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Pratylenchus smoliki, a new nematode species (Pratylenchidae: Tylenchomorpha) from the Great Plains region of North America

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Abstract

Pratylenchus smoliki is a new species of root-lesion nematode described from corn-soybean production fields in the Central Great Plains of North America. It is characterized by populations with relatively abundant males, two lip annuli, females with a round functional spermatheca and a conoid to subcylindrical tail with a non-crenate, smooth terminus. In host preference tests, corn and wheat produce the largest nematode populations, whereas sorghum and soybeans produce less than 20% the numbers observed on corn. Scanning electron microscopy reveals that the *en face* patterns compare to those seen in *Pratylenchus pseudocoffeae*, *P. scribneri*, *P. hexincisus*, and *P. alleni*. The pattern is described as rectangular to trapezoidal subdorsal and subventral lips adjoining oral disc, but with a clear demarcation between the oral disc and the subdorsal and subventral sectors. A Maximum Likelihood COI tree recognizes *P. smoliki* as a moderately-well-supported clade with several haplotype subgroups. A Maximum Likelihood partial 28S tree provides strong support for the *P. smoliki* clade and reinforces the close relationships between species with similar *en face* patterns. Topotype specimens of *P. alleni* were demonstrably different from *P. smoliki* using DNA markers. The geographic range of *P. smoliki* overlaps with the ranges of *P. alleni*, *P. scribneri*, *P. neglectus*, *P. hexicisus*, and *P. dakotaensis*. The observed host range (corn, rye, sunflower, and wheat) suggests that *P. smoliki* may be native to the tallgrass prairie region of the Great Plains.

Keywords

COI, Nematode distribution, Phylogeny, Plant-parasitic nematodes, Root-lesion nematode, Taxonomy.

Globally, there are 103 described species of *Pratylenchus* according to a recent accounting (Nguyen et al., 2019). A regional survey of the Central Great Plains of North America, conducted using a DNA barcoding approach, recorded nine described species and a roughly equivalent number of potentially undescribed species (Ozbayrak et al., 2019). One of those undescribed species, previously referred to as *Pratylenchus* sp. 1 in Ozbayrak et al. (2019) was described as *Pratylenchus dakotaensis* (Handoo

et al., 2021). In this manuscript, we describe a second of those undescribed species, previously referred to as *Pratylenchus* sp. 2 in Ozbayrak et al. (2019), herein described as *Pratylenchus smoliki* n. sp. The new species has been collected from two Great Plains States: Kansas and Nebraska. All positive collections were obtained from fields in a corn-soybean rotation. Notably *P. smoliki* n. sp. reproduces sexually and males make up approximately 30% of the adult population. The possibility that the species

identity might be *Pratylenchus alleni* (Ferris, 1961), another two-lipped species with males, led to the collection of that species at its type locality in Saline County, Illinois. *Pratylenchus alleni* has been given a pest rating of “A” by the California Department of Agriculture, suggesting that the species is an organism of known economic importance and subject to action enforced by the state (<http://blogs.cdffa.ca.gov/Section3162/?p=1955>). Therefore, the comparison of *P. alleni* and *P. smoliki* n. sp. has economic as well as scientific relevance.

DNA barcoding with the COI mitochondrial gene has been an accurate approach for species identification in *Pratylenchus* (Troccoli et al., 2016; Singh et al., 2018; Nguyen et al., 2019; Ozbayrak et al., 2019; Handoo et al., 2021). Since COI provides differentiation at the population level as well as species-level discrimination, it is necessary to generate an estimate of within-species genetic variation for the mitochondrial marker. The determination of species relationships, however, requires additional genetic markers, an examination of morphological characters, and an understanding of ecological and physiological characteristics. Presently, empirical evidence supports a strong correspondence between COI haplotype groups and described species boundaries in *Pratylenchus*. In this study, we have constructed a maximum likelihood tree of COI that includes *P. smoliki* n. sp., 22 other described species that are represented by a minimum of two specimens, and nine other described *Pratylenchus* species that are represented by a single specimen. Sequence characterization by 28S and ITS1 is also presented. Species delimitation and host associations for the major *Pratylenchus* species of the Central Great Plains were included in Ozbayrak et al. (2019). Mean genetic distances between species and within species COI diversity have been recalculated to incorporate new specimens. The further characterization of *Pratylenchus smoliki* n. sp. from that publication and the formal description of this species is the primary objective of this study.

Materials and methods

Nematode populations

Nematodes included in this study were collected as part of a series of plant disease surveys as described in Ozbayrak et al. (2019). *Pratylenchus smoliki* n. sp. was collected from Buffalo County, Nebraska and Shawnee County, Kansas as part of a wheat and corn soil survey. Comparative host tests and most morphological analyses were conducted on specimens cultured at Kansas State University, originally collected

from the type locality. The original isolation of *P. smoliki* n. sp. was from corn root samples collected from experimental seed treatment trials at the Kansas River Valley Experiment Field located 3.5 miles east of Silver Lake, Kansas, U.S.A (Shawnee County). Nematode populations were subsequently maintained at the Kansas State University Throckmorton greenhouse complex in Eudora silt loam planted with the corn hybrid DKC60-69RIB. Nematodes for morphological and molecular analyses were extracted from root incubations as described by Georgi et al. (1983) and shipped as living nematodes in tap water to the Powers lab at the University of Nebraska-Lincoln. Sample collection sites (county locations), nematode identification (NID) numbers, and host information for each analyzed specimen are presented in Table 1.

Morphological analysis

Nematodes isolated from soil were first examined using a dissecting stereomicroscope. Individual nematodes were mounted in water on temporary glass slides, measured, and digitally photographed using a Leica DMLB light microscope with differential interference contrast optics and a Leica DC300 video camera. A set of 26 standard measurements were taken on individual specimens allowing for the combined retention of morphological and molecular characters. Temporary slides were dismantled after morphological analysis, the nematode was crushed in 18 μ l of sterile water with a micropipette tip and frozen in individual PCR microfuge tubes. Both adult females and males, as well as juveniles, were subjected to morphological and molecular analyses. Voucher specimens were fixed in 4% formaldehyde plus 2% glycerol, and the slow evaporation method was used prior to mounting.

Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 4% glutaraldehyde followed by post-fixation with 2% Osmium Tetroxide, dehydration in a graded series of alcohol to 100% ethyl alcohol, critical point drying, mounting on SEM specimen stubs, and coating with silver. Images were obtained on a Hitachi S4700 Field-Emission scanning electron microscope. Microscopic images of all specimens were stored in an in-house database in the Department of Plant Pathology at University of Nebraska-Lincoln.

Molecular analysis

Polymerase chain reaction amplifications were performed using three different markers: the cytochrome oxidase subunit I (COI), the internal transcribed spacer I (ITS1), and the large-subunit ribosomal RNA (28S)

Table 1. *Pratylenchus* specimens used for phylogenetic analysis in this study.

| NID # | Species | Stage | Locality | Host | Marker | GenBank# |
|--------|--------------------------|-------|------------------|------|----------|----------|
| N3873 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | MK878313 |
| N3873 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | rDNA LSU | OK490313 |
| N3880 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | MK878314 |
| N3880 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | ITS1 | OK490336 |
| N8136 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | MK878315 |
| N8137 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878316 |
| N8139 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878317 |
| N8139 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490314 |
| N8145 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878318 |
| N8145 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490315 |
| N8146 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878319 |
| N8148 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878320 |
| N8154 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878321 |
| N8156 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878322 |
| N8170 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | MK878323 |
| N8170 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490316 |
| N9793 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489823 |
| N9793 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490317 |
| N9793 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA SSU | OK490342 |
| N9795 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | rDNA SSU | OK490343 |
| N9809 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | ITS1 | OK490337 |
| N10037 | <i>P. smoliki</i> n. sp. | F | Buffalo Co., NE | Corn | COI | MK878324 |
| N10039 | <i>P. smoliki</i> n. sp. | F | Buffalo Co., NE | Corn | COI | MK878325 |
| N10053 | <i>P. smoliki</i> n. sp. | F | Buffalo Co., NE | Corn | COI | MK878326 |
| N11506 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489824 |
| N11506 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490318 |
| N11507 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489825 |
| N11508 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489826 |
| N11509 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489827 |
| N11509 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490319 |
| N11510 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489828 |
| N11592 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489829 |
| N11593 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489830 |
| N11593 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490320 |
| N11594 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA SSU | OK490344 |
| N11595 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489831 |
| N11595 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490321 |
| N11600 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489832 |
| N11602 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489833 |
| N11603 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490322 |

Pratylenchus smoliki description: Powers et al.

| | | | | | | |
|--------|--------------------------|---|--------------------|---------|----------|----------|
| N11605 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489834 |
| N11607 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489835 |
| N11612 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489836 |
| N11613 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489837 |
| N11614 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489838 |
| N11614 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490323 |
| N11618 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489839 |
| N12448 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489840 |
| N12451 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489841 |
| N12451 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489842 |
| N12453 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489843 |
| N12453 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | rDNA LSU | OK490324 |
| N12454 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | ITS1 | OK490338 |
| N12457 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489844 |
| N12458 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489845 |
| N12459 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489846 |
| N12463 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489847 |
| N12463 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | rDNA LSU | OK490325 |
| N12464 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489848 |
| N12465 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489849 |
| N12466 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489850 |
| N12467 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489851 |
| N12468 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489852 |
| N12469 | <i>P. smoliki</i> n. sp. | M | Shawnee Co., KS* | Corn | COI | OK489853 |
| N12470 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489854 |
| N12470 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | rDNA LSU | OK490326 |
| N12471 | <i>P. smoliki</i> n. sp. | F | Shawnee Co., KS* | Corn | COI | OK489855 |
| N3717 | <i>P. alleni</i> | M | Madison Co., NE | Corn | COI | MK877458 |
| N3717 | <i>P. alleni</i> | M | Madison Co., NE | Corn | rDNA LSU | OK490293 |
| N7381 | <i>P. alleni</i> | F | Saline Co., IL** | Soybean | COI | MK877459 |
| N7382 | <i>P. alleni</i> | M | Saline Co., IL** | Soybean | COI | MK877460 |
| N10848 | <i>P. alleni</i> | J | Saline Co., IL** | Soybean | rDNA LSU | OK490294 |
| N10850 | <i>P. alleni</i> | F | Saline Co., IL** | Soybean | COI | MK877461 |
| N10850 | <i>P. alleni</i> | F | Saline Co., IL** | Soybean | rDNA LSU | OK490295 |
| N8539 | <i>P. crenatus</i> | F | County Galway, IRE | Pasture | rDNA LSU | OK490296 |
| N9774 | <i>P. crenatus</i> | J | Rwanda | - | COI | OK489810 |
| N6350 | <i>P. dakotaensis</i> | F | Atchison Co., KS | Corn | rDNA LSU | OK490297 |
| N6351 | <i>P. dakotaensis</i> | J | Atchison Co., KS | Corn | rDNA LSU | OK490298 |
| N6438 | <i>P. hexincisus</i> | F | Graham Co., KS | Corn | rDNA LSU | OK490299 |
| N7726 | <i>P. hexincisus</i> | F | Big Horn Co., WY | Drybean | rDNA LSU | OK490300 |
| N10849 | <i>P. hexincisus</i> | J | Saline Co., IL | Soybean | rDNA LSU | OK490301 |
| N10756 | <i>P. neglectus</i> | F | Seward Co., KS | Corn | rDNA LSU | OK490302 |
| N11481 | <i>P. neglectus</i> | F | Gallatin Co., MT | Corn | COI | OK489811 |
| N11483 | <i>P. neglectus</i> | F | Gallatin Co., MT | Corn | COI | OK489812 |

