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 Sutton¹ and Thomas Powers¹*

¹Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE, 68583-0722.
²Department of Plant Pathology, Kansas State University, Manhattan, KS, 66502.
*E-mail: tpowers1@unl.edu

This paper was edited by Erik J. Ragsdale.

Received for publication May 24, 2019.

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. hexinensis*, *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 Stekhoven, 1941 and *P. penetrans* (Orn 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 cm³ 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-voucheder specimens were removed from temporary slides and smashed in an 18 µl 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′-GGDTRACWTTHTAYCCNCC-3′) developed by the UNL nematology laboratory, and COI-JB5 (5′-AGCACTAACCTTTAACATAAGAATG-3′) (Derycke et al., 2005), resulting in a fragment length of 727 to 739bp for genetic analysis after trimming the primers from the amplified product. On occasion, forward primer JB3 (5′-TTTTTTTGGGCA TCCTGAGGTTAT-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′-ACAAGTACCGTAGGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (De Ley et al. 1999). PCR was conducted in a total volume of 30μl reaction mix consisting of 1.2μl molecular biology grade water (Sigma-Aldrich), 2.4μl of each primer, and 15μl of 2x JumpStart REDTaq ReadyMix Reaction Mix (Sigma-Aldrich), and 9μl 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 250M 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 V1.4 (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 × 10⁻⁷ 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 V1.4 (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. thomei, 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
DNA barcoding of Pratylenchus species: Ozbayrak et al.

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

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

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. hexicusus* entries of 99.7%. Specimens hypothesized to be *P. hexicusus* had no close COI match identified as *P. hexicusus* (Table 2; Ozbayrak et al. in prep). For the D2-D3 domains, those specimens exhibited a relatively close match to accessions representing *P. hexicusus* (KX828320–100% 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.

