields (56.50% in Nebraska, 23.80% in North Dakota, and 93.75% in Wyoming).

Phylogenetic analysis and nematode identification

For DNA analysis, nematodes from fields in Nebraska, Kansas, Wyoming, North Dakota, Montana, and Colorado were chosen to maximize geographic and host coverage across the region (Table 1). Samples from outside the Great Plains were added for phylogenetic context including topotype specimens of *P. alleni* isolated from a soybean field five miles north of Eldorado City, in Saline County in Illinois. A total of 915 specimens of *Pratylenchus* were sequenced for a 727 to 739-bp fragment of the COI gene. The sequence length was 730-bp for most of the specimens, except *P. neglectus* (727-bp), and *Pratylenchus* sp. 9 and *Pratylenchus* sp. 10 (739-bp). The initial set of 915 *Pratylenchus* COI haplotypes was reduced to a 143 unique-sequence data set by the removal of redundant sequences. Maximum likelihood, neighbor-joining, and Bayesian phylogenetic trees each identified 19 distinct COI haplotype groups that were well-supported by bootstrap values (BS: 96-100% based on tree construction method), intra- vs interspecific genetic distance and posterior probabilities (PP:100 for all groups) (Fig. 2). These haplotype groups were tentatively labeled as *P. neglectus*, *P. scribneri*, *P. thornei*, *P. hexincisus*, *P. alleni*, *P. penetrans*, *P. zeae* Graham, 1951, *P. crenatus* Loof, 1960, *P. vulnus* Allen & Jensen, 1951, *Pratylenchus* sp. 1 to sp. 10, including one unnamed singleton, NID 6402 from

Table 1. Number of fields sampled from states of the Great Plains region used in DNA barcoding study of *Pratylenchus* spp.

States	Corn	Wheat	Dry beans	Potatoes	Barley	Alfalfa	Sugar beets	Total field	Field %
Nebraska	97	8	0	5	0	0	0	110	44.72
Kansas	43	19	0	0	0	1	0	63	25.61
Wyoming	0	0	18	8	3	0	0	29	11.79
North Dakota	0	19	0	0	0	0	0	19	7.72
Montana	6	0	0	1	4	4	0	15	6.10
Colorado	5	0	0	2	0	1	2	10	4.07
Total field	151	46	18	16	7	6	2	246	
Field %	61.38	18.70	7.32	6.50	2.85	2.44	0.81		

Kansas. Species identity was based on a combination of factors including distinctive morphological features, agreement with GenBank vouchers, congruence between independent markers, and in the case of *P. alleni*, topotype specimens. Four haplotype groups in Figure 2 consisted exclusively of specimens collected outside the Great Plains. These were *P. crenatus* from Ireland, Poland, and Puerto Rico, *P. vulnus* from California, and species 9 and 10, both exclusively represented by specimens from Arkansas. Among the 19 haplotype groups, males were recorded in seven haplotype groups including *P. penetrans*, *P. alleni*, *P. crenatus* and haplotype groups sp. 1, sp. 2, sp. 9, and sp. 10. In total, 14 haplotype groups and one singleton (NID 6402) were associated with agroecosystems of the Great Plains Region.

DNA sequences of the D2-D3 region of the 28S rRNA were evaluated in a phylogenetic tree using 24 specimens previously sequenced for the COI gene together with 70 additional sequences retrieved from GenBank (Supplementary Figure 1). Of the 24 sequences, 10 were placed within well-supported clades representing named species of *Pratylenchus*. These include *P. alleni* (NIDs 10848, 10850, 3717), *P. crenatus* (NID 8539), *P. thornei* (NIDs 7566, 7567), *P. neglectus* (NID 10756), and *P. penetrans* (NID 7091, 6260, 6261). Sequences of the D2–D3 region for the *Pratylenchus* species sp. 1, sp. 2, sp. 5, sp. 8, and sp. 9 were not closely associated with known *Pratylenchus* species in the NJ, ML, or Bayesian phylogenetic trees.

A BLAST search on GenBank with *Pratylenchus* COI sequences generated in this study helped to determine species identity through near identical matches (99-100%) for seven of the COI haplotype groups (Table 2). These well-supported haplotype groups corresponded to *P. neglectus*, *P. penetrans*, *P. thornei*, *P. alleni*. *P. zeae*, *P. crenatus*, and *P. vulnus*. *Pratylenchus scribneri*, the second most abundant *Pratylenchus* species in this survey, provided conflicting results in GenBank BLAST searches. For specimen NID 7839, the percentage identity of the top 9 matches ranged from 98.5% (*P. scribneri* KY424092) to 99.5% (*P. scribneri* MH016378) and bracket two close matches to *P. hexicusus* of 98.7% (KY828320 and KY828322). Similarly, for the D2-D3 domains of the 28S rRNA, the top 25 matches for *P. scribneri* (percentage identity range from 99.7% to 98.1%), encompassed a range that included three *P. hexincisus* entries of 99.7%. Specimens hypothesized to be *P. hexincisus* had no close COI match identified as *P. hexincisus* (Table 2; Ozbayrak et al. in prep). For the D2-D3 domains, those specimens exhibited a relatively close match to accessions representing *P. hexincisus* (KY828291–98.9% similarity) and *P. scribneri* (KX842632–100% similarity). In total, 10 haplotype groups with no clear taxonomic affinities based on DNA sequences were solely identified as *Pratylenchus* 'sp.' These specimens had low percentage identity scores for the COI gene and moderate or ambiguous scores for the D2–D3 domains.

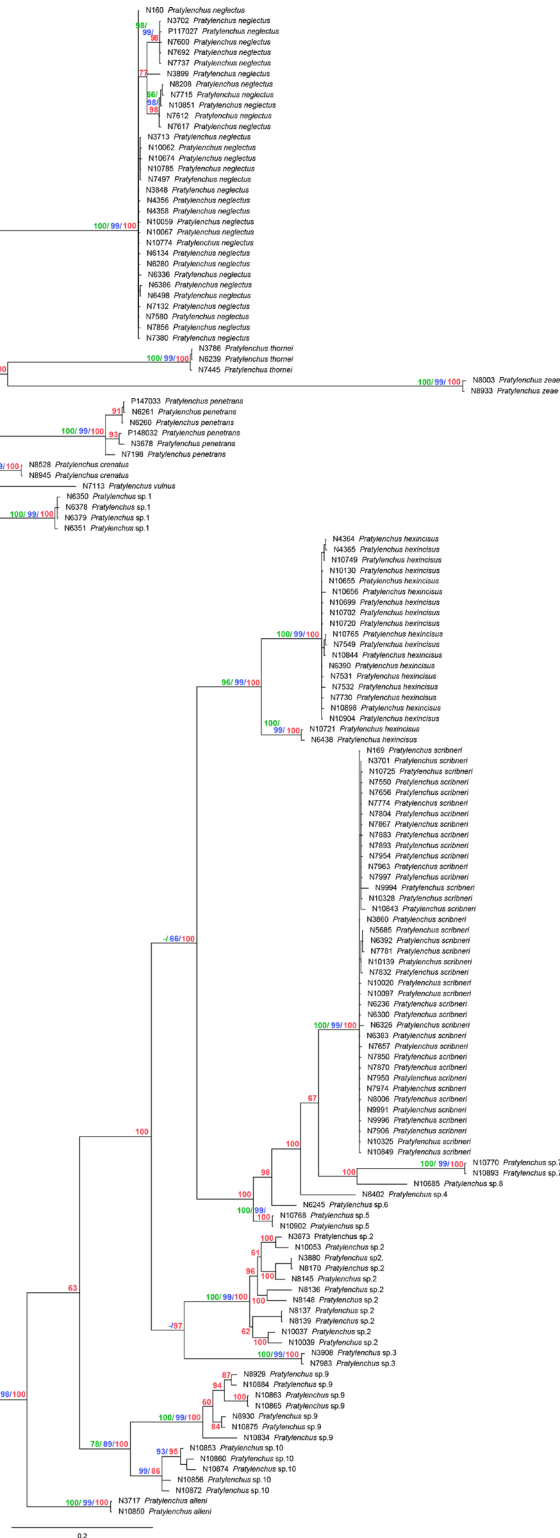Intragroup and intergroup genetic distance matrices are presented in Table 3. Estimated mean

Figure 2: Bayesian tree of unique COI haplotypes derived from 915 *Pratylenchus* specimen data set. Nodes are labeled according to tree building approach, with bootstrap values indicated in green for neighbor-joining, blue for maximum-likelihood, and red for posterior probability in Bayesian analysis. The absence of a value reflects the absence of that node, or bootstrap values below 50 in a particular tree building approach. Terminal nodes are labeled by Nematode Identification Numbers (NIDs) and taxon name.