| | | | | | | |
|---------|--------------------------|---|-----------------------|-------------------|----------|----------|
| N11485 | <i>P. neglectus</i> | F | Broadwater Co., MT | Corn | COI | OK489813 |
| N11487 | <i>P. neglectus</i> | F | Broadwater Co., MT | Corn | COI | OK489814 |
| N11489 | <i>P. neglectus</i> | F | Broadwater Co., MT | Corn | COI | OK489815 |
| N11491 | <i>P. neglectus</i> | F | Treasure Co., MT | Corn | COI | OK489816 |
| N11499 | <i>P. neglectus</i> | F | Yellowstone Co., MT | Corn | COI | OK489817 |
| N6260 | <i>P. penetrans</i> | M | Fairbanks Co., AK | Peony | rDNA LSU | OK490303 |
| N6261 | <i>P. penetrans</i> | J | Fairbanks Co., AK | Peony | rDNA LSU | OK490304 |
| N7091 | <i>P. penetrans</i> | F | Fairbanks Co., AK | Peony | rDNA LSU | OK490305 |
| N11414 | <i>P. penetrans</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489818 |
| N11415 | <i>P. penetrans</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489819 |
| N11416 | <i>P. penetrans</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489820 |
| N11417 | <i>P. penetrans</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489821 |
| N11424 | <i>P. penetrans</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489822 |
| N6383 | <i>P. scribneri</i> | F | Marshall Co., KS | Corn | rDNA LSU | OK490306 |
| N7833 | <i>P. scribneri</i> | J | Custer Co., NE | Corn | rDNA LSU | OK490307 |
| N7839 | <i>P. scribneri</i> | J | Custer Co., NE | Corn | rDNA LSU | OK490308 |
| N7845 | <i>P. scribneri</i> | F | Custer Co., NE | Corn | rDNA LSU | OK490309 |
| N10274 | <i>P. scribneri</i> | F | Kearney Co., NE | Corn | rDNA LSU | OK490310 |
| N10301 | <i>P. scribneri</i> | F | Phelps Co., NE | Corn | rDNA LSU | OK490311 |
| N10306 | <i>P. scribneri</i> | F | Phelps Co., NE | Corn | rDNA LSU | OK490312 |
| P130021 | <i>P. scribneri</i> | - | Maryland | - | ITS1 | OK490333 |
| P156024 | <i>P. scribneri</i> | F | Florida | - | ITS1 | OK490334 |
| P344041 | <i>P. scribneri</i> | - | - | - | ITS1 | OK490335 |
| N7566 | <i>P. thornei</i> | F | Treasure Co., MT | Corn | rDNA LSU | OK490327 |
| N7567 | <i>P. thornei</i> | F | Treasure Co., MT | Corn | rDNA LSU | OK490328 |
| N11495 | <i>P. thornei</i> | F | Yellowstone Co., MT | Corn | COI | OK489856 |
| N11497 | <i>P. thornei</i> | F | Yellowstone Co., MT | Corn | COI | OK489857 |
| N11422 | <i>P. vulnus</i> | F | British Columbia, CAN | <i>Prunus</i> sp. | COI | OK489858 |
| N10764 | <i>Pratylenchus</i> sp. | F | Sumner Co., KS | Corn | rDNA LSU | OK490329 |
| N12456 | <i>Pratylenchus</i> sp. | M | Shawnee Co., KS | Corn | COI | OK489859 |
| N10685 | <i>Pratylenchus</i> sp. | F | Seward Co., KS | Corn | rDNA LSU | OK490330 |
| N8930 | <i>Pratylenchus</i> sp. | F | Lincoln Co., AR | Soybean | rDNA LSU | OK490331 |
| N10841 | <i>Pratylenchus</i> sp. | J | Cross Co., AR | Soybean | rDNA LSU | OK490332 |
| P156016 | <i>Pratylenchus</i> sp. | F | Lancaster Co., NE | Leadplant | ITS1 | OK490339 |
| P156019 | <i>Pratylenchus</i> sp. | F | Gage Co., NE | Big Bluestem | ITS1 | OK490340 |
| P201006 | <i>Pratylenchus</i> sp. | - | Idaho | - | ITS1 | OK490341 |
| N84 | <i>Nacobbus aberrans</i> | M | Xalatlaco, MEX | - | COI | OK489860 |
| N85 | <i>Nacobbus aberrans</i> | M | Xalatlaco, MEX | - | COI | OK489861 |
| N194 | <i>Nacobbus aberrans</i> | J | Sioux Co., NE | Sugarbeet | COI | OK489862 |
| N452 | <i>Nacobbus aberrans</i> | - | Zacatecas, MEX | Drybean | COI | OK489863 |
| N537 | <i>Nacobbus aberrans</i> | J | Chacra, ARG | Tomato | COI | OK489864 |
| N658 | <i>Nacobbus aberrans</i> | J | Lisandro Olmos, ARG | - | COI | OK489865 |

Note: *Type locality for *Pratylenchus smoliki* n. sp. Kansas River Valley Experiment Field, within the 80 acre Paramore Unit, located 3.5 miles east of Silver Lake on U.S. Highway 24, then 1 mile south of Kiro, and 1.5 miles east on 17th Street. **Type locality for *Pratylenchus alleni*. Soybean field 5 miles north of Eldorado, Illinois. Ferris, V. R. (1961).

Table 2. Primers.

| Marker | Primer set | Amplicon Size (kb) | Primer Sequence (5' → 3') | Direction | Reference |
|-----------|------------|--------------------|---|-----------|------------------------|
| COIa | PsmoF4 | 0.42 | 5'-ATY GCS CCC GCC TTT GG-3' | Forward | This manuscript |
| COI | JB5 | | 5'-AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG-3' | Reverse | Derycke et al. (2005) |
| COI | JB3 | 0.43 | 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' | Forward | Bowles et al., (1992) |
| COI | JB5 | | 5'-AGCACCTAAACTTAAAACATAATGA AAATG-3' | Reverse | Derycke et al. (2005) |
| COI | F1KF | 0.95 | 5'- CCTACTATGATTGGTGGTTTTGGTA ATTG-3' | Forward | Kanzaki & Futai (2002) |
| COI | JB5 | | 5'-AGCACCTAAACTTAAAACATAATGA AAATG-3' | Reverse | Derycke et al. (2005) |
| COI | F7bPrat | 0.78 | 5'-GGDTGRACWTHHTAYCCNCC-3' | Forward | Ozbayrak et al. (2019) |
| COI | JB5 | | 5'-AGCACCTAAACTTAAAACATAATGA AAATG-3' | Reverse | Derycke et al. (2005) |
| ITS1b | rDNA2 | 0.62 | 5'-TTGATTACGTCCCTGCCCTTT-3' | Forward | Vrain et al. (1992) |
| ITS1 | rDNA1.58Sa | | 5'-ACGAGCCGAGTGATCCACC-3' | Reverse | Cherry et al. (1997) |
| rDNA LSUc | D2A | 0.75 | 5'-ACAAGTACCGTGAGGGAAAGTTG-3' | Forward | De Ley et al. (1999) |
| rDNA LSU | D3B | | 5'-TCGGAAGGAACCAGCTACTA-3 | Reverse | De Ley et al. (1999) |
| rDNA LSU | 28s-PratF3 | 0.48 | 5'-TTTGCAAGTGGAGTGCGT-3' | Forward | This manuscript |
| rDNA LSU | 28s-PratR1 | | 5'-AATAGTTCACCATCTTTTCGGG -3' | Reverse | This manuscript |

Note: ^aCytochrome oxidase subunit I. ^bInternal transcribed spacer 1. ^crDNA Large Subunit.

(Table 2). The PCR reaction mixtures and thermocycler conditions for each marker were as follows.

For a final PCR reaction mixture volume of 30µl/ amplification, 5-10 µl of nematode template were added to each reaction mixture of 1.6 µM final concentration for both forward and reverse primers and a 0.05U/µl final concentration of Sigma 2X JumpStart RED Taq ReadyMix. After loading the thermocycler with the reaction mixtures at a Hotstart (94°C), the thermocycler PCR conditions were: one cycle of initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 sec; annealing for 30 sec; and extension at 72°C for 90 sec. Annealing temperatures were 50°C, 55°C, and 48°C for COI, ITS1, and 28S amplifications, respectively. A

one-cycle final extension stage at 72°C ran for 5 min before the thermocycler program settled at 24°C.

All PCR reactions were conducted on a Techne Prime Thermal Cycler, 60 × 0.5ml (Bibby Scientific Ltd., Staffordshire, UK). To evaluate amplifications, 3 µl of each PCR product was loaded into 1% Low EEO agarose gels and stained with 1% ethidium bromide. Gels were placed into electrophoresis with 0.5X Tris-Borate-EDTA (TBE) running buffer for 35 min at 155V. UV-visualized gel images were digitally recorded. Successful PCR amplifications were purified with a Gel/PCR DNA Fragment Extraction Kit (IBI Scientific, Dubuque, IA) following the manufacturer's guidelines. Purified DNA amplicons were sequenced in both directions by the UCDNA

Sequencing Facility at the University of California-Davis or ETON BioSciences Inc. Sequences were edited and aligned on CodonCode Aligner Version 9.0 (CodonCode Corp, Centerville, Massachusetts). The nucleotide sequences obtained in this study were deposited into the GenBank database (NCBI) under the accession numbers OK489810-OK489865 (COI) and OK490293-OK490344 (28S, ITS1, and 18S).

Host range trials

Host trials were conducted in 0.5 l Deepots (Stuewe & Sons Inc.) containing pasteurized Eudora silt loam soil from the type location and inoculated with 1,000 to 1,500 nematodes extracted from field-grown corn roots. Trials consisted of (1) commercial corn hybrids, (2) agronomic crops, with two cultivars each of corn, sorghum, soybean, and wheat, and (3) diverse cover crops with corn as the control. Host status was determined by root incubation of eight-week-old plants followed by nematode extraction as described by Georgi et al. (1983). All trials were conducted as randomized complete block designs with four to five replications, and repeated in time once or twice.

Results

*Pratylenchus smoliki** n. sp. (Figs. 1, 2, 3, 4, 5)

*The specific epithet is proposed to honor Dr. James Smolik

Figure 1. (Plate of *Pratylenchus smoliki* n. sp. light micrograph)

Figures 2–5. (Plates of SEM *Pratylenchus smoliki* n. sp.—Plate of heads, plate of profiles, plate of mid-body, plate of tails)

Measurements see Table 3. (Table 3. Morphometric parameters of live specimens of *Pratylenchus smoliki* n. sp. from corn, Shawnee County, KS, USA. Measurements are in μm and in the form $\pm\text{SD}$ (range)).

Description

Females

Body slender, vermiform, tapering anteriorly, ventrally arcuate to slightly sinuate when relaxed. Body annuli approximately 1 to 2 μm wide at mid-body. Lateral field mostly with four lines, but sometimes supplemented with additional inner lines or striae from midbody to the vulval region, giving the appearance under the light microscope of 5, 6 or even 10 lines. Lateral field beginning 7.1 ± 1.5 (4-11) μm from the anterior end and 7.2 ± 1.6 (5-11) μm wide, occupying

23 to 45% of body diameter at mid-body. Areolation not readily observed in light microscopy, but more apparent in SEM. Lateral field extending 83 to 91% of tail length, terminating 2.6 ± 0.6 (2-4) μm from the tail tip.