<table>
<thead>
<tr>
<th>Haplotype group (this study)</th>
<th>Blasted NID No. (this study)</th>
<th>COI Match Accession No.</th>
<th>Corresponding GenBank Name</th>
<th>Identity%</th>
<th>D2–D3 Match Accession No.</th>
<th>Corresponding GenBank Name</th>
<th>Identity%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. neglectus</em></td>
<td>N10756</td>
<td>KY424103</td>
<td><em>P. neglectus</em></td>
<td>99.8</td>
<td>MG906766</td>
<td><em>P. neglectus</em></td>
<td>99.7</td>
</tr>
<tr>
<td><em>P. penetrans</em></td>
<td>N6260</td>
<td>KY816936</td>
<td><em>P. penetrans</em></td>
<td>99.8</td>
<td>JX046986</td>
<td><em>P. penetrans</em></td>
<td>98.9</td>
</tr>
<tr>
<td><em>P. thornei</em></td>
<td>N7566</td>
<td>KY828316</td>
<td><em>P. thornei</em></td>
<td>99.5</td>
<td>KYT213559</td>
<td><em>P. thornei</em></td>
<td>100</td>
</tr>
<tr>
<td><em>P. alleni</em></td>
<td>N3717</td>
<td>MK045330</td>
<td><em>P. alleni</em></td>
<td>99.3</td>
<td>MN251270</td>
<td><em>P. alleni</em></td>
<td>100</td>
</tr>
<tr>
<td><em>P. zeae</em></td>
<td>N8934</td>
<td>KY424056</td>
<td><em>P. zeae</em></td>
<td>99.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. crenatus</em></td>
<td>N8539</td>
<td>KY816943</td>
<td><em>P. crenatus</em></td>
<td>100</td>
<td>KY468865</td>
<td><em>P. crenatus</em></td>
<td>98.1</td>
</tr>
<tr>
<td><em>P. vulnus</em></td>
<td>N7113</td>
<td>GQ332425</td>
<td><em>P. vulnus</em></td>
<td>99.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. scribneri</em></td>
<td>N7839</td>
<td>MH016378</td>
<td><em>P. scribneri</em></td>
<td>99.5</td>
<td>MK292133</td>
<td><em>P. scribneri</em></td>
<td>99.7</td>
</tr>
<tr>
<td><em>P. hexincisus</em></td>
<td>N7726</td>
<td>MH016378</td>
<td><em>P. scribneri</em></td>
<td>81.0</td>
<td>KY828290</td>
<td><em>P. hexincisus</em></td>
<td>99.7</td>
</tr>
<tr>
<td><em>P. hexincisus</em></td>
<td>N7726</td>
<td>MH016378</td>
<td><em>P. scribneri</em></td>
<td>81.0</td>
<td>EU130841</td>
<td><em>P. agilis</em></td>
<td>99.7</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 1</td>
<td>N6350</td>
<td>KU198944</td>
<td><em>Pratylenchus</em> sp.</td>
<td>83.0</td>
<td>MN251269</td>
<td><em>Pratylenchus</em> sp.</td>
<td>99.6</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 2</td>
<td>N8139</td>
<td>MK045330</td>
<td><em>P. alleni</em></td>
<td>79.3</td>
<td>MN251273</td>
<td><em>Pratylenchus</em> sp.</td>
<td>100</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 3</td>
<td>N3908</td>
<td>MK045330</td>
<td><em>P. alleni</em></td>
<td>83.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 4</td>
<td>N8402</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>88.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 5</td>
<td>N10767</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>87.3</td>
<td>MH730449</td>
<td><em>P. scribneri</em></td>
<td>99.2</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 6</td>
<td>N6245</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>88.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 7</td>
<td>N10770</td>
<td>KY424093</td>
<td><em>P. scribneri</em></td>
<td>85.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 8</td>
<td>N10685</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>89.1</td>
<td>KT175531</td>
<td><em>P. pseudocoffeae</em></td>
<td>96.2</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 9 (1)</td>
<td>N10841</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>80.1</td>
<td>KT175531</td>
<td><em>P. pseudocoffeae</em></td>
<td>95.8</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 9 (2)</td>
<td>N8930</td>
<td>KY424092</td>
<td><em>P. scribneri</em></td>
<td>79.7</td>
<td>KT175531</td>
<td><em>P. pseudocoffeae</em></td>
<td>95.7</td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 10 (1)</td>
<td>N10856</td>
<td>MK045330</td>
<td><em>P. alleni</em></td>
<td>78.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pratylenchus</em> sp. 10 (2)</td>
<td>N10853</td>
<td>MK045330</td>
<td><em>P. alleni</em></td>
<td>79.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Estimates of evolutionary divergence (p-distances) over sequence pairs within (bold) and between groups.