Table 2. Haplotype group representatives and their highest identity matches from GenBank as of August 29, 2019.

Haplotype group (this study)	Blasted NID No. (this study)	COI Match Accession No.	Corresponding GenBank Name	Identity%	D2–D3 Match Accession No.	Corresponding GenBank Name	Identity%
P. neglectus	N10756	KY424103	P. neglectus	99.8	MG906766	P. neglectus	99.7
P. penetrans	N6260	KY816936	P. penetrans	99.8	JX046986	P. penetrans	98.9
P. thornei	N7566	KY828316	P. thornei	99.5	KYT213559	P. thornei	100
P. alleni	N3717	MK045330	P. alleni	99.3	MN251270	P. alleni	100
P. zeae	N8934	KY424056	P. zeae	99.5			
P. crenatus	N8539	KY816943	P. crenatus	100	KY468865	P. crenatus	98.1
P. vulnus	N7113	GQ332425	P. vulnus	99.3			
P. scribneri	N7839	MH016378	P. scribneri	99.5	MK292133 KY828290 EU130841	P. scribneri P. hexincisus P. agilis	99.7 99.7 99.7
P. hexincisus	N7726	MH016378	P. scribneri	81.0	KX842632 EU130841 KY828291	P. scribneri P. agilis P. hexincisus	100 98.8 98.9
Pratylenchus sp. 1	N6350	KU198944	Pratylenchus sp.	83.0	MN251269	Pratylenchus sp.	99.6
Pratylenchus sp. 2	N8139	MK045330	P. alleni	79.3	MN251273	Pratylenchus sp.	100
Pratylenchus sp. 3	N3908	MK045330	P. alleni	83.4			
Pratylenchus sp. 4	N6402	KY424092	P. scribneri	88.6			
Pratylenchus sp. 5	N10767	KY424092	P. scribneri	87.3	MH730449	P. scribneri	99.2
Pratylenchus sp. 6	N6245	KY424092	P. scribneri	88.5			
Pratylenchus sp. 7	N10770	KY424093	P. scribneri	85.2			
Pratylenchus sp. 8	N10685	KY424092	P. scribneri	89.1	KT175531	P. pseudocoffeae	96.2
Pratylenchus sp. 9 (1)	N10841	KY424092	P. scribneri	80.1	KT175531	P. pseudocoffeae	95.8
Pratylenchus sp. 9 (2)	N8930	KY424092	P. scribneri	79.7	KT175531	P. pseudocoffeae	95.7
Pratylenchus sp. 10 (1)	N10856	MK045330	P. alleni	78.7			
Pratylenchus sp. 10 (2)	N10853	MK045330	P. alleni	79.5			

Table 3. Estimates of evolutionary divergence (p-distances) over sequence pairs within (bold) and between groups.

	P. alleni	P. crenatus	P. hexincisus	P. neglectus	P. penetrans	P. scribneri	P. thornei	P. vulnus	P. zeae	sp. 1	sp. 2	sp. 3	sp. 4	sp. 5	sp. 6	sp. 7	sp. 8	sp. 9	sp. 10
P. alleni	**0**																		
P. crenatus	0.308	**0.001**																	
P. hexincisus	0.216	0.355	**0.022**																
P. neglectus	0.28	0.33	0.362	**0.009**															
P. penetrans	0.322	0.348	0.399	0.298	**0.038**														
P. scribneri	0.172	0.327	0.199	0.317	0.351	**0.003**													
P. thornei	0.332	0.357	0.391	0.3	0.319	0.359	**0**												
P. vulnus	0.235	0.328	0.321	0.317	0.325	0.29	0.33	**0.007**											
P. zeae	0.33	0.377	0.399	0.334	0.354	0.367	0.329	0.348	**0.002**										
sp. 1	0.209	0.3	0.283	0.304	0.332	0.231	0.326	0.263	0.327	**0.005**									
sp. 2	0.228	0.357	0.222	0.373	0.396	0.232	0.389	0.342	0.409	0.294	**0.077**								
sp. 3	0.182	0.352	0.231	0.324	0.357	0.189	0.359	0.291	0.344	0.223	0.205	**0.004**							
sp. 4	0.174	0.316	0.207	0.316	0.331	0.118	0.348	0.274	0.356	0.219	0.233	0.186	**n/c[a]**						
sp. 5	0.195	0.33	0.181	0.334	0.352	0.125	0.359	0.302	0.361	0.253	0.209	0.199	0.132	**0**					
sp. 6	0.187	0.334	0.197	0.315	0.358	0.108	0.354	0.296	0.364	0.236	0.223	0.191	0.112	0.079	**0**				
sp. 7	0.171	0.299	0.212	0.3	0.333	0.153	0.327	0.256	0.341	0.199	0.235	0.185	0.16	0.163	0.159	**0**			
sp. 8	0.165	0.308	0.221	0.299	0.345	0.12	0.327	0.278	0.359	0.204	0.254	0.196	0.127	0.158	0.132	0.136	**0**		
sp. 9	0.202	0.33	0.244	0.338	0.353	0.206	0.347	0.265	0.361	0.237	0.246	0.221	0.2	0.201	0.202	0.207	0.206	**0.048**	
sp. 10	0.195	0.342	0.214	0.35	0.35	0.211	0.351	0.262	0.372	0.25	0.212	0.226	0.207	0.208	0.21	0.216	0.213	0.156	**0.034**

The number of base differences per site from averaging over all sequence pairs between groups are shown. The analysis involved 915 nucleotide sequences. [a]Not possible to estimate evolutionary distances.

genetic raw distance (p-distances) for all haplotype pairs was 0.20. Pairwise genetic distances within most haplotype groups ranged from 0 to 0.048. Haplotypes groups *Pratylenchus* sp. 2 and *Pratylenchus* sp. 9 exhibited the highest intraspecific diversity with distance values of 0.077 and 0.048, respectively. Interspecific variability among haplotype groups was exceptionally high, ranging from a low of 0.079 between haplotypes *Pratylenchus* sp. 5 and *Pratylenchus* sp. 6 to 0.409 between haplotypes *P. zeae* and *Pratylenchus* sp. 2.

Species delimitation

Molecular species delimitation using ABGD suggested 19 to 61 haplotype groups in the recursive partitioning based on differing intraspecific divergence priors (Fig. 3). The last three partitions settled on 19 groups with *P*-values of 0.0359, 0.0599, and 0.100, respectively. ABGD derived groupings at these partitions were congruent with the haplotype groups on phylogenetic trees.

Both GYMC and TCS identified multiple subdivisions within the 19 groups derived by ABGD and the highly supported clades in the phylogenetic trees. The single threshold GMYC analysis revealed 22 ML clusters (CI 22–23) and 39 ML entities (CI 39–42) when using a strict molecular clock and yule tree prior (Fig. 3). The likelihood for the null model was 972.166 whereas the maximum likelihood of the GMYC model was 998.4352. The likelihood ratio (52.5385) test rejected the null hypothesis for the models tested, and thus the assumption that all sequences belonged to the same species (LR test: *P* < 0.001). TCS analysis resulted in 36 and 32 haplotype networks at connection limits of 95 and 90%, respectively, for the complete data set (Fig. 3).

The estimation of divergence time of the major haplotype groups was investigated using BEAST using two different molecular clock rates to calibrate dates of lineage divergence (Fig. 4). The nematode molecular clock rate derived from *Caenorhabditis briggsae*, using an assumption of two generations per year for *Pratylenchus*, resulted in younger node ages than the insect substitution rate. Divergence time estimation based on the nematode clock rate revealed an early split of *Pratylenchus* clades occurred at an estimated 6.28 Mya (CI: 5.12–7.59 Mya) in the late Miocene epoch. *Pratylenchus neglectus* may have diverged from *P. penetrans* as early as 4.73 Mya (CI: 3.56–5.61 Mya), and the split of *P. thornei* from *P. zeae* at an estimated 5.39 Mya (CI: 3.38–5.47 Mya).

Later *Pratylenchus* divergences occurred in the Pliocene epoch at approximately 2.7 Mya, prior to the onset of the ice ages. The most recent common ancestor (MRCA) of *P. hexincisus* and *P. scribneri* emerged in the mid-Pleistocene approximately 1.46 Mya (CI: 1.24–1.69 Mya). The MRCA of *P. scribneri*, a relatively young clade, emerged in the late Pleistocene epoch at 0.65 Mya (0.48–0.73 Mya). Three lineages of sexually reproducing species, *Pratylenchus* sp. 9, *Pratylenchus* sp. 10, and *P. alleni*, all diversified during early to mid-stages of the ice ages between 2 million and 500,000 years ago (Fig. 4).