Lip region cap-like, narrower than the succeeding body contour, with two lip annuli; 2.3 ± 0.3 (2-3) μm high and 7.3 ± 0.5 (6-8) μm wide, anterior margin truncate with rounded edges. Head immediately posterior to second lip annulus 9.4 ± 0.7 (8-11) μm wide. Cephalic framework moderately developed.

En face view characterized by a divided face with rectangular to trapezoidal subdorsal and subventral lips adjoining oral disc, but with a clear demarcation between the oral disc and the subdorsal and subventral sectors. The lateral lip sectors are separated from the disc and subventral/subdorsals by two incisures doubly bent, forming an obtuse angle (pattern “I” sensu Subbotin et al., 2008). This lip pattern configuration is intermediate between groups 2 and 3, according to the classification scheme of Corbett and Clark (1983), differing especially in the separate oral disc.

Stylet short, robust; knobs rounded to rhomboid, flat or slightly indented anteriorly and 4.5 ± 0.4 (4-5) μm wide. Dorsal pharyngeal gland orifice located 2.7 ± 0.3 (2-3) μm posterior to base of knobs. Median bulb muscular, round to ovoid, 13.1 ± 1.3 (11-16) μm long \times 10.7 ± 0.7 (9-12) μm wide, occupying 51-73% of the corresponding body diameter. Cuticularized valve plates prominent. Nerve ring encircling isthmus, 66.7 ± 4.5 (58-75) μm from anterior end. Isthmus 16.5 ± 3.7 (10-22) μm long, about 2 μm wide. Hemizonid 3.1 ± 0.3 (2-4) μm long, located up to 7 μm anterior to secretory-excretory pore, but usually within 2 to 5 μm . Secretory-excretory pore usually anterior to pharyngo-intestinal junction. Pharyngeal glands in tandem, elongate, overlapping intestine ventrally, 43.9 ± 7.5 (28-68) μm long; pharyngeal gland nuclei in tandem. Intestine lacking fasciculi. Reproductive system monodelphic, prodelfic, 169.7 ± 36.1 (91-258) μm long, ovary outstretched with single row of oocytes, vulva usually slightly less than 80% of total body length from anterior end, vulval lips usually slightly protruding, occasionally slightly sunken; lateral flaps and epiptygma absent. Spermatheca rounded to ovoid, 13.9 ± 2.8 (8-19) μm long by 13.1 ± 2.3 (7-18) μm wide, containing spherical sperm, often with distinct nuclei. Post-vulval uterine sac 18.3 ± 4.2 (10-27) μm long, 0.9 times anal body diameter or about 22.5% of the vulva-anus distance. Distance from vulva to anus 82.9 ± 11.9 (63-98) μm . Tail short, conoid to subcylindrical, sometimes slightly ventrally arcuate, with 14 to 26 annuli. Tail tip blunt, subhemispherical to



Figure 1: Light microscope images of *Pratylenchus smoliki* n. sp. specimens. (A) NID 8139 female head, (B) NID 8139 female tail, (C) NID 8139 spermatheca, vulva and post-uterine sac, (D) NID 8170 female reproductive system, (E) NID 8170 female entire body, (F) NID 8137 male entire body, (G) NID 8154 spicules and bursa, (H) NID 8154 male head, (I) NID 8137 spicules and bursa, (J) NID 8154 midbody lateral lines in male.

truncate, smooth or slightly irregular. Phasmid pore-like, located 7 to 11 annules posterior to anus, 27 to 51% of total tail length. Hyaline portion of tail terminus 1.8 ± 0.5 (1-3) µm thick.

Males

Abundant, morphology generally similar to that of female, [including *en face* morphology] and sexual

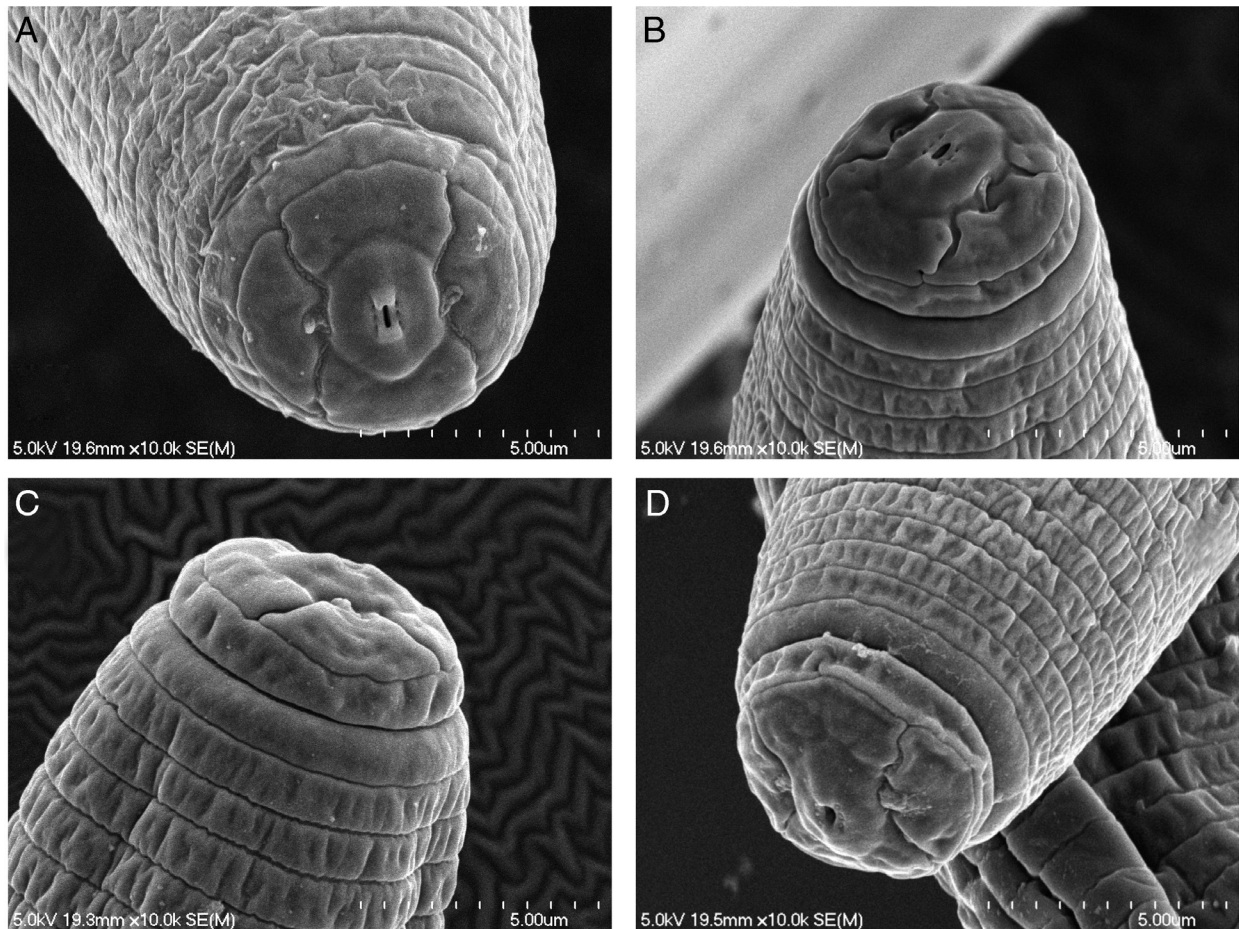


Figure 2: *P. smoliki* n. sp. SEM in *en face* view, different NID numbers specify different individual specimens. (A) NID 4808 Trapezoidal subdorsal and subventral lips adjoining oral disc with demarcation between sectors, six inner labial sensilla flank oral opening, (B) NID 4801 Lateral lip sectors clearly separated from oral disc and subventral/subdorsal sectors, (C) NID 4805 and (D) NID 4820 Anterior region profiles displaying two lip annuli.

dimorphism. Lip region characters as in female, but more rounded in outline. Some individuals observed with two lip annules on one side (dorsal or ventral) and three on the other. Lateral fields marked by four lines, without apparent areolation when viewed by light microscopy; additional lines or striae not observed. Reproductive system consisting of a single testis anteriorly outstretched, extending 201.4 ± 25.5 (157-273) μm anterior to cloaca. Spicules arcuate, cephalated; gubernaculum slightly curved. Tail conical, ventrally arcuate or bent, sometimes slightly digitate, with finely rounded to bluntly pointed terminus. Bursa 33.5 ± 3.8 (26-40) μm long, varying in extent from 80 to 137% of tail length. Ventral surface of bursa slightly to moderately crenate, occasionally notched. Phasmid pore-like, 9.1 ± 2.1 (4-14) μm posterior to anus, at 31 to 60% of total tail length.

Type locality and habitat

Holotype Specimen Measurements (nematode identification number [NID] 9793) from the Kansas River Valley (KRV) Experiment Field, near Silver Lake, Kansas.

Type material

Holotype tissue from NID9793 has been deposited with accession number P-2021-052 and catalog number HWML-112258 in the Harold W. Manter Laboratory of Parasitology, W-529 Nebraska Hall, University of Nebraska State Museum curated by Dr. Scott Gardner. Three additional paratype slides, each containing two females and two males, were distributed to the United States Department of

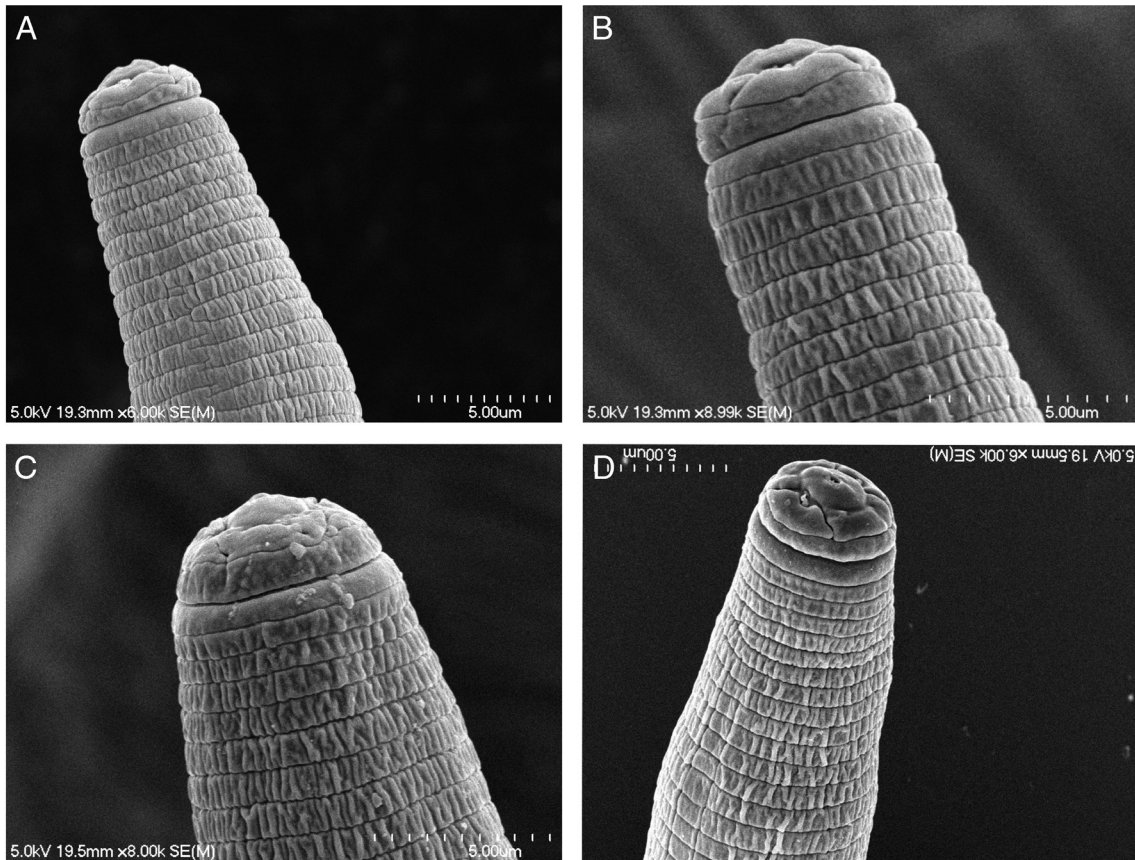


Figure 3: *P. smoliki* n. sp. SEM of anterior region with beginning of lateral field, different NID numbers specify different individual specimens. (A) NID 4815, (B) NID 4812, (C) NID 4816, (D) NID 4817.