<table>
<thead>
<tr>
<th></th>
<th>P. alleni</th>
<th>P. crenatus</th>
<th>P. hexincisus</th>
<th>P. neglectus</th>
<th>P. penetrans</th>
<th>P. scribneri</th>
<th>P. thornei</th>
<th>P. vulnus</th>
<th>P. zeae</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. alleni</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. crenatus</td>
<td>0.308</td>
<td>0.355</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. hexincisus</td>
<td>0.28</td>
<td>0.33</td>
<td>0.362</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. neglectus</td>
<td>0.322</td>
<td>0.348</td>
<td>0.399</td>
<td>0.298</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. penetrans</td>
<td>0.172</td>
<td>0.327</td>
<td>0.199</td>
<td>0.317</td>
<td>0.351</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. scribneri</td>
<td>0.332</td>
<td>0.357</td>
<td>0.391</td>
<td>0.3</td>
<td>0.319</td>
<td>0.359</td>
<td>0</td>
<td>0.007</td>
<td>0.002</td>
</tr>
<tr>
<td>P. thornei</td>
<td>0.235</td>
<td>0.328</td>
<td>0.321</td>
<td>0.317</td>
<td>0.325</td>
<td>0.29</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. vulnus</td>
<td>0.33</td>
<td>0.377</td>
<td>0.399</td>
<td>0.334</td>
<td>0.354</td>
<td>0.367</td>
<td>0.329</td>
<td>0.348</td>
<td>0.327</td>
</tr>
<tr>
<td>P. zeae</td>
<td>sp. 1</td>
<td>0.209</td>
<td>0.3</td>
<td>0.283</td>
<td>0.304</td>
<td>0.332</td>
<td>0.231</td>
<td>0.263</td>
<td>0.364</td>
</tr>
<tr>
<td>sp. 2</td>
<td>0.228</td>
<td>0.357</td>
<td>0.222</td>
<td>0.373</td>
<td>0.396</td>
<td>0.232</td>
<td>0.389</td>
<td>0.342</td>
<td>0.409</td>
</tr>
<tr>
<td>sp. 3</td>
<td>0.182</td>
<td>0.352</td>
<td>0.231</td>
<td>0.324</td>
<td>0.357</td>
<td>0.189</td>
<td>0.359</td>
<td>0.291</td>
<td>0.344</td>
</tr>
<tr>
<td>sp. 4</td>
<td>0.174</td>
<td>0.316</td>
<td>0.207</td>
<td>0.316</td>
<td>0.331</td>
<td>0.118</td>
<td>0.348</td>
<td>0.274</td>
<td>0.356</td>
</tr>
<tr>
<td>sp. 5</td>
<td>0.195</td>
<td>0.33</td>
<td>0.181</td>
<td>0.334</td>
<td>0.352</td>
<td>0.125</td>
<td>0.359</td>
<td>0.302</td>
<td>0.361</td>
</tr>
<tr>
<td>sp. 6</td>
<td>0.187</td>
<td>0.334</td>
<td>0.197</td>
<td>0.315</td>
<td>0.358</td>
<td>0.108</td>
<td>0.354</td>
<td>0.296</td>
<td>0.364</td>
</tr>
<tr>
<td>sp. 7</td>
<td>0.171</td>
<td>0.299</td>
<td>0.212</td>
<td>0.3</td>
<td>0.333</td>
<td>0.153</td>
<td>0.327</td>
<td>0.256</td>
<td>0.341</td>
</tr>
<tr>
<td>sp. 8</td>
<td>0.165</td>
<td>0.308</td>
<td>0.221</td>
<td>0.299</td>
<td>0.345</td>
<td>0.12</td>
<td>0.327</td>
<td>0.278</td>
<td>0.359</td>
</tr>
<tr>
<td>sp. 9</td>
<td>0.202</td>
<td>0.33</td>
<td>0.244</td>
<td>0.338</td>
<td>0.353</td>
<td>0.206</td>
<td>0.347</td>
<td>0.265</td>
<td>0.361</td>
</tr>
<tr>
<td>sp. 10</td>
<td>0.195</td>
<td>0.342</td>
<td>0.214</td>
<td>0.35</td>
<td>0.35</td>
<td>0.211</td>
<td>0.351</td>
<td>0.262</td>
<td>0.372</td>
</tr>
</tbody>
</table>

The number of base differences per site from averaging over all sequence pairs between groups are shown. The analysis involved 915 nucleotide sequences. *Not possible to estimate evolutionary distances.
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 $7.2 \times 10^{-8}$ 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.
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
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 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.
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 *Pratylenchus* survey; (B) colors indicate the host in the field at time of the survey.

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.
DNA barcoding of *Pratylenchus* species: Ozbayrak et al.

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

Thanks to the Nebraska Corn Board for financial assistance, Nebraska and Kansas Departments of Agriculture, USDA Cooperative Agricultural Pest Survey Program (CAPS), USDA Multistate Project 4186 and the numerous collaborators and colleagues who have contributed nematode specimens for analysis.

References


DNA barcoding of Pratylenchus species: Ozbayrak et al.


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.


Appendix

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