COI haplotype group host associations and distribution

The most frequently sampled haplotype group in the Great Plains was *Pratylenchus neglectus*, comprising 53% of all specimens. *P. neglectus* was identified in 96, 90, and 83% of all wheat, potato, and dry bean fields, respectively. On the other hand, this haplotype group was encountered in corn fields less frequently, collected in 42% of the corn fields across the Great Plains (Fig. 5). Four distinct haplotype networks labeled A, B, C, and D were recognized for *P. neglectus* at both the 95 and 90% connection limit. These four *P. neglectus* subgroups were also detected in GMYC delimitation methods. Network A consisted of eight unique haplotypes (or subgroups), occurring in 10 states of North America and Canada (Fig. 6A). The most abundant haplotypes were neg1, neg2, and neg3 within network A. Haplotype neg1 specimens were found in six states and were most often associated with corn, but also associated with dry bean, wheat, alfalfa, potatoes, and cereal rye. Haplotype neg2 and neg3 specimens were found in 10 and 9 states, respectively, as well as Canada, and were most frequently recovered from wheat, but also associated with other crops (Fig. 6B). A total of six haplotypes from networks B, C, and D were located in western and northwestern states of Colorado, Wyoming, Idaho, Montana, and North Dakota with only a few specimens from Nebraska and Kansas included in these networks (Fig. 6A). Specimens in networks B, C, and D were associated with barley, potatoes, alfalfa, dry beans, corn, wheat, and sugar beets (Fig. 6B).

The second most abundant haplotype group in the Great Plains, comprising 30% of all specimens, was *P. scribneri*. This haplotype group appeared as a single TCS network comprised of 17 closely related haplotypes (or subgroups) (Fig. 7). These haplotypes were recovered from 104 fields (45 counties) and were associated most often with corn fields in 4 states; 78 in Nebraska, 14 in Kansas, 2 in South Dakota, and 1 in Montana. Also, they were recorded
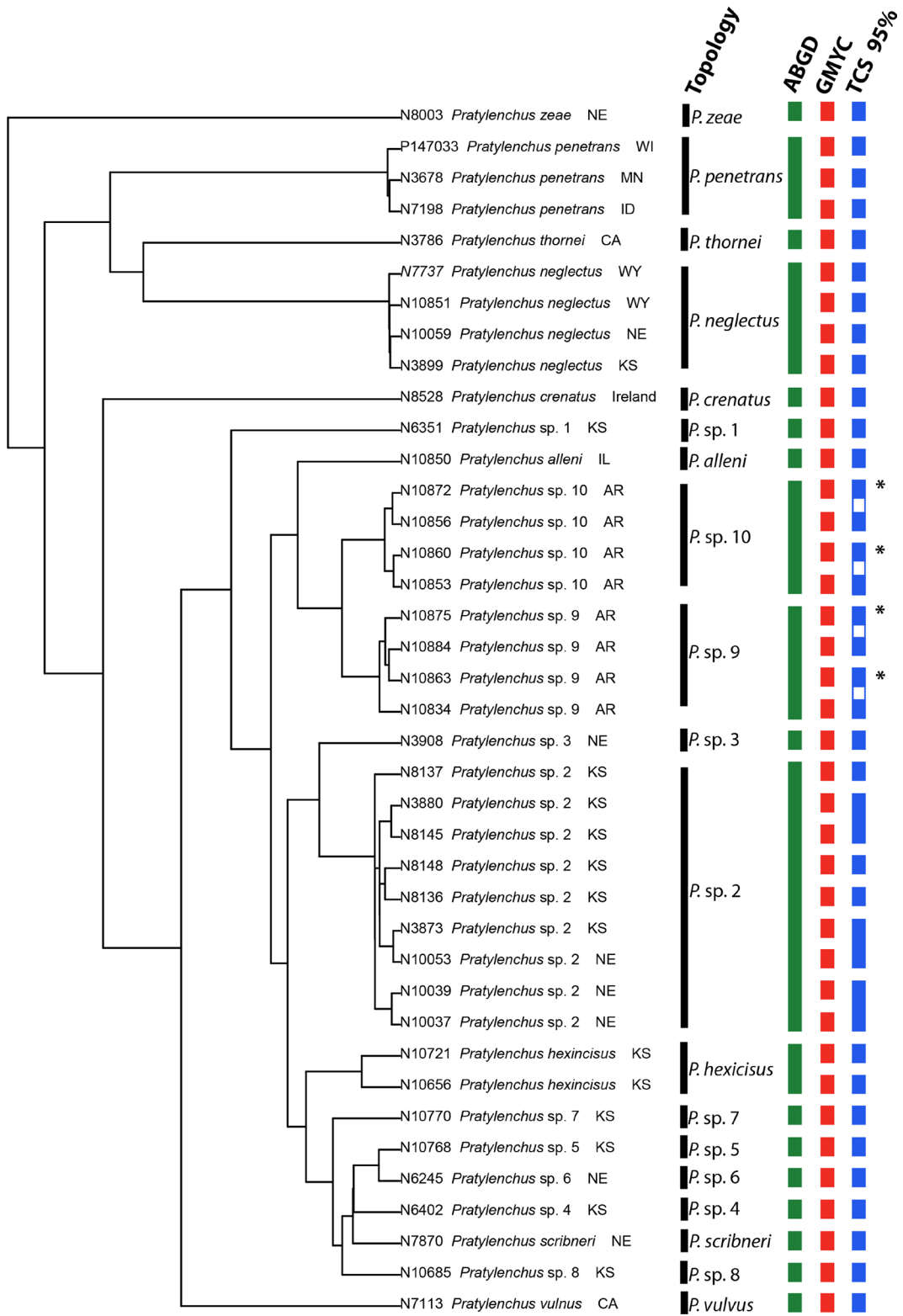
Figure 3: Maximum likelihood tree of haplotype groups reflecting group membership support based on consensus tree topology, and species delimitation methods. These methods are indicated by colored bars: Automatic Barcoding Gap Discovery (ABGD) green bar, Generalized Mixed Yule Coalescent (GMYC) red bar, and statistical parsimony analysis (TCS) blue bar. TCS groups marked with an asterisk indicate a merging of two groups at a 90% connection limit.
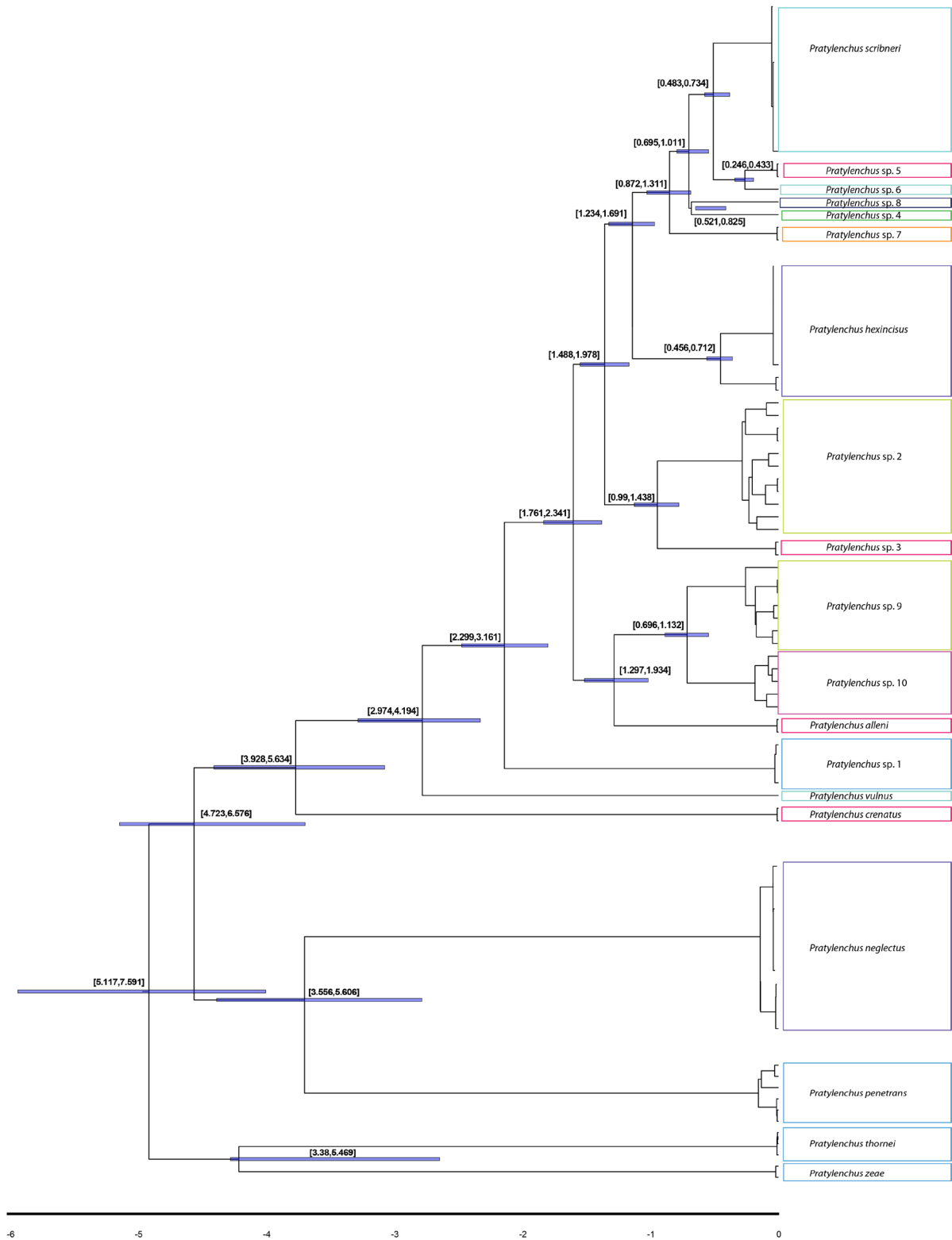
Figure 4: Bayesian evolutionary analysis by sampling trees (BEAST) analysis of estimated divergence times using the mitochondrial substitution genome rate of 7.2×10^{-8} per site per generation, a calibration originally calculated for the nematode *Caenorhabditis briggsae* (Howe et al., 2010). The rate assumes two *Pratylenchus* generations per year. Divergence age confidence intervals of 95% are provided at nodes.
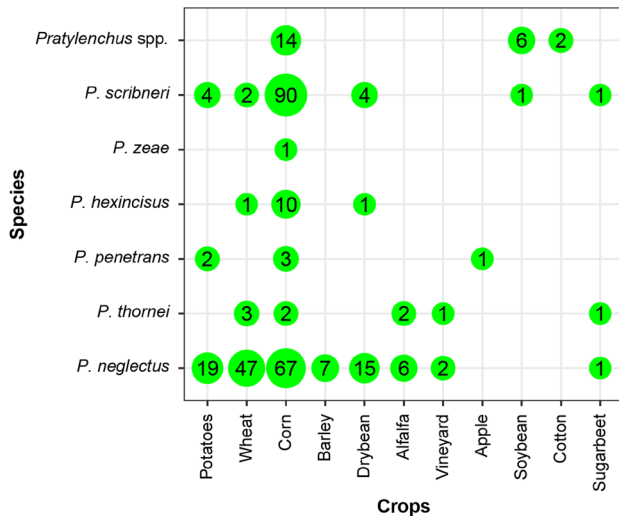
Figure 5: Matrix exhibiting *Pratylenchus* species of the Great Plains and their host associations. The number of fields with a specific plant host-*Pratylenchus* species combination is indicated by the number within the green circles.