Agriculture Nematode Collection, Beltsville, MD, USA. As required by the International Commission on Zoological Nomenclature, the ZooBank registration number for the new Linnaean binomials is LSID urn:lsid:zoobank.org:pub:9B64E021-C835-420A-A479-F5E688DC61B3.

Voucher material

Two slides, each containing two females and two males, of the reference *P. smoliki* n. sp. (Silver Lake, Kansas) populations were distributed to each of the following collections: University of California Riverside Nematode Collection, Riverside, USA and University of California Davis Nematode Collection, Davis, USA.

Differential diagnosis

Pratylenchus smoliki n. sp. Is characterized by an offset lip region bearing two annuli which are distinctly narrower than the head region immediately posterior,

SEM face divided into clearly-demarcated oral disc, with separate lateral and submedial lip sectors forming an obtuse, doubly bent angle with the labial disc, corresponding to pattern I in Subbotin et al. (2008), stylet 15 (14-16) μm long, with rounded knobs, pharyngeal overlap fairly short (1-2 corresponding body diameters in length), lateral field faintly and occasionally areolated as viewed in light microscopy, and with four incisures plus additional striae, especially at midbody, body annulation moderate, spermatheca round, filled with sperm, vulval lips slightly protruding, tail conoid to subcylindrical with blunt, usually smooth terminus, males common, with slightly smaller stylet (14 [13-15] μm), lateral field with 4 lines, spicules 17 (15-19) μm in length and ventrally curved, bluntly pointed tail.

The matrix code of the new species, according to Castillo and Vovlas (2007) is: A1, B2, C2, D2, E2(3), F2(34), G(2)3, H1, I1(23), J1(23), K1.

The main morphological characters distinguishing *Pratylenchus smoliki* n. sp. include the two lip annuli,

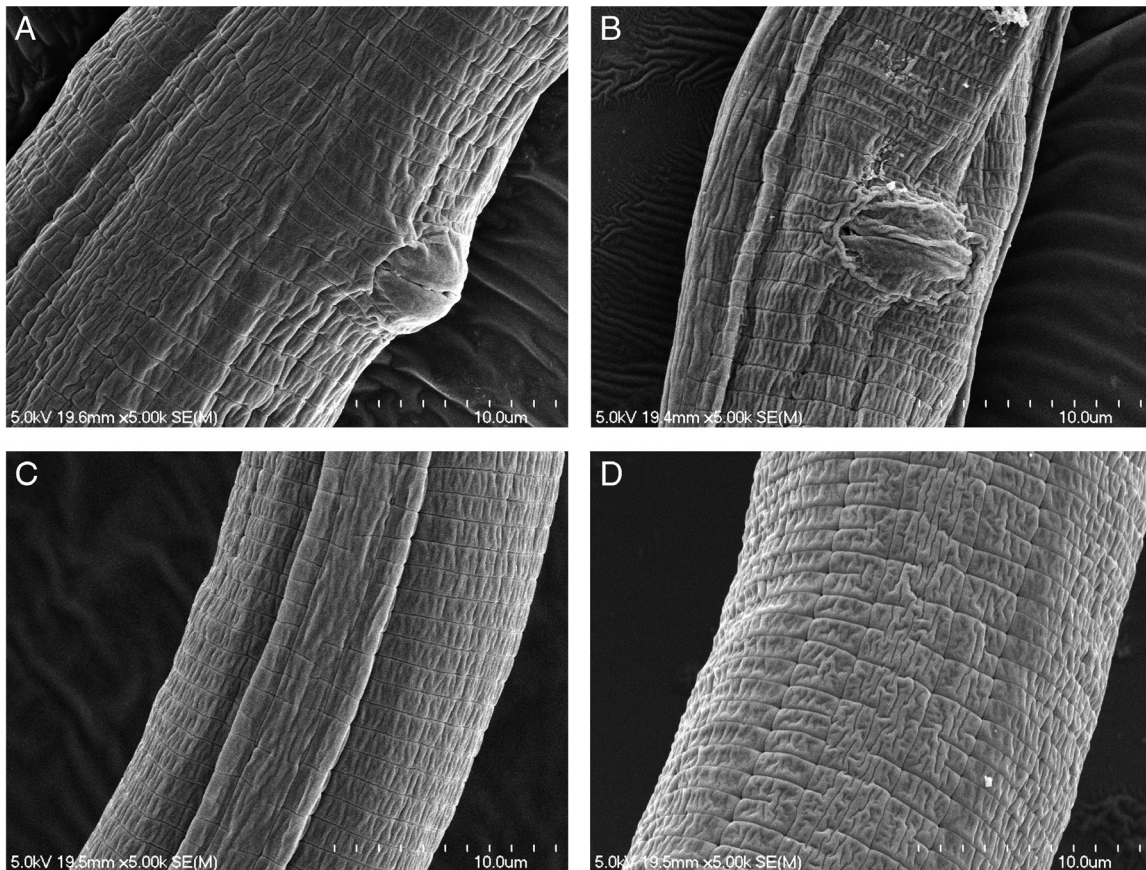


Figure 4: *P. smoliki* n. sp. SEM of midbody region, different NID numbers specify different individual specimens. (A) NID 4808 and (B) NID 4818 slightly protruding vulval lips, (C) NID 4807 and (D) NID 4803 lateral field with irregularly areolated lines at midbody.

functional, rounded spermatheca, stylet about 15 μm in length, more posterior vulva ($V > 75\%$), conoid to subcylindrical tail with smooth terminus, and abundant males. Similar *Pratylenchus* species, based on this combination of characters, along with the results of our phylogenetic analyses, include *P. alleni* Ferris, 1961, *P. hexincisus* Taylor & Jenkins, 1957, *P. pseudocoffeae* Mizukubo, 1992, and *P. scribneri* Steiner in Sherbakoff & Stanley, 1943. The latter three species all have basically the same face pattern (type I in Subbotin et al., 2008) as *P. smoliki* n. sp. when viewed with SEM. The face pattern of *P. alleni* has not been clearly established, but preliminary results of our SEM studies indicate a similar general appearance.

Pratylenchus smoliki n. sp. differs from *P. alleni* mainly by the longer body (usually $> 500 \mu\text{m}$ vs maximum $440 \mu\text{m}$), and in the shorter pharyngeal overlap ($< 30 \mu\text{m}$ vs $> 40 \mu\text{m}$), along with generally shorter post-vulval uterine sac (PUS), relative to vulval body width (PUS/vbw; 0.9 vs 1.1) and slightly more

anterior vulva ($< 80\%$ vs $> 80\%$), although the value ranges for these characters overlap (Figure 6).

From *P. hexincisus*, the new species differs in having numerous males (vs rare) and a functional, rounded spermatheca (vs. spermatheca absent or reduced), along with greater body length and c ratio, and lower b and c' values (mean values: $511 \mu\text{m}$ vs $453 \mu\text{m}$, 22.1 vs 18.4, 5.6 vs 6.1, and 1.9 vs 2.3, respectively). *P. smoliki* n. sp. also usually displays fewer lateral field lines in the advulval region (4-6 vs 6-8).

Pratylenchus smoliki n. sp. can be differentiated from *P. pseudocoffeae* based on spermatheca shape (round vs oval), and shorter PUS ($< 20 \mu\text{m}$ vs $> 25 \mu\text{m}$). Overall, *P. smoliki* n. sp. has lower values for a, c', V and stylet length (mean values: 24.4 vs 27.6, 1.9 vs 2.2, 79.2 vs 81.0, and 15.1 vs 16.0 μm , respectively), and a higher mean c value (22.1 vs 19.3).

Pratylenchus smoliki n. sp. differs from *P. scribneri* in spermatheca shape (round vs oval), shorter PUS ($< 20 \mu\text{m}$, PUS/vbw 0.9 vs $> 25 \mu\text{m}$, PUS/vbw 1.3),

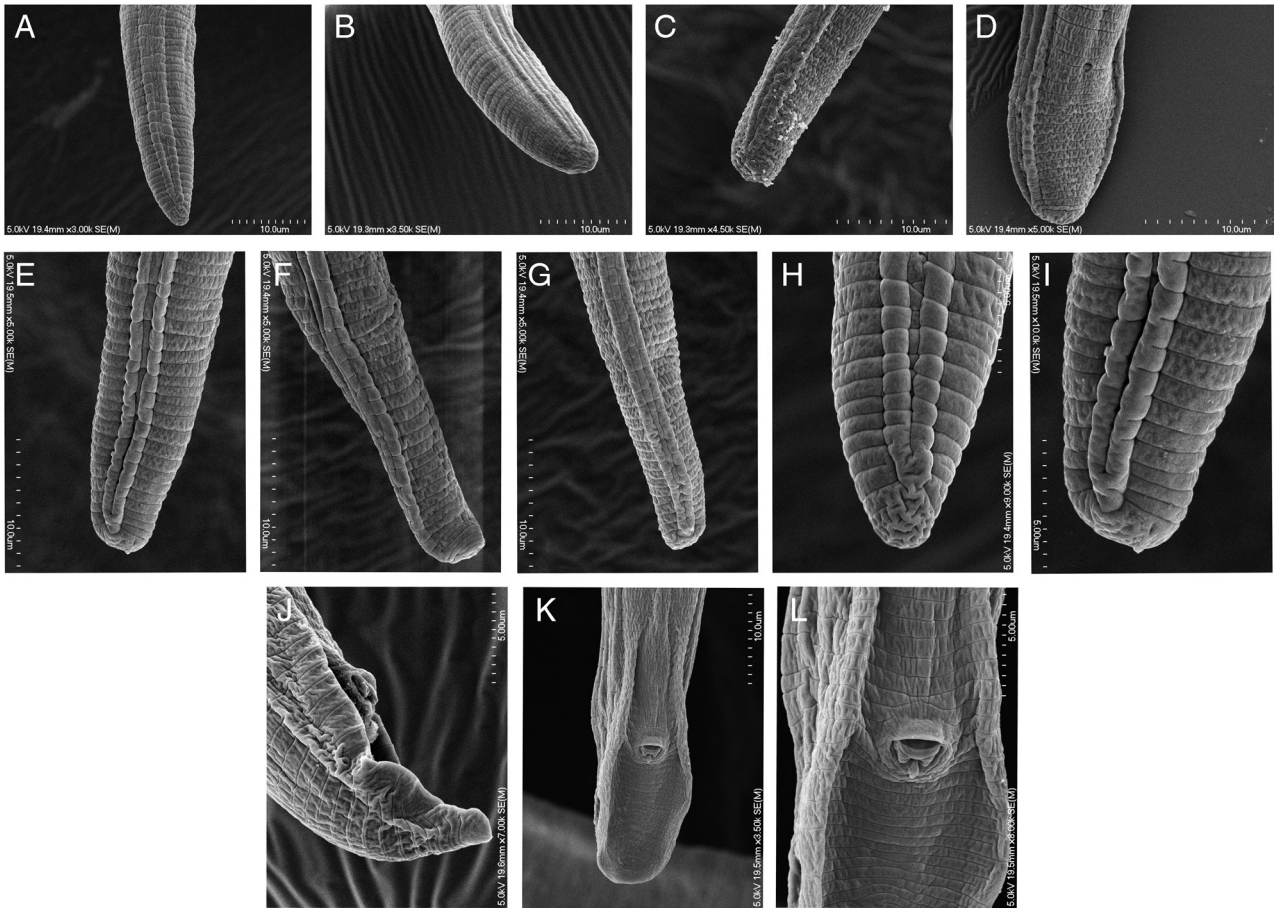


Figure 5: SEM images of *P. smoliki* n. sp. tails, different NID numbers specify different individual specimens. A–I exhibit the variation in tail termini, J–L illustrate male tails. (A) NID 4803, (B) NID 4819, (C) NID 4813, (D) NID 4818, (E) NID 4805, (F) NID 4811, (G) NID 4809, (H) NID 4803, (I) NID 4805, (J) NID 4817, (K) NID 4816, (L) NID 4816.

shorter pharyngeal overlap (<30 vs >30 µm), lower mean *a* value (<25 vs >25) and a generally shorter tail (mean *c* 22.1 vs 17.5, mean *c'* 1.9 vs 2.4).