from four potato fields in Nebraska, one wheat field in both Nebraska and Texas, and a sugar beet field in Colorado (Fig. 5).

Pratylenchus thornei was comprised of a single haplotype distributed across six different states (Fig. 8A). *P. thornei* was primarily associated with wheat in Kansas but was also collected from corn and alfalfa in Montana, corn in Oklahoma, a single plot in a cover crop (cereal rye) experiment in Nebraska, in a vineyard in California, and a sugar beet field rotated with barley in Colorado.

Pratylenchus hexincisus specimens were split into two separate groups in TCS networks and GMYC methods (Fig. 8B), but ABGD only recognized a second network at *P*-values between 0.008 and 0.022 (Fig. 8). *Pratylenchus hexincisus* was primarily associated with corn fields in eastern Kansas, Nebraska, and South Dakota, as well as from dry beans in Wyoming and wheat in North Dakota.

Four haplotype groups with male specimens, *P. penetrans*, *Pratylenchus* sp. 2, sp. 9, and sp. 10, displayed diverse TCS networks and multiple haplotype subgroups in GMYC methods. The final partition in ABGD, however, recognized these four sexual groups as four distinct entities. For *P. penetrans*, ABGD revealed two distinct groups at *P*-values of 0.0359, 0.0599, and 0.100. Six haplotypes were detected, suggesting three distinct networks (networks not shown) in TCS analyses at both 90 and

95% cutoff values, and three entities were revealed in GMYC analysis. *Pratylenchus penetrans* was not common in the agronomic crops sampled in the Great Plains region. It was recovered from one south central cornfield in Nebraska, an apple orchard in eastern Nebraska, and two corn fields in Montana. Species less commonly found in this Great Plains survey included *P. zeae* and *P. alleni*, both collected from single corn fields in Nebraska.

Eleven *Pratylenchus* sp. 2 haplotypes comprised six networks at 95 and 90% connection limits, respectively, and GMYC methods exhibited nine entities. *Pratylenchus* sp. 9 and sp. 10 had four networks at 95% cutoff and two at 90% cutoff, respectively. GMYC analysis revealed four entities for both groups. Species delimitation results for other groups exhibited one network per haplotype per entity due to the existence of few specimens in these groups.

Nearly all haplotypes representing unknown *Pratylenchus* species were associated with corn, with the exception of one wheat, one soybean, and one cotton field. Barcoding also revealed that 44 of the 439 *Pratylenchus*-infested fields had a mixed population of at least two *Pratylenchus* species. Mixtures were recorded in 19.5, 10.6, and 27.8% of the corn, wheat, and dry bean fields, respectively. Five corn fields in Kansas and 23 corn fields in Nebraska had a mixture of different haplotype groups. The most common combination of species was *P. neglectus* and *P. scribneri*, which were recovered together from approximately 55% of all mixed fields (Fig. 9).

Discussion

This study is the first comprehensive survey using COI DNA barcode to evaluate *Pratylenchus* species diversity and haplotype associations with the major agroecosystems of the Great Plains region of North America. This study also provides details on phylogenetic membership in haplotype groups, relationships among *Pratylenchus* haplotype groups, their geographic distribution and host associations with crops from 11 states across the region. Earlier studies reported the presence of nine described *Pratylenchus* species associated with prairie and agricultural fields (Orr and Dickerson, 1967; Thorne and Malek, 1968; Smolik and Evenson, 1987; Yan et al., 2016; Al-Khafaji, 2018). This DNA barcoding survey detected seven distinct haplotype groups representing known *Pratylenchus* species: *P. neglectus, P. scribneri, P. thornei, P. hexincisus, P. alleni, P. penetrans, P. zeae,* and eight unnamed haplotype groups and one unnamed singleton, associated with Great Plains agroecosystems. Posterior probability, bootstrap
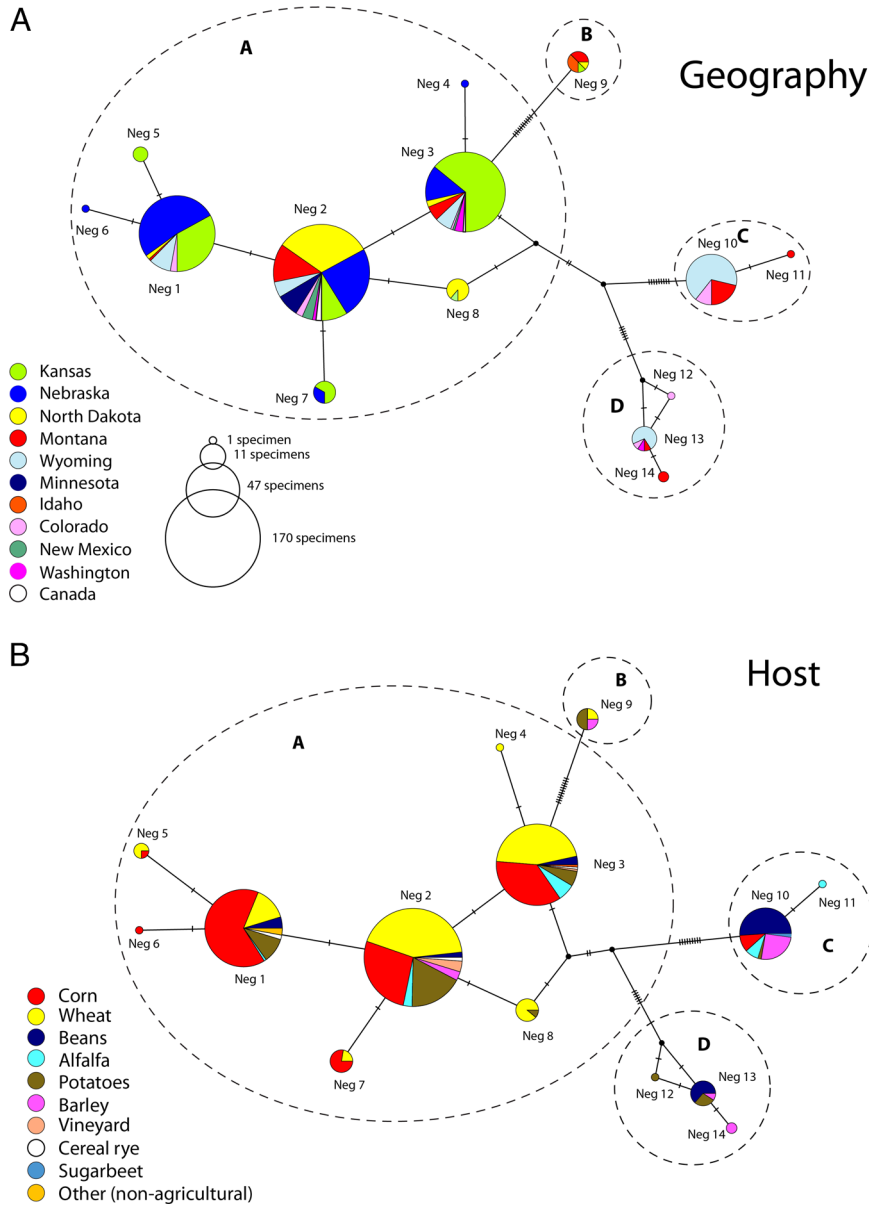
Figure 6: TCS networks depicting *Pratylenchus neglectus* haplotype abundance and relationships among haplotypes. Dashed lines encircling haplotypes indicate 95% connection limits. Hash marks between haplotypes indicate number of mutational steps between haplotypes. Relative size of the circles reflects abundance of specimens exhibiting that specific haplotype. (A) Colors indicate geographic origin of the haplotypes in the *Pratylenchus* survey; (B) colors indicate the host in the field at time of the survey.

values, genetic distances, and calculations of lineage age strongly supported the genetic distinction of the haplotype groups. Group membership remained constant with different tree building methods, although relationships at deeper nodes in the tree varied slightly. Intraspecific genetic divergence was found to be low for most of the haplotype groups, but substantial

genetic differentiation within haplotype groups was recognized by GMYC and TCS approaches. Four haplotype groups with males (*P. penetrans*, *Pratylenchus* sp. 2, sp. 9, and sp. 10) had relatively high within-group genetic variability. The overall mean intergroup divergence was high, suggesting a relatively long period since the *Pratylenchus* lineages
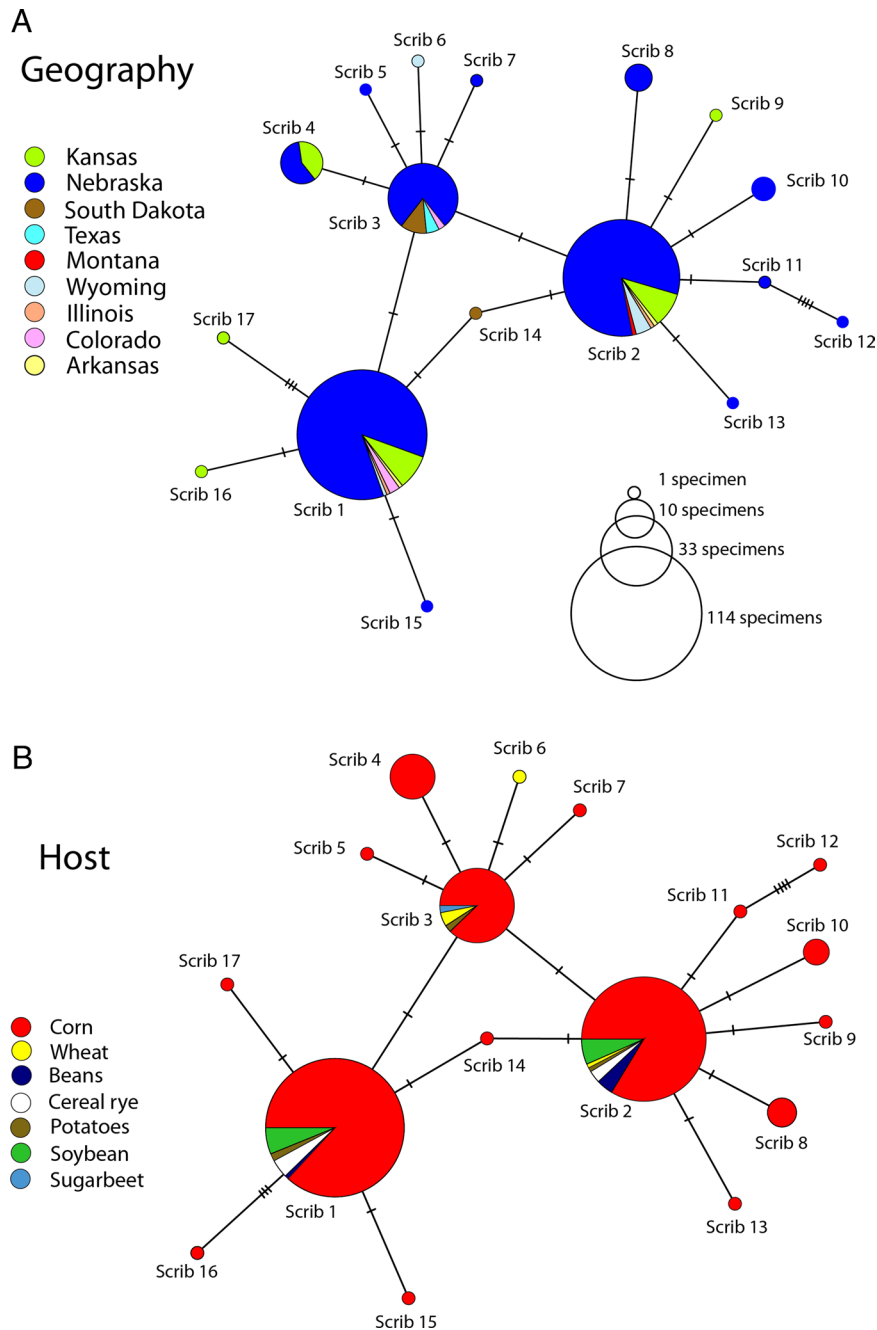
Figure 7: TCS network at 95% connection limit depicting *Pratylenchus scribneri* haplotype abundance and relationships among haplotypes. Hash marks between haplotypes indicate number of mutational steps between haplotypes. Relative size of the circles reflects abundance of specimens exhibiting that specific haplotype. (A) Colors indicate geographic origin of the haplotypes in the *Pratylenchus* survey; (B) colors indicate the host in the field at time of the survey.

diverged. Divergence times of *Pratylenchus* species in this study were estimated using a higher evolutionary rate than is commonly used in COI mitochondrial DNA studies (Brower, 1994; Howe et al., 2010). The

higher substitution rate was derived from studies with *C. briggsae* and resulted in a rate higher than the standard rate derived from insects. Still, the calculated rate of evolution supported the age of divergence
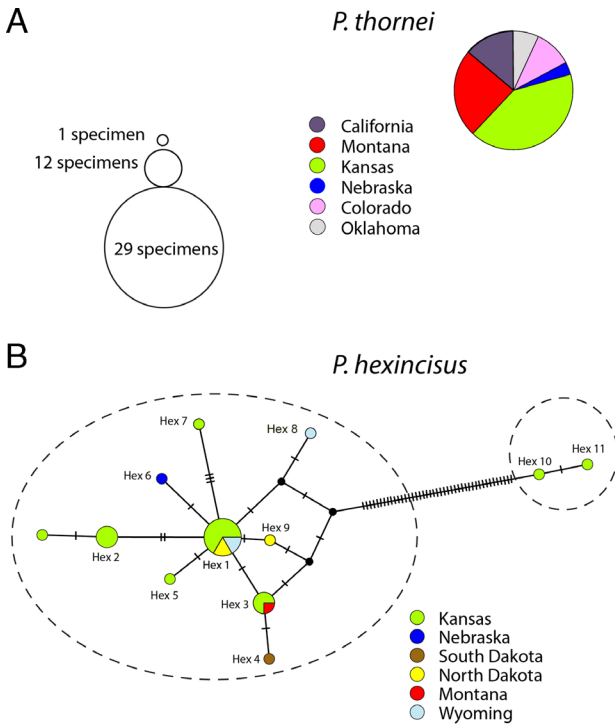
Figure 8: TCS networks depicting (A) the single haplotype of *Pratylenchus thornei* and (B) the two networks exhibited by *Pratylenchus hexincisus*. Dashed lines encircling haplotypes indicate 95% connection limits. Hash marks between haplotypes indicate number of mutational steps between haplotypes. Relative size of the circles reflects abundance of specimens exhibiting that specific haplotype. Colors indicate geographic origin of the haplotypes in the *Pratylenchus* survey.

among the major species lineages in the Great Plains at approximately 1 to 5 million years ago.