It should be noted, however, that overlap of the range values of all of these morphological characters makes the separation of these species by morphology alone difficult and unreliable.

Other *Pratylenchus* species with different SEM face patterns, but sharing with *P. smoliki* n. sp. the combination of two lip annuli, functional spermatheca, presence of males, and lateral field non-areolated with four lines at vulval region include *P. coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941, *P. flakkensis* Seinhorst, 1968, *P. loosi* Loof, 1960, and *P. silvaticus* Brzeski, 1998.

P. smoliki n. sp. can be differentiated from *P. coffeae* by its shorter stylet (mean stylet length 15.1 vs 16.3 µm) and pharyngeal overlap (<30 vs > 30 µm), round (vs oval) spermatheca, and shorter PUS (<20 vs

>35 µm). Overall, *P. smoliki* n. sp. has lower values for *L*, *a*, *b*, *c'* and *V* (mean values 511, 24.4, 5.6, 1.9, 79.2 vs 578, 28.6, 6.4, 2.3, 80.3, respectively). The face pattern for *P. coffeae* shows a mostly set off labial disc, but no clear separation of the lateral or submedial lips and thus no angle formed between the laterals and the labial disc (pattern J of Subbotin et al., 2008).

From *P. flakkensis*, *P. smoliki* n. sp. differs in its shorter tail (mean *c* 22.1 vs 17.2, mean *c'* 1.9 vs 2.5) with smooth (vs annulated) terminus and shorter stylet (< 16 vs > 16 µm). In *P. flakkensis*, the lateral and submedial lips are not fused and the laterals form an obtuse, doubly-bent angle, similar to the pattern in *P. smoliki* n. sp, but the labial disc is not set off (pattern H in Subbotin et al., 2008).

Compared with *P. loosi*, the new species differs in having a rounded (vs rectangular) spermatheca, more anterior vulva (mean *V* value 79.2% vs 82.0%), smooth

Table 3. Measurements of *P. smoliki* n. sp. specimens.

| | Holotype | N | Females | N | Males | N | Juveniles |
|---------------|----------|----|------------------------|----|-------------------------|---|------------------------|
| L | 491 | 22 | 511.4 + 50.6 (407-604) | 25 | 456.8 + 32.7 (415-560) | 4 | 320.5 + 38.1 (289-385) |
| a | 21.0 | 22 | 24.4 + 3.0 (18.8-31.9) | 25 | 27.1 + 1.7 (23.8-31.7) | 4 | 20.5 + 2.3 (18.0-24.1) |
| b | 5.4 | 21 | 5.6 + 0.4 (4.8-6.6) | 25 | 5.3 + 0.4 (4.4-5.9) | 4 | 3.7 + 0.5 (3.3-4.5) |
| b' | 4.2 | 22 | 4.3 + 0.4 (3.5-4.9) | 25 | 4.1 + 0.3 (3.6-4.6) | 4 | 3.0 + 0.3 (2.5-3.4) |
| c | 19.5 | 22 | 22.1 + 2.8 (17.7-31.5) | 25 | 22.6 + 3.9 (16.7-34.1) | 4 | 17.7 + 2.2 (15.7-21.5) |
| c' | 1.9 | 22 | 1.9 + 0.2 (1.6-2.2) | 24 | 1.6 + 0.2 (1.2-2.2) | 4 | 1.8 + 0.1 (1.7-1.9) |
| V % | 79.4 | 22 | 79.2 + 1.4 (76.8-82.0) | | | | |
| Lip annules | 2 | 22 | 2.0 + 0 (2-2) | 25 | 2.0 + 0 (2-2) | 4 | 2.0 + 0 (2-2) |
| St | 16 | 22 | 15.1 + 0.6 (14-16) | 25 | 14.2 + 0.6 (13-15) | 4 | 12.7 + 0.6 (12-14) |
| M % | 50 | 22 | 48.3 + 1.6 (44.9-51.7) | 25 | 47.7 + 2.9 (43.1-54.7) | 4 | 44.9 + 2.5 (41.9-48.7) |
| DGO | 3 | 22 | 2.7 + 0.3 (2-3) | 25 | 2.9 + 0.1 (3-3) | 4 | 2.3 + 0.1 (2-3) |
| O % | 17.3 | 22 | 17.7 + 1.9 (12.7-20.3) | 25 | 20.0 + 1.2 (18.3-23.0) | 4 | 18.3 + 1.3 (16.5-20.0) |
| MB % | 45.1 | 22 | 44.1 + 2.5 (39.4-51.7) | 25 | 44.6 + 2.6 (39.8-50.1) | 4 | 42.0 + 3.2 (39.2-47.1) |
| Overlap | | 22 | 29.1 + 6.5 (16-41) | 25 | 26.2 + 6.4 (14-39) | 4 | 19.4 + 8.3 (7-27) |
| Body ann W | 1.6 | 22 | 1.4 + 0.2 (1.0-2.0) | 24 | 1.3 + 0.1 (1.0-1.5) | 4 | 1.2 + 0.1 (1.0-1.3) |
| LFL | 4 | | | 24 | 4.0 + 0.0 (4-4) | | |
| PUS/Bw | 0.6 | 22 | 0.9 + 0.2 (1-1) | | | | |
| PUS/V-A % | 16.1 | 22 | 22.5 + 6.4 (13.5-32.8) | | | | |
| Sthc L/W | | 22 | 1.1 + 0.1 (0.9-1.4) | | | | |
| T % | | | | 25 | 44.1 + 4.8 (35.0-55.0) | | |
| Spicule | | | | 25 | 16.8 + 0.8 (15-19) | | |
| Gubernaculum | | | | 24 | 4.3 + 0.6 (3-6) | | |
| Bursa: % tail | | | | 25 | 96.5 + 9.2 (80.5-136.5) | | |
| Tail | 25 | 22 | 23.4 + 2.8 (17-29) | 25 | 20.7 + 3.2 (13-28) | 4 | 18.2 + 0.4 (18-19) |
| T/VA | 0.3 | 22 | 0.3 + 0.0 (0.2-0.4) | | | | |
| Ran | 21 | 22 | 19.7 + 3.0 (14-26) | 6 | 18.6 + 3.0 (15-24) | 4 | 18.0 + 1.2 (16-19) |

and blunt tail terminus (vs pointed) and shorter stylet (average length 15.1 vs 16.0 μ m). In general, *P. smoliki* n. sp. is shorter (mean L 511 vs 588 μ m), stouter (mean a 24.4 vs 29.4), with a shorter tail (mean c 22.1 vs 19.3, mean c' 1.9 vs 2.8) and slightly lower b ratio (mean value 5.6 vs 6.4). The face pattern for *P. loosi* is similar to that of *P. coffeae* (above).

P. smoliki n. sp. differs from *P. silvaticus* by its shorter pharyngeal overlap (<30 vs >30 μ m), longer body (mean L 511 vs 450 μ m), more posterior vulva (mean V <80% vs >80%), and shorter tail (mean c' 1.9 vs. 2.4). In addition, average values for b (5.6 vs 6.3) and PUS relative to vulval body width (0.9 vs 1.3) are

lower in *P. smoliki* n. sp. No *en face* pattern data for *P. silvaticus* are available.

Also morphologically similar is *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941, which generally lacks males, except in rare instances. *P. smoliki* n. sp. females have a prominent, functional spermatheca (vs. absent or reduced in *P. neglectus*), longer PUS (> 16 vs < 16 μ m), more anterior vulva (mean V of 79.2% vs 82.0%), shorter stylet (mean stylet length 15.1 vs 16.5 μ m), and shorter tail (mean c value 22.1 vs 19.8, mean c' 1.9 vs 2.2). In *en face* view, the labial disc of *P. neglectus* is not offset, and forms a simple (single inflection), obtuse angle with the lateral lip sectors.