Species delimitation analysis displayed variation in determining the number of putative species. GMYC and TCS methods generally yielded similar results and supported the recognition of subgroups as species within the haplotype groups in Figure 3. Conversely ABGD generally mirrored the tree topologies at *P* value of 0.0359, 0.0599, and 0.100. GMYC and TCS identified *P. scribneri, P. thornei, P. alleni, P. crenatus*, and most of the unknown groups as independent lineages or evolutionary entities but split *P. neglectus*, *P. penetrans*, *Pratylenchus* sp. 2, *Pratylenchus* sp. 9, and *Pratylenchus* sp. 10 into two or more putative species.

Some *Pratylenchus* species previously reported from the Great Plains were not observed in this study. *Pratylenchus tenuis* is known only from its type locality of Avon, South Dakota (Thorne and Malek, 1968). Handoo and Golden (1989) re-described the species, based on two female type specimens. The distinctive characters of this species were high, narrow tulip-shaped stylet knobs and an unusually elongate esophageal lobe three times the body width. It was not possible to assign any of the haplotype groups to this species based on those characters. *Pratylenchus flakkensis*, reported from corn in Iowa by Norton (1983), is represented in GenBank by two partial COI sequences that did not match any COI sequence generated in this survey.

There are several key conclusions that can be drawn from this DNA barcode-based survey on *Pratylenchus* diversity in the Great Plains. First, *P. neglectus* is the most widespread and abundant lesion nematode across the region. It was recorded from field soils currently producing alfalfa, barley, corn, dry beans, potato, wheat, and sugar beets. Although presence in the field is not direct evidence of parasitism on the current crop, and most agronomic crops in the region are grown in rotation with other plants, it is a safe assumption that active *Pratylenchus* populations found around the roots during a growing season are feeding on those roots. This observation complements the findings of Al-Khafaji (2018) and May et al. (2016) concerning the widespread presence of *P. neglectus* on wheat in Montana, as well as the studies of Todd and Oakley (1996) and Todd et al. (2014) that documented the high incidence of *P. neglectus* in corn and wheat fields in Kansas. *Pratylenchus neglectus* was also observed as a frequent member of mixed species populations, often associated with *P. scribneri,* another common inhabitant of corn fields in the region (Smolik and Evenson, 1987; Todd, 1991). It is unknown to what extent a mixed population will compromise management strategies, but it makes sense that understanding host relationships and damage potential of all species in a population will lead to better predictive models for pest management.

Pratylenchus scribneri is the second most prevalent nematode in the region, and the lesion species most likely to be recovered in the corn-soybean cropping rotations of Nebraska and Kansas. In contrast, it was not recovered frequently from wheat, suggesting a reduced reproductive capacity or an inability to successfully compete with *P. neglectus* on wheat. Another species known to reproduce on wheat, but not frequently encountered in the Great Plains region is *P. thornei*. This species was not common in Kansas wheat samples, collected from cereal rye in a single experimental
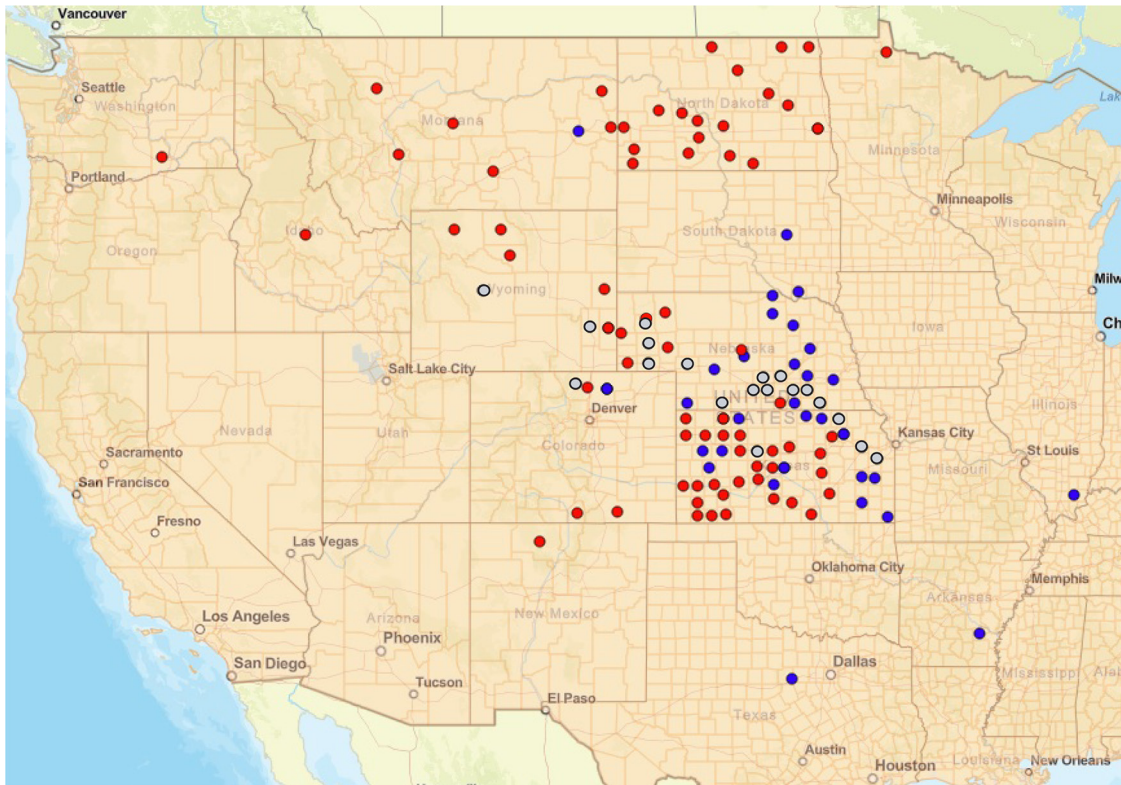
Figure 9: County location of surveyed fields with *Pratylenchus neglectus* (red), *P. scribneri* (blue), and fields with a mixture of both species (gray).

plot in central Nebraska, found in Colorado in a mixed planting of oats and alfalfa, and was not recovered at all in North Dakota wheat fields despite widespread sampling in the state. Smiley et al. (2005) found *P. thornei* exclusively in 6% of wheat fields in the Pacific Northwest, and in combination with *P. neglectus* in 30% of the soils. In the Great Plains, *P. thornei* may be limited by soil factors as suggested by Thompson et al. (2010), or possibly there has been insufficient time for the species to spread across the region since its introduction. The latter explanation is supported by the lack of sequence polymorphism among Great Plains specimens of *P. thornei*. *Pratylenchus penetrans* is another species that is surprisingly limited in its distribution within the Great Plains. Among Great Plains agronomic crops, *P. penetrans* was only recovered from a single field of corn in Nebraska. Two other species with highly localized distributions were *P. zeae* and *P. alleni*. *Pratylenchus zeae* was collected from a single corn field in Keith County, Nebraska, and outside the Great Plains region it occurred in Arkansas corn fields. *Pratylenchus alleni* was collected from a soybean field in Illinois on its type host at its type locality in

Saline County. In spite of extensive production of corn and soybeans grown in rotation throughout the region, outside of the type locality, *P. alleni* was only found in a single corn field in Madison County, Nebraska. It is possible that a focused survey on soybeans and potato will increase the documented distribution of both *P. alleni* and *P. penetrans* within the Great Plains.

Some of the COI haplotype groups revealed by the phylogenetic analysis were not easily associated with a known species of *Pratylenchus*. For example, the species we tentatively identified as *P. hexincisus* had no close match for COI in GenBank, and D2–D3 sequences provide moderately close matches to both *P. scribneri* and *P. hexincisus*. This haplotype group was collected from six states in the Great Plains associated with beans, corn, and wheat. A more extensive taxonomic analysis of this species is underway (Ozbayrak et al., in prep). Eight other haplotype groups and one singleton were not readily identified as previously described *Pratylenchus* species. One haplotype group, represented by 14 specimens collected from corn in Shawnee County in Kansas and Buffalo County in Nebraska,

was characterized by the relatively frequent presence of males. These specimens superficially resemble *P. penetrans* and may be the species previously reported as *P. penetrans* from eastern Kansas (Orr and Dickerson, 1967; Todd et al., 2014).

This mosaic of *Pratylenchus* species distributed across the Great Plains raises a question about the necessity of identifying species composition in agricultural fields. Management options of *Pratylenchus* species generally fall into four main categories: fallow, crop rotation, genetic resistance, and genetic tolerance (Smiley, 2015). If all these haplotype groups responded in a similar fashion to environmental and physiological conditions, then a common management strategy could be applied for lesion nematodes. However, evidence suggests these haplotype groups may differ in their host preferences, environmental tolerances, and possibly their competitive interactions. The frequency of *P. scribneri* in corn and its absence from most wheat fields indirectly suggests the existence of host preferences (Smiley et al., 2005; Smiley, 2015; Todd and Powers, 2018). More complete characterization of these haplotype groups will require the establishment of pure cultures and analysis of reproductive capabilities on hosts grown in the Great Plains region.

Acknowledgments

References

Acosta, N. and Malek, R. B. 1979. Influence of temperature on population development of eight species of *Pratylenchus* on soybean. Journal of Nematology 11(3):229–32, available at: https://doi.org/10.1016/S1386-1425(02)00236-6

Al-Khafaji, R. T. 2018. An assessment of nematodes affecting wheat in Montana. doctoral dissertation, Montana State University, Bozeman, MT.

Araya, T. Z., Padilla, W. P., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Liébanas, G., Palomares-Rius, J. E. and Castillo, P. 2016. Root lesion nematodes of the genus *Pratylenchus* (Nematoda: Pratylenchidae) from Costa Rica with molecular identification of *P. gutierrezi* and *P. panamaensis* topotypes. European Journal of Plant Pathology 145(4):973–98, available at: https://doi.org/10.1007/s10658-016-0884-z

Askary, T. H., Banday, S. A., Iqbal, U., Khan, A. A., Mir, M. M. and Waliullah, M. I. S. 2012. Plant parasitic nematode diversity in pome, stone and nut fruits, in Lichtfouse, E. (Ed.), Agroecology and strategies for climate change, Springer, Dordrecht, pp. 237–68, available at: https://doi.org/10.1007/978-94-007-1905-7

Bélair, G., Dauphinais, N., Benoit, D. L. and Fournier, Y. 2007. Reproduction of *Pratylenchus penetrans* on 24 common weeds in potato fields in Quebec. Journal of Nematology 39(4):321–6, available at: http://journals.fcla.edu/jon/article/view/67745

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Suchard, M. A., Rambaut, A. and Drummond, A. J. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLOS Computational Biology 10(4):e1003537, available at: https://doi.org/10.1371/journal.pcbi.1003537

Brower, A. V. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences 91(14):6491–5, available at: https://doi.org/10.1073/pnas.91.14.6491

Brown, M. J., Riedel, R. M. and Rowe, R. C. 1980. Species of *Pratylenchus* associated with *Solanum tuberosum* cv Superior in Ohio. Journal of Nematology 12(3):189–92.

Castillo, P. and Vovlas, N. 2007. Pratylenchus (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management, Brill, Boston, MA, available at: https://doi.org/10.1007/s13398-014-0173-7.2

Clement, M., Snell, Q., Walke, P., Posada, D. and Crandall, K. 2002. TCS: estimating gene genealogies. Proceedings 16th International Parallel and Distributed Processing Symposium 2:184, available at: https://doi.org/10.1109/IPDPS.2002.1016585

De Ley, P., Nadler, S., Thomas, W. K., Frisse, L., Felix, M. A. and Sternberg, P. 1999. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1(6):591–612, available at: https://doi.org/10.1163/156854199508559

De Luca, F., Troccoli, A., Duncan, L. W., Subbotin, S. A., Waeyenberge, L., Moens, M. and Inserra, R. N. 2010. Characterisation of a population of *Pratylenchus hippeastri* from bromeliads and description of two related new species, *P. floridensis* n. sp. and *P. parafloridensis* n. sp., from grasses in Florida. Nematology 12(6):847–68, available at: https://doi.org/10.1163/138855410X495809

De Luca, F., Troccoli, A., Duncan, L. W., Subbotin, S. A., Waeyenberge, L., Coyne, D. L., Brentu, F.C. and Inserra, R. N. 2012. *Pratylenchus speijeri* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode pest of plantain in West Africa. Nematology 14(8):987–1004, available at: https://doi.org/10.1163/156854112X638424

Derycke, S., Remerie, T., Vierstraete, A., Backeljau, T., Vanfleteren, J. R., Vincx, M. and Moens, T. 2005. Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. Marine Ecology Progress Series 300:91–103, available at: https://doi.org/10.3354/meps300091

Dickerson, O. J. 1979. The effects of temperature on *Pratylenchus scribneri* and *P. alleni* populations on soybeans and tomatoes. Journal of Nematology 11(1):23–6.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5):1792–7, available at: https://doi.org/10.1093/nar/gkh340

Fanelli, E., Troccoli, A., Capriglia, F., Lucarelli, G., Vovlas, N., Greco, N. and De Luca, F. 2018. Correction to: sequence variation in ribosomal DNA and in the nuclear hsp90 gene of *Pratylenchus penetrans* (Nematoda: Pratylenchidae) populations and phylogenetic analysis. European Journal of Plant Pathology 152:573–4, available at: https://doi.org/10.1007/s10658-018-1489-5

Ferris, V. R. 1961. A new species of *Pratylenchus* (Nemata-Tylenchida) from roots of soybeans. Proceedings of the Helminthological Society of Washington, 28:109–11.

Flis, L., Dobosz, R., Rybarczyk-Mydłowska, K., Wasilewska-Nascimento, B., Kubicz, M. and Winiszewska, G. 2018. First report of the lesion nematodes: *Pratylenchus brachyurus* and *Pratylenchus delattrei* on tomato (*Solanum lycopersicum* L.) plants in Cape Verde. Helminthologia 55(1):88–94, available at: https://doi.org/10.1515/helm-2017-0053

Fujisawa, T. and Barraclough, T. G. 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62(5):707–24, available at: https://doi.org/10.1093/sysbio/syt033

Hammas, C. N., Hedfi, L. H., Regaieg, H., Larayedh, A., Badiss, A., Qing, Y. and Najet, H. R. 2018. First report of *Pratylenchus vulnus* associated with apple in Tunisia. Journal of Nematology 50(4):579–86, available at: https://doi.org/10.21307/jofnem-2018-056

Handoo, Z. A. and Golden, A. M. 1989. A key and diagnostic compendium to the species of the genus *Pratylenchus* Filipjev, 1936 (lesion nematodes). Journal of Nematology 21(2):202–18.

Howe, D. K., Baer, C. F. and Denver, D. R. 2010. High rate of large deletions in *Caenorhabditis briggsae* mitochondrial genome mutation processes. Genome Biology and Evolution 2(1):29–38, available at: https://doi.org/10.1093/gbe/evp055

Huelsenbeck, J. P. and Ronquist, F. 2001. MrBAYES; Bayesian inference for phylogeny. Bioinformatics 17(8):754–5, available at: https://doi.org/10.1093/bioinformatics/17.8.754

Inserra, R. N., Troccoli, A., Gozel, U., Bernard, E. C., Dunn, D. and Duncan, L. W. 2007. *Pratylenchus hippeastri* n. sp. (Nematoda: Pratylenchidae) from amaryllis in Florida with notes on *P. scribneri* and *P. hexincisus*. Nematology 9(1):25–42, available at: https://doi.org/10.1163/156854107779969754

Janssen, T., Karssen, G., Orlando, V., Subbotin, S. A. and Bert, W. 2017. Molecular characterization and species delimiting of plant-parasitic nematodes of the genus *Pratylenchus* from the penetrans group (Nematoda: Pratylenchidae). Molecular Phylogenetics and Evolution 117:30–48, available at: https://doi.org/10.1016/j.ympev.2017.07.027

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48(9):692, available at: www.cabdirect.org/cabdirect/abstract/19650801105

Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Kikuchi, T., Manzanilla-Lopez, R., Palomares-Rius, J. E., Wesemael, W. M. and Perry, R. N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14(9):946–61, available at: https://doi.org/10.1111/mpp.12057

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870–4, available at: https://doi.org/10.1093/molbev/msw054

Leigh, J. W. and Bryant, D. 2015. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9):1110–6, available at: https://doi.org/10.1111/2041-210X.12410

MacGuidwin, A. E. and Bender, B. E. 2015. Development of a damage function model for *Pratylenchus penetrans* on corn. Plant Disease 100(4):764–9, available at: https://doi.org/10.1094/pdis-06-15-0663-re

May, D. B., Johnson, W. A., Zuck, P. C., Chen, C. C. and Dyer, A. T. 2016. Assessment and management of root lesion nematodes in Montana wheat production. Plant disease 100(10):2069–79.