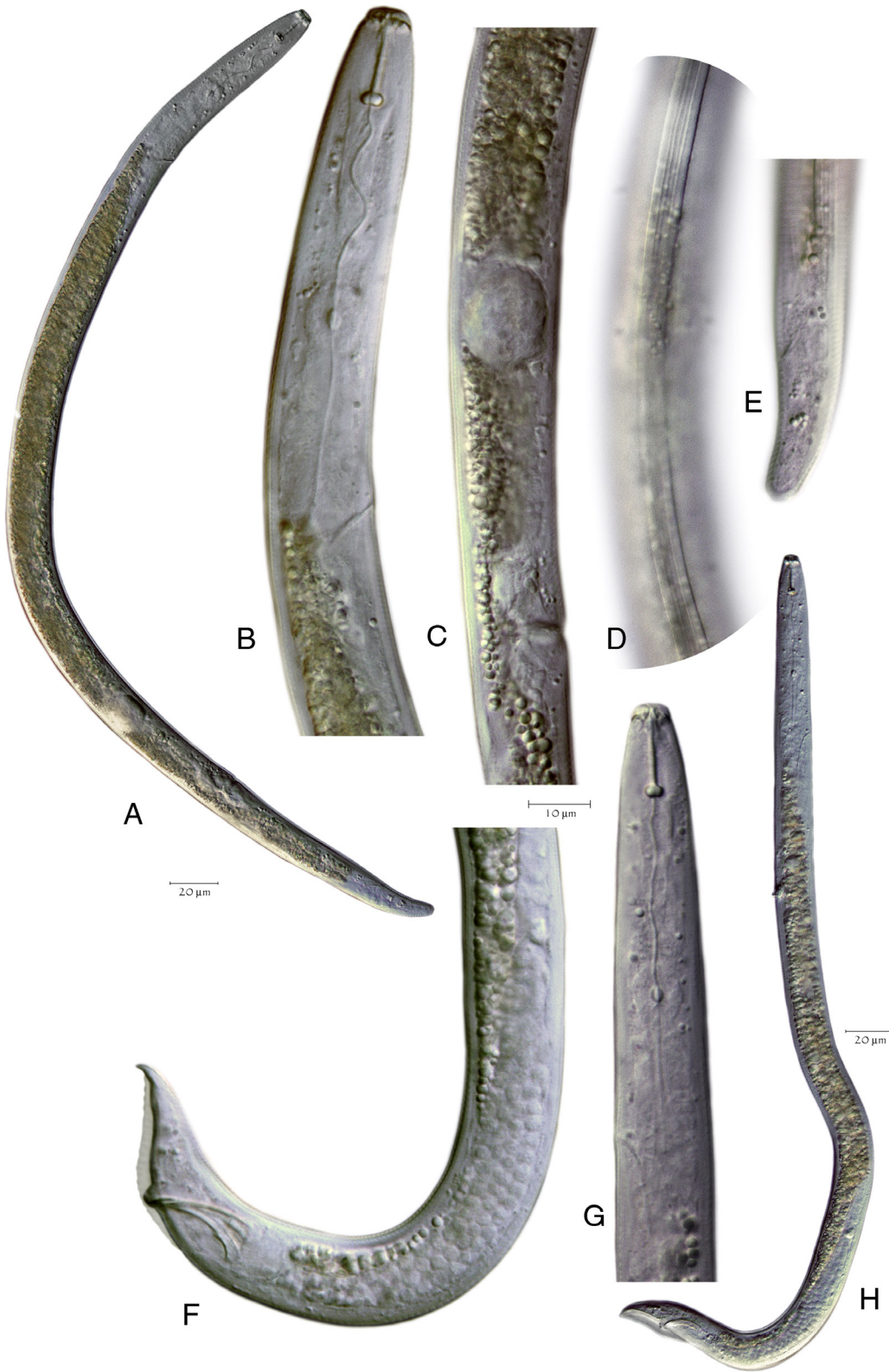
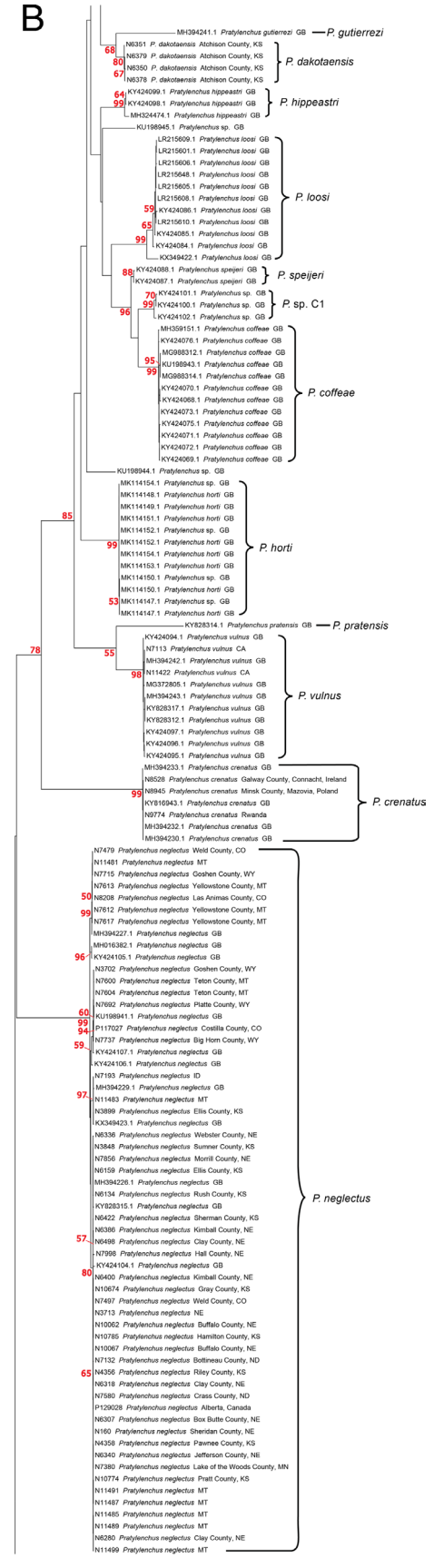


Figure 6: Light microscope images of *Pratylenchus allenii* specimens from the type locality Saline County, Illinois. (A–E) female NID 7381, (F–H) male NID 7382. (A) Entire body, (B) Head and anterior region, (C) Reproductive region, (D) Midbody lateral lines, (E) Tail, (F) Tail, bursa and spicules, (G) Head and anterior portion, (H) Entire body

A



B



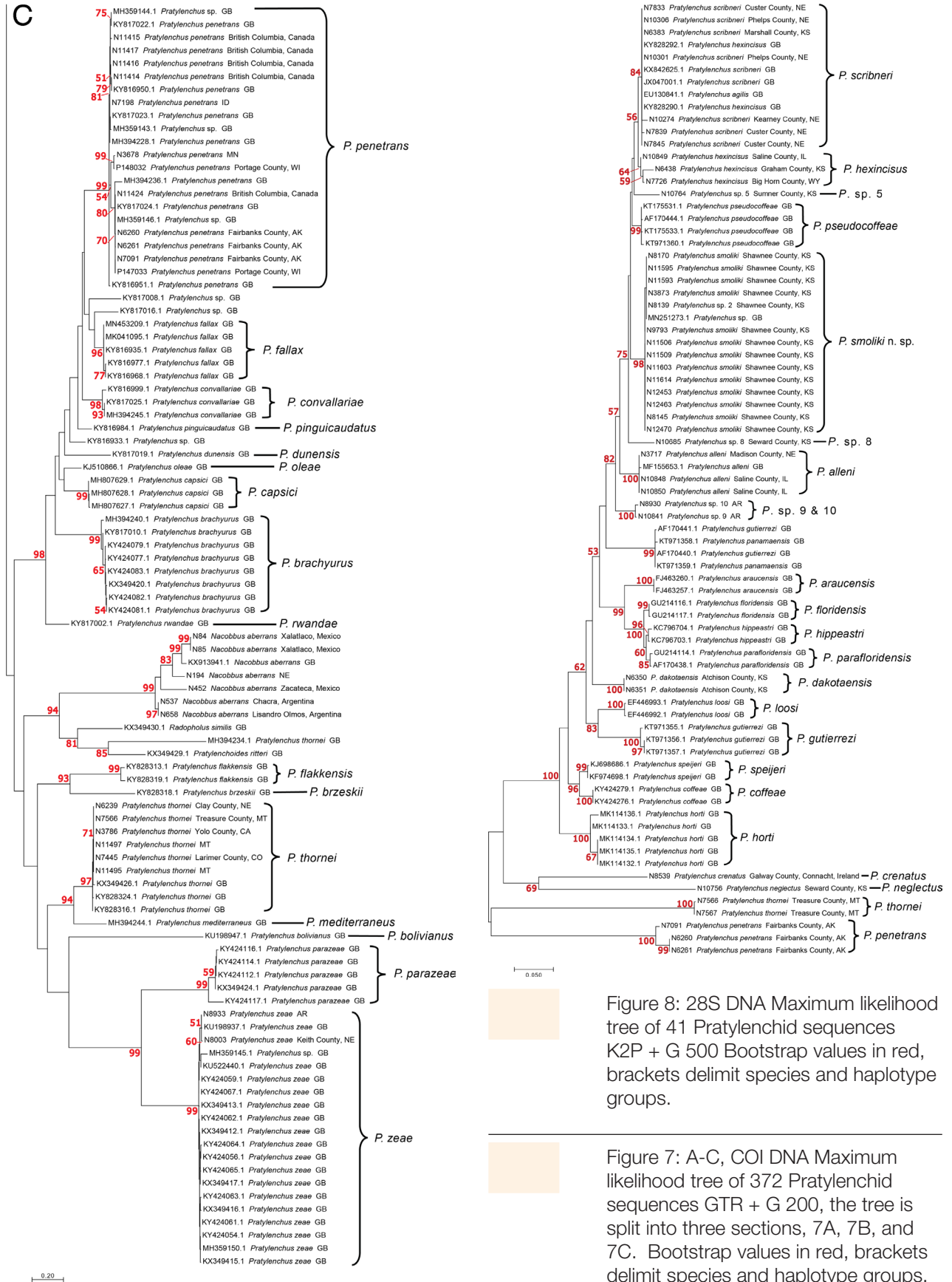


Figure 8: 28S DNA Maximum likelihood tree of 41 Pratylenchid sequences K2P + G 500 Bootstrap values in red, brackets delimit species and haplotype groups.

Figure 7: A-C, COI DNA Maximum likelihood tree of 372 Pratylenchid sequences GTR + G 200, the tree is split into three sections, 7A, 7B, and 7C. Bootstrap values in red, brackets delimit species and haplotype groups.

Table 4. Estimates of evolutionary divergence (p-distances) over sequence pairs within (bold) and between groups.

| | <i>N.</i> | <i>P.</i> <i>aberrans</i> | <i>P.</i> <i>alleni</i> | <i>P.</i> <i>crenatus</i> | <i>P.</i> <i>hexicisus</i> | <i>P.</i> <i>neglectus</i> | <i>P.</i> <i>penetrans</i> | <i>P.</i> <i>scribneri</i> | <i>P.</i> <i>sp. 3</i> | <i>P.</i> <i>Clay</i> | <i>P.</i> <i>dakotaensis</i> | <i>P.</i> <i>smoliki</i> | <i>P.</i> <i>sp. 9</i> | <i>P.</i> <i>sp. 7</i> | <i>P.</i> <i>sp. 5</i> | <i>P.</i> <i>thornei</i> | <i>P.</i> <i>vulnus</i> | <i>P.</i> <i>zeae</i> | | |
|--------------------------|-----------|------------------------------|----------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---------------------------|--------------------------|---------------------------------|-----------------------------|---------------------------|---------------------------|---------------------------|-----------------------------|----------------------------|--------------------------|--|--|
| <i>Nacobbus aberrans</i> | 0.105 | | | | | | | | | | | | | | | | | | | |
| <i>P. alleni</i> | 0.328 | 0.003 | | | | | | | | | | | | | | | | | | |
| <i>P. crenatus</i> | 0.358 | 0.316 | 0.001 | | | | | | | | | | | | | | | | | |
| <i>P. hexicisus</i> | 0.416 | 0.217 | 0.36 | 0.029 | | | | | | | | | | | | | | | | |
| <i>P. neglectus</i> | 0.314 | 0.3 | 0.36 | 0.381 | 0.02 | | | | | | | | | | | | | | | |
| <i>P. penetrans</i> | 0.325 | 0.323 | 0.32 | 0.379 | 0.31 | 0.032 | | | | | | | | | | | | | | |
| <i>P. scribneri</i> | 0.36 | 0.172 | 0.32 | 0.198 | 0.329 | 0.334 | 0.007 | | | | | | | | | | | | | |
| <i>P. sp. 3</i> | 0.365 | 0.181 | 0.356 | 0.23 | 0.339 | 0.348 | 0.187 | 0.004 | | | | | | | | | | | | |
| <i>P. sp. Clay</i> | 0.373 | 0.188 | 0.329 | 0.196 | 0.329 | 0.339 | 0.112 | 0.191 | 0 | | | | | | | | | | | |
| <i>P. dakotaensis</i> | 0.335 | 0.216 | 0.306 | 0.286 | 0.322 | 0.307 | 0.242 | 0.228 | 0.247 | 0.078 | | | | | | | | | | |
| <i>P. smoliki</i> n. sp. | 0.412 | 0.219 | 0.381 | 0.21 | 0.377 | 0.362 | 0.227 | 0.19 | 0.221 | 0.298 | 0.101 | | | | | | | | | |
| <i>P. sp. 9 & 10</i> | 0.378 | 0.204 | 0.336 | 0.23 | 0.355 | 0.35 | 0.21 | 0.226 | 0.205 | 0.254 | 0.229 | 0.105 | | | | | | | | |
| <i>P. sp. 7</i> | 0.351 | 0.171 | 0.303 | 0.215 | 0.311 | 0.321 | 0.142 | 0.188 | 0.15 | 0.212 | 0.235 | 0.212 | 0.091 | | | | | | | |
| <i>P. sp. 5</i> | 0.38 | 0.195 | 0.333 | 0.179 | 0.358 | 0.349 | 0.127 | 0.2 | 0.079 | 0.26 | 0.206 | 0.204 | 0.161 | 0 | | | | | | |
| <i>P. thornei</i> | 0.311 | 0.34 | 0.372 | 0.386 | 0.298 | 0.311 | 0.355 | 0.352 | 0.348 | 0.32 | 0.373 | 0.349 | 0.328 | 0.366 | 0.002 | | | | | |
| <i>P. vulnus</i> | 0.335 | 0.241 | 0.321 | 0.3 | 0.333 | 0.305 | 0.265 | 0.287 | 0.276 | 0.241 | 0.319 | 0.259 | 0.246 | 0.297 | 0.316 | 0.051 | | | | |
| <i>P. zeae</i> | 0.326 | 0.326 | 0.346 | 0.398 | 0.315 | 0.331 | 0.352 | 0.332 | 0.34 | 0.314 | 0.394 | 0.36 | 0.331 | 0.36 | 0.317 | 0.327 | 0.012 | | | |
| <i>P. horti</i> | 0.331 | 0.26 | 0.331 | 0.296 | 0.317 | 0.31 | 0.243 | 0.23 | 0.244 | 0.217 | 0.308 | 0.246 | 0.228 | 0.277 | 0.295 | 0.254 | 0.299 | | | |
| <i>P. fallax</i> | 0.279 | 0.302 | 0.292 | 0.359 | 0.278 | 0.153 | 0.311 | 0.332 | 0.321 | 0.271 | 0.39 | 0.342 | 0.297 | 0.346 | 0.272 | 0.296 | 0.309 | | | |
| <i>P. pseudocoffeae</i> | 0.326 | 0.18 | 0.339 | 0.22 | 0.326 | 0.326 | 0.193 | 0.181 | 0.173 | 0.243 | 0.229 | 0.222 | 0.184 | 0.197 | 0.345 | 0.277 | 0.331 | | | |
| <i>P. coffeae</i> | 0.314 | 0.231 | 0.28 | 0.302 | 0.324 | 0.293 | 0.229 | 0.249 | 0.237 | 0.224 | 0.291 | 0.24 | 0.224 | 0.257 | 0.304 | 0.244 | 0.303 | | | |
| <i>P. hippeastri</i> | 0.307 | 0.234 | 0.318 | 0.3 | 0.325 | 0.307 | 0.247 | 0.246 | 0.255 | 0.201 | 0.278 | 0.256 | 0.228 | 0.267 | 0.326 | 0.246 | 0.317 | | | |
| <i>P. loosi</i> | 0.301 | 0.223 | 0.311 | 0.302 | 0.281 | 0.302 | 0.242 | 0.236 | 0.246 | 0.215 | 0.301 | 0.248 | 0.235 | 0.263 | 0.298 | 0.271 | 0.296 | | | |
| <i>P. convallariae</i> | 0.315 | 0.337 | 0.316 | 0.385 | 0.316 | 0.167 | 0.329 | 0.349 | 0.333 | 0.291 | 0.389 | 0.346 | 0.308 | 0.351 | 0.31 | 0.305 | 0.323 | | | |
| <i>P. sp</i> | 0.324 | 0.222 | 0.287 | 0.284 | 0.309 | 0.309 | 0.228 | 0.242 | 0.254 | 0.204 | 0.288 | 0.242 | 0.227 | 0.252 | 0.32 | 0.25 | 0.296 | | | |
| <i>P. speijeri</i> | 0.306 | 0.235 | 0.29 | 0.274 | 0.307 | 0.312 | 0.216 | 0.253 | 0.233 | 0.198 | 0.3 | 0.236 | 0.224 | 0.248 | 0.305 | 0.231 | 0.306 | | | |
| <i>P. capsici</i> | 0.299 | 0.313 | 0.329 | 0.365 | 0.296 | 0.194 | 0.312 | 0.319 | 0.317 | 0.277 | 0.368 | 0.342 | 0.306 | 0.339 | 0.296 | 0.3 | 0.326 | | | |
| <i>P. brachyurus</i> | 0.316 | 0.323 | 0.339 | 0.396 | 0.31 | 0.233 | 0.341 | 0.341 | 0.366 | 0.296 | 0.388 | 0.371 | 0.328 | 0.369 | 0.283 | 0.317 | 0.328 | | | |
| <i>P. fiakkensis</i> | 0.317 | 0.342 | 0.377 | 0.392 | 0.352 | 0.305 | 0.356 | 0.349 | 0.343 | 0.324 | 0.384 | 0.354 | 0.336 | 0.371 | 0.292 | 0.335 | 0.332 | | | |

| | | | | | | | | | | | | | | | | | |
|-------------------------|-------|------------------|-------|-------------------|---------------------|-----------------|------------------------|------------------|--------------------|-------------------|----------------------|----------------------|--------------------|-------|-------|-------|-------|
| <i>P. parazeae</i> | 0.386 | 0.368 | 0.394 | 0.398 | 0.366 | 0.385 | 0.368 | 0.355 | 0.368 | 0.353 | 0.398 | 0.388 | 0.359 | 0.374 | 0.346 | 0.359 | 0.264 |
| <i>P. horti</i> | | <i>P. fallax</i> | | <i>P. coffeae</i> | <i>P. hippeastr</i> | <i>P. loosi</i> | <i>P. convallariae</i> | <i>P. sp. C1</i> | <i>P. speijeri</i> | <i>P. capsici</i> | <i>P. brachyurus</i> | <i>P. flakkensis</i> | <i>P. parazeae</i> | | | | |
| <i>P. horti</i> | 0.001 | | | | | | | | | | | | | | | | |
| <i>P. fallax</i> | 0.292 | 0.007 | | | | | | | | | | | | | | | |
| <i>P. pseudocoffeae</i> | 0.248 | 0.325 | 0 | | | | | | | | | | | | | | |
| <i>P. coffeae</i> | 0.229 | 0.291 | 0.252 | 0.008 | | | | | | | | | | | | | |
| <i>P. hippeastr</i> | 0.224 | 0.304 | 0.256 | 0.185 | 0.023 | | | | | | | | | | | | |
| <i>P. loosi</i> | 0.221 | 0.305 | 0.249 | 0.193 | 0.209 | 0.026 | | | | | | | | | | | |
| <i>P. convallariae</i> | 0.305 | 0.145 | 0.331 | 0.279 | 0.297 | 0.306 | 0.021 | | | | | | | | | | |
| <i>P. sp. C1</i> | 0.238 | 0.298 | 0.27 | 0.143 | 0.197 | 0.193 | 0.296 | 0.018 | | | | | | | | | |
| <i>P. speijeri</i> | 0.215 | 0.294 | 0.241 | 0.118 | 0.164 | 0.181 | 0.283 | 0.109 | 0.005 | | | | | | | | |
| <i>P. capsici</i> | 0.276 | 0.169 | 0.334 | 0.295 | 0.278 | 0.284 | 0.187 | 0.292 | 0.28 | 0.006 | | | | | | | |
| <i>P. brachyurus</i> | 0.308 | 0.202 | 0.356 | 0.308 | 0.316 | 0.296 | 0.229 | 0.298 | 0.279 | 0.211 | 0.014 | | | | | | |
| <i>P. flakkensis</i> | 0.314 | 0.292 | 0.356 | 0.299 | 0.291 | 0.292 | 0.337 | 0.335 | 0.333 | 0.289 | 0.315 | 0.04 | | | | | |
| <i>P. parazeae</i> | 0.352 | 0.377 | 0.389 | 0.361 | 0.373 | 0.37 | 0.389 | 0.353 | 0.357 | 0.357 | 0.353 | 0.356 | 0.049 | | | | |

Recently another new species of *Pratylenchus*—*P. dakotaensis* Handoo, Yan, Kantor, Chowdhury, Plaisance, Bauchan & Mowery, 2021—from North Dakota (USA) has been described. DNA sequence data readily differentiate *P. dakotaensis* from *P. smoliki* n. sp., despite the biome similarity of Great Plains type localities. In addition, *P. smoliki* n. sp. differs from *P. dakotaensis* in number of lip annules (2 vs 3), tail terminus morphology (smooth under LM vs crenate), and lateral field characteristics (outer bands generally plain under LM in *P. smoliki* n. sp. vs areolated in *P. dakotaensis*). The two species also differ in *en face* pattern: as discussed above, *P. smoliki* n. sp. displays a pattern similar to type I of Subbotin et al. (2008), with a more set-off labial disc, while the face of *P. dakotaensis* conform to type H, similar to that of *P. flakkensis*, with the labial disc not demarcated.

Phylogeny: The maximum likelihood COI tree of *Pratylenchus* species includes 47 specimens from Kansas and Nebraska that belong to a clade representing *Pratylenchus smoliki* n. sp (Figs. 6 and 7). The clade is supported by a bootstrap support value of 81, with subgroups within the clade that indicate differentiation at a subspecific level. The *P. smoliki* n. sp. clade includes specimens of all life stages. Thirteen of the *P. smoliki* n. sp. specimens on the COI tree are also represented on 28S ML tree (Fig. 8). A bootstrap support value of 98 characterizes the *P. smoliki* n. sp. clade on the 28S tree, and a GenBank sequence from Wisconsin suggests that the geographic range of the species may extend to three U.S. states. The 28S tree has support at deeper nodes in the tree and allows recognition of *P. smoliki* n. sp. as a member of a group consisting of four related described species (*P. scribneri*, *P. hexincisus*, *P. pseudocoffeae*, *P. allenii*) and a putative undescribed species labeled as haplotype groups 9 and 10 in Figure 7. This grouping of species is consistently supported by 28S, 18S, and ITS trees in the literature (Inserra et al., 2007; Subbotin et al., 2008; Araya et al., 2016; Troccoli et al., 2016; Janssen et al., 2017; Singh et al., 2018; Nguyen et al., 2019; Ozbayrak et al., 2019; Handoo et al., 2021) and is highly congruent with grouping based on lip patterns. A maximum likelihood ITS1 tree also illustrates the distinctiveness of *P. smoliki* n. sp. (Supplementary Fig. S1). Although higher evolutionary rates in the COI gene constrains its use for deeper divergences on the tree, it is often the case that COI haplotype groups of described species are consistent with species boundaries established by other morphological and molecular characters. However, within the genus *Pratylenchus* a considerable amount of mitochondrial genetic differentiation has occurred, and for some

species such as *P. smoliki* n. sp., the population-level variation is relatively high (Table 4; Ozbayrak et al., 2019 Table 3). Low amounts of haplotype diversity and a simple population structure may reflect recent dispersal characteristic of an introduced species (Clavero et al., 2016). *P. thornei* is a good example of a potentially introduced species to North America, where a single haplotype is spread across six western states in the U.S. (Ozbayark et al., 2019). Conversely, multiple genetic subgroups may reflect a native origin with a higher level of differentiation among regional populations (Grenier et al., 2010, Powers et al., 2016). *Pratylenchus* isolates from native plant communities will help resolve the issue of natives versus introduced status for *Pratylenchus* species.