Miller, M. A., Pfeiffer, W. and Schwartz, T. 2011. The CIPRES Science Gateway: a community resource for phylogenetic analyses, Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery, July 18–21, ACM, New York, NY, available at: https://doi.org/10.1145/2016741.2016785

Morgan, G. D., MacGuidwin, A. E., Zhu, J. and Binning, L. K. 2002. Population dynamics and distribution of root lesion nematode (*Pratylenchus penetrans*) over a three year potato crop rotation. Agronomy Journal 94(5):1146–55, available at: https://doi.org/10.2134/agronj2002.1146

Nicol, J. M., Turner, S. J., Coyne, D. L., Den Nijs, L. and Hockland, S. 2011. Current nematode threats to world agriculture, in Jones, J. (Ed.), Genomics and molecular genetics of plant-nematode interactions

Springer, Dordrecht, pp. 21–43, available at: https://doi.org/10.1007/978-94-007-0434-3

Norton, D. C. 1983. Occurrence and distribution of plant parasitic nematodes associated with maize in Iowa. Processing of the Iowa Academy of Science 90(3):88–92.

Olabiyi, T. I., Olayiwola, A. O. and Oyediran, G. O. 2009. Influence of soil textures on distribution of phytonematodes in the South Western Nigeria, World Journal of Agricultural Sciences 5(5):557–60, available at: www.idosi.org/wjas/wjas5(5)/6.pdf

Orr, C.C. and Dickerson, O. J. 1967. Nematodes in true prairie soils of Kansas. Transactions of the Kansas Academy of Science 69(3):317–34.

Palomares-Rius, J. E., Guesmi, I., Horrigue-Raouani, N., Cantalapiedra-Navarrete, C., Liébanas, G. and Castillo, P. 2014. Morphological and molecular characterisation of *Pratylenchus oleae* n. sp. (Nematoda: Pratylenchidae) parasitizing wild and cultivated olives in Spain and Tunisia. European Journal of Plant Pathology 140(1):53–67, available at: https://doi.org/10.1007/s10658-014-0443-4

Paradis, E., Claude, J. and Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20(2):289–90, available at: https://doi.org/10.1093/bioinformatics/btg412

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D. and Vogler, A. P. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55(4):595–609, available at: https://doi.org/10.1080/10635150600852011

Puillandre, N., Lambert, A., Brouillet, S. and Achaz, G. 2012. ABGD, Automatic barcode gap discovery for primary species delimitation. Molecular Ecology 21(8):1864–77, available at: https://doi.org/10.1111/j.1365-294X.2011.05239.x.

Qing, X., Bert, W., Gamliel, A., Bucki, P., Duvrinin, S., Alon, T. and Braun Miyara, S. 2019. Phylogeography and molecular species delimitation of *Pratylenchus capsici* n. sp., a new root lesion nematode in Israel on pepper (*Capsicum annuum*). Phytopathology 109(5):847–58.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G. and Suchard, M. A. 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. Systematic Biology 67(5):901–4, available at: https://doi.org/10.1093/sysbio/syy032

Reboish, R.V. and Golden, A. M. 1985. Pathogenicity and reproduction of *Pratylenchus agilis* in field microplots of soybeans, corn, tomato, or cornsoybean cropping systems. Plant Disease 69:927–9.

Russell, C. C. 1987. A space-saving root incubation system. Journal of Nematology 19:554.

Singh, P. R., Nyiragatare, A., Janssen, T., Couvreur, M., Decraemer, W. and Bert, W. 2018. Morphological and molecular characterisation of *Pratylenchus rwandae* n. sp. (Tylenchida: Pratylenchidae) associated with maize in Rwanda. Nematology 20:781–94, available at: https://doi.org/10.1163/15685411-00003175

Smiley, R. W. 2015. Root-lesion nematodes: biology and management in Pacific Northwest wheat cropping systems. A Pacific Northwest Extension Publication 617:14.

Smiley, R. W., Whittaker, R. G., Gourlie, J. A. and Easley, S. A. 2005. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. Plant Disease 89(9):958–68, available at: https://doi.org/10.1094/PD-89-0958

Smolik, J. 1977. Nematodes associated with irrigated corn in South Dakota. Proceedings of the South Dakota Academy of Science 56:93–9.

Smolik, J. and Lewis, J. 1982. Effect of range condition on density and biomass of nematodes in a mixed prairie ecosystem. Journal of Range Management 35(5):657–63, available at: www.jstor.org/stable/10.2307/3898658

Smolik, J. D. and Evenson, P. D. 1987. Relationship of yields and *Pratylenchus* spp. population densities in dryland and irrigated corn. Journal of Nematology 19(1):71–3, available at: www.ncbi.nlm.nih.gov/pubmed/19290280

Subbotin, S. A., Ragsdale, E. J., Mullens, T., Roberts, P. A., Mundo-Ocampo, M. and Baldwin, J. G. 2008. A phylogenetic framework for root lesion nematodes of the genus *Pratylenchus* (Nematoda): evidence from 18S and D2-D3 expansion segments of 28S ribosomal RNA genes and morphological characters. Molecular Phylogenetics and Evolution 48(2):491–505, available at: https://doi.org/10.1016/j.ympev.2008.04.028

Templeton, A. R., Crandall, K. and Sing, C. F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132(2):619–33, available at: https://doi.org/c:/Docs/Bayes

Thompson, J. P., Clewett, T. G., Sheedy, J. G., Reen, R. A., O'Reilly, M. M. and Bell, K. L. 2010. Occurrence of root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) and stunt nematode (*Merlinius brevidens*) in the northern grain region of Australia. Australasian Plant Pathology 39(3):254–64, available at: https://doi.org/10.1071/AP09094

Thorne, G. and Malek, R. (1968), Nematodes of the Northern Great Plains: Part 1 Tylenchida [Nemata Secernentea], Technical Bulletin TB31, Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota.

Todd, T. and Powers, T. 2018. Distribution and importance of plant nematodes, in Nebraska, Kansas and Colorado, in Subbotin, S. A. and Chitambar, J. J. (Eds), Plant parasitic nematodes in sustainable agriculture of North America: Vol. 2 – Northeastern, Midwestern and Southern USA Springer, Cham, pp. 109–23, available at: https://doi.org/10.1007/978-3-319-99588-5_5

Todd, T. C. 1991. Effect of cropping regime on populations of *Belonolaimus* sp. and *Pratylenchus scribneri* in sandy soil. Journal of Nematology 23(4):646–51.

Todd, T. C. and Oakley, T. R. 1996. Seasonal dynamics and yield relationships of *Pratylenchus* spp. in corn roots. Journal of Nematology 28(4):676–81.

Todd, T. C., Appel, J. A., Vogel, J. and Tisserat, N. A. 2014. Survey of plant parasitic nematodes in Kansas and Eastern Colorado wheat fields. Plant Health Progress 15(3):112–7, available at: https://doi.org/10.1094/PHP-RS-14-0003

Wallace, M. K. and MacDonald, D. H. 1979. Nematodes in Minnesota apple orchards. Plant Disease Reporter 63(12):1063–7.

Waterhouse, A. M., Martin, D. M. A., Barton, G. J., Procter, J. B. and Clamp, M. 2009. Jalview Version 2: a multiple sequence alignment editor and analysis workbench. Bioinformatics 25(9):1189–91, available at: https://doi.org/10.1093/bioinformatics/btp033

Yan, G. P., Plaisance, A., Huang, D., Chowdhury, I. A. and Handoo, Z. A. 2017a. First report of the new root lesion nematode *Pratylenchus* sp. on soybean in North Dakota. Plant Disease 101(8):1554, available at: https://doi.org/10.1094/PDIS-04-17-0594-PDN

Yan, G. P., Plaisance, A., Huang, D., Handoo, Z. A. and Chitwood, D. J. 2017b. First report of a new, unnamed lesion nematode *Pratylenchus* sp. infecting soybean in North Dakota. Plant Disease 101(8):1555, available at: https://doi.org/10.1094/PDIS-12-16-1749-PDN

Yan, G. P., Plaisance, A., Huang, D., Gudmestad, N. C. and Handoo, Z. A. 2015. First report of the root lesion nematode *Pratylenchus scribneri* infecting potato in North Dakota. Plant Disease 100(5):1023, available at: https://doi.org/10.1094/PDIS-10-15-1227-PDN

Yan, G. P., Paisance, A., Huang, D., Liu, Z., Chapara, V. and Handoo, Z. A. 2016. First report of the root lesion nematode *Pratylenchus neglectus* on wheat (*Triticum aestivum*) in North Dakota. Plant Disease 100(8):1794–2016, available at: https://doi.org/10.1094/PDIS02160260PDN

Appendix

Online Supplementary available at: https://doi.org/10.32873/unl.dr.20191202