Topotype specimens of *P. allenii* from Saline County, Illinois grouped with *P. allenii* specimens collected from Wisconsin and Nebraska in both COI and 28S trees. This species does not occur frequently in Great Plains corn/soybean rotations compared to the occurrence of *P. scribneri* and *P. neglectus* in these cropping systems. *P. allenii* does appear very similar to *P. smoliki* n. sp. and *P. pseudocoffeae* in SEM face, body, and tail views (Fig. 6; Deimi et al., 2009; Araya et al., 2016) supporting their relatively close genetic relationships.

Host range trials

Host range trials were conducted to assess the reproductive potential of *P. smoliki* n. sp. Host status varied with corn hybrid ($p < 0.001$), agronomic crop ($p = 0.016$), and cover crop ($p < 0.0001$). Mean

numbers of nematodes recovered from corn roots in corn hybrid trials ranged from 6,169 per pot to 14,864 per pot, with a standard error of 1,782 (Fig. 9). In agronomic crop trials, the largest populations were recovered from corn and wheat, with populations recovered from sorghum and soybean averaging less than 20% of the mean number of nematodes recovered from corn roots (Fig. 10). There was no evidence of a cultivar within crop effect ($p = 0.98$). In cover crop trials, the largest populations were recovered from corn and rye, followed by sunflower and wheat, with less than 30% of the mean number of nematodes recovered from corn roots (Fig. 11). Alfalfa, pea, and radish were associated with the lowest recovered nematode populations, averaging less than 10% of the mean number of nematodes recovered from corn roots.

Root-lesion nematodes are typically characterized as “polyphagous”, with wide host ranges (Castillo and Vovlas, 2007). Nevertheless, large ranges in host suitability have been reported for *Pratylenchus* species, including *P. penetrans* (Belair et al., 2007), and *P. neglectus* and *P. thornei* (Taylor et al., 2000; Vanstone and Russ, 2001; Smiley et al., 2014). While *P. smoliki* n. sp. was recovered from the two-month-old roots of every crop plant investigated in the present study, there was a similarly large range in host suitability among those crops, with important implications for nematode management. Corn appears to be the primary agronomic host for *P. smoliki* n. sp., although it should be noted that corn hybrids exhibited a continuum in host

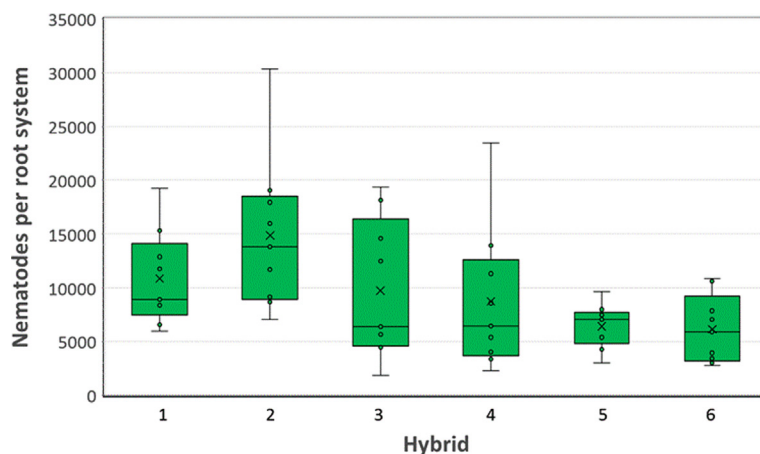


Figure 9: Box and whisker plots of the distribution of numbers of *Pratylenchus smoliki* n. sp. recovered from roots of six eight-week old corn hybrids. The lower bound of the box indicates the first quartile, the central line is the median, and the upper bound is the third quartile. The lower and upper whiskers represent minimum and maximum values. Outliers that are more than 1.5 times the interquartile range (third quartile minus first quartile) from the lower or upper quartile, respectively, are indicated with dots. The mean is indicated by the symbol “x”.

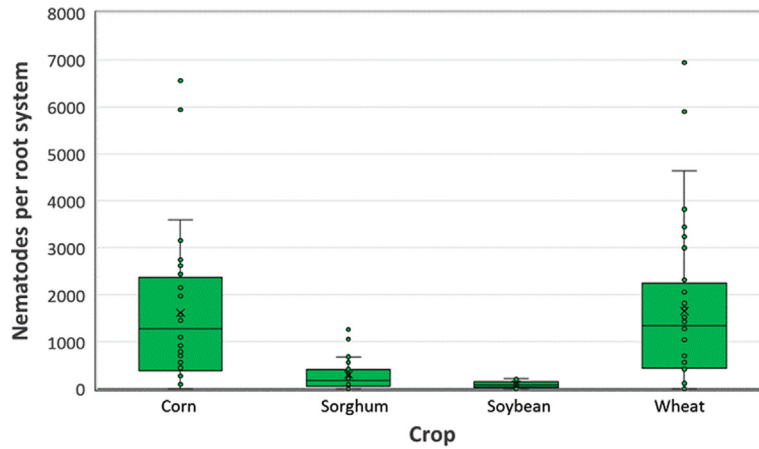


Figure 10: Box and whisker plots of the distribution of numbers of *Pratylenchus smoliki* n. sp. recovered from roots of four eight-week old agronomic crop plants. The lower bound of the box indicates the first quartile, the central line is the median, and the upper bound is the third quartile. The lower and upper whiskers represent minimum and maximum values. Outliers that are more than 1.5 times the interquartile range (third quartile minus first quartile) from the lower or upper quartile, respectively, are indicated with dots. The mean is indicated by the symbol “x”.

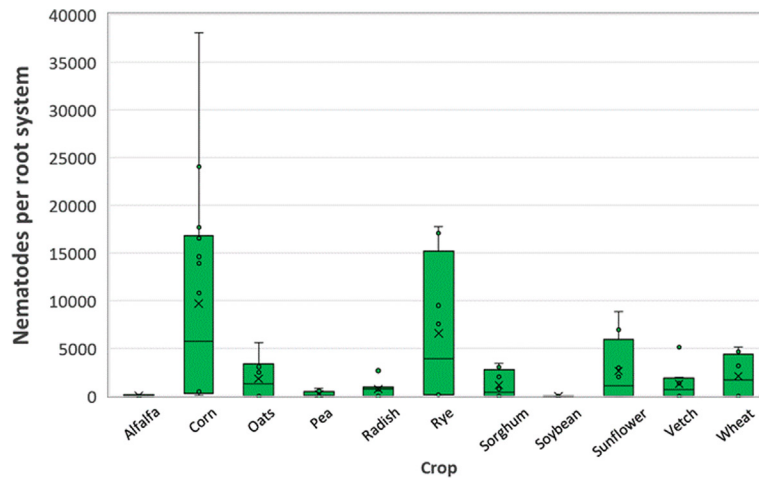


Figure 11: Box and whisker plots of the distribution of numbers of *Pratylenchus smoliki* n. sp. recovered from roots of 11 eight-week old cover crops. The lower bound of the box indicates the first quartile, the central line is the median, and the upper bound is the third quartile. The lower and upper whiskers represent minimum and maximum values. Outliers that are more than 1.5 times the interquartile range (third quartile minus first quartile) from the lower or upper quartile, respectively, are indicated with dots. The mean is indicated by the symbol “x”.

suitability. Wheat, rye, and sunflower are also suitable hosts based on the observed nematode population increases, while alfalfa, sorghum, and soybean were consistently associated with little or no nematode population increase. These latter agronomic crops could be recommended as rotational crops for managing this nematode in corn. Additionally, the host

status of cover crops (as well as weeds) needs to be considered, as several of these have been reported to be good hosts for agronomically important species of *Pratylenchus* (Miller, 1978; Belair et al., 2007). At least one cover crop (rye) was identified as a risk in terms of host suitability in the present study. Finally, although phylogenetic signals in pathogen host

ranges have been suggested to be predictive of novel host associations (Gilbert and Parker, 2016), this signal does not appear to be robust in *P. smoliki* n. sp., with the possible exception of the Triticeae. The observed host range might alternatively be explained by ecological fitting (Agosta, 2006), where each of the most suitable crop host species are members of plant families common to the tallgrass prairie: Andropogoneae (e.g. *Andropogon gerardi*), Triticeae (e.g. *Elymus canadensis*), and Asteraceae (e.g. *Helianthus petiolaris*). If this is the case, it suggests that *P. smoliki* n. sp. is native to the eastern Great Plains region. The COI phylogeny provides moderate support for this suggestion. The data reported here provide a preliminary record of the host range of *P. smoliki* n. sp., however, further research is needed to ascertain the existence of (1) intraspecific variation in host range among *P. smoliki* n. sp. populations and (2) varietal differences in host suitability among crop species.

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In memory of James D. Smolik, Pioneer of nematode prairie ecology, colleague of Gerald Thorne, Nematologist at South Dakota State University, Brookings, SD 57007

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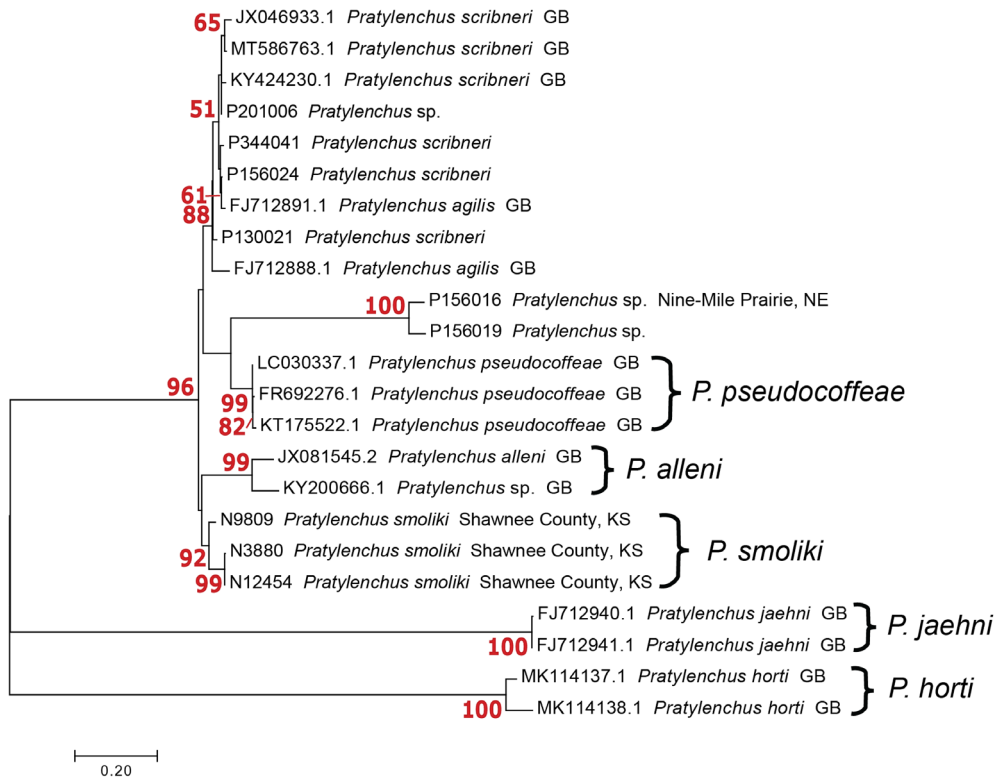


Figure S1: Maximum likelihood tree of ITS1 sequences from *P. smoliki* n. sp. and closely related species. Bootstrap values in red.