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Diversity of Plant-Parasitic Nematodes of the Genus *Pratylenchus* (Nematoda: Pratylenchidae) in California: Descriptions of Two New Species and Insights from Phylogenetic Analyses †

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Abstract

Root-lesion nematodes of the genus *Pratylenchus* are among the most economically significant plant-parasitic nematodes worldwide. During nematological surveys conducted in natural and agricultural ecosystems in California, several known species, *P. bolivianus*, *P. hippeastri*, *P. pinguicaudatus*, *P. scribneri*, *P. thornei*, and *P. vulnus*, as well as two undescribed species, *P. chizhovi* sp. n. and *P. gracilis* sp. n., were recovered. *Pratylenchus chizhovi* sp. n. is characterized by a slightly offset lip region with three annuli narrowing anteriorly, a short stylet (14.5–17.0 µm) with rounded knobs, a lateral field with four incisures, a spherical spermatheca lacking sperm, a vulva with somewhat prominent lips, a subcylindrical tail with a truncate-rounded, smooth terminus, and unknown males. *Pratylenchus gracilis* sp. n. is characterized by a lip region offset with three annuli narrowing anteriorly, a short stylet (15–18 µm) with rounded knobs, a lateral field with four incisures, an oval to rounded spermatheca containing some sperm cells, a vulva with somewhat prominent lips, a subcylindrical tail with a rounded, smooth terminus, and males present. Phylogenetic relationships of California *Pratylenchus* species and other members of the genus were inferred using 18S rRNA, D2–D3 of 28S rRNA, and the mitochondrial *COI* gene sequences. This study provides an updated assessment of *Pratylenchus* species diversity in California, highlighting both previously described taxa and newly discovered species.

Keywords: 18S rRNA gene; *COI* gene; D2–D3 of 28S rRNA gene; morphology; morphometrics; new species; phylogeny; systematics; taxonomy

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1. Introduction

The root-lesion nematode, genus *Pratylenchus* Filipjev, 1936, is among the most economically significant genera of plant-parasitic nematodes worldwide [1]. Its importance derives from its exceptionally broad host range across both natural and agricultural ecosystems, as well as its cosmopolitan distribution, with reported occurrences even in Antarctic soils [2].

Species of *Pratylenchus* are migratory endoparasites that penetrate and move through the root cortex, where their feeding activity results in reduced root growth, cortical lesions, necrosis, tissue browning, and ultimately cell death [1]. The mechanical and physiological damage caused by these nematodes also predisposes plants to secondary infections by soil-borne pathogens, including fungi and bacteria [3], thereby exacerbating their overall impact on plant health.

In their monography of the genus, Castillo and Vovlas [1] recognized 68 valid species of *Pratylenchus*. Subsequent taxonomic studies increased this number to 98 [4], and current assessments indicate that the genus now comprises more than 100 described species. Despite this documented diversity, several new species have been described within the last six years [5–14], underscoring the continued need for integrative taxonomic approaches combining morphology and molecular data.

Up to now, the diversity of *Pratylenchus* reported from California included 15 valid species occurring in both natural ecosystems and agroecosystems: *P. alleni* Ferris, 1961; *P. brachyurus* (Godfrey, 1929) Filipjev and Schuurmans Stekhoven, 1941, *P. coffeae* (Zimmermann, 1898) Filipjev and Schuurmans Stekhoven, 1941, *P. crenatus* Loof, 1960, *P. hexincisus* Taylor and Jenkins, 1957, *P. hippeastri* Inserra, Troccoli, Gozel, Bernard, Dunn and Duncan, 2007, *P. morettoi* Luc, Baldwin and Bell, 1986, *P. macrostylus* Wu, 1971, *P. neglectus* (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941, *P. penetrans* (Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941, *P. pseudopratensis* Seinhorst, 1968 (reported as *P. sefaensis*), *P. scribneri* Steiner in Sherbakoff and Stanley, 1943, *P. thornei* Sher and Allen, 1953, *P. vulnus* Allen and Jensen, 1951, and *P. zeae* Graham, 1951 (Table 1). Among these, *P. brachyurus*, *P. penetrans*, *P. vulnus*, *P. scribneri*, and *P. hexincisus* are recognized as root-lesion nematodes of major economic importance on almond, beet, citrus, corn, cotton, grape, onion, strawberry, tomato, walnut, and others in California [15]. *Pratylenchus vulnus*, *P. neglectus*, and *P. thornei* are considered the most common and widely distributed species in the region. It is also noteworthy that *P. morettoi*, *P. thornei*, and *P. vulnus* were originally described from California.

Table 1. *Pratylenchus* species that have been reported in California.

***Pratylenchus* Species**	**References**
P. alleni	[16]
P. chizhovi sp. n. *	This study
P. bolivianus *	This study
P. brachyurus *	[15,17–21]
P. coffeae	[17]
P. crenatus	[15,17]
P. gracilis sp. n. *	This study
P. hexincisus	[15,20,22]
P. hippeastri *	[23], this study
P. morettoi	[16]
P. macrostylus	[24]
P. neglectus *	[15,21,25]
P. penetrans *	[15,17,18,20,21,25]
P. pinguicaudatus *	This study
P. pseudopratensis	[24]
P. scribneri *	[15,17–21,25], this study
P. thornei *	[15,17–21,25,26], this study
P. vulnus *	[15,17–21,25,27–29], this study
P. zeae	[30]
Pratylenchus sp. 2 *	[21]

*—species identified using molecular methods.

During nematological surveys carried out in California, in natural ecosystems and agroecosystems, several known and two undescribed species of *Pratylenchus* were recovered. The main objective of this study is to describe these two new species using an integrative taxonomic framework and characterize them molecularly based on sequences of the 18S rRNA gene, D2–D3 expansion segments of the 28S rRNA gene, and a partial fragment of the mitochondrial *COI* gene. These datasets are also used to infer the phylogenetic relationships of the newly described species within the genus *Pratylenchus*.

2. Materials and Methods

2.1. Nematode Samples and Extraction

The nematodes examined in this study were recovered from soil and root samples collected during 2010–2024. Nematodes were extracted from soil using the centrifugal flotation method [31]. The list of species and populations studied, including their locations, is provided in Table 2 and Figure 1.



Figure 1. Geographic locations of *Pratylenchus* species characterized in this study across California.

Table 2. *Pratylenchus* species from California and Mexico were sequenced and analyzed in this study.

Species	Location, GPS Coordinates, Plant	Sample Code	GenBank Accession Number		
			18S rRNA Gene	D2–D3 of 28S rRNA Gene	*COI* Gene
P. bolivianus	California, Mendocino County, Van Damme State Park; 39°16.528′, −123°46.242′, unknown plants.	BC991, BC1002	-	PX657230	PX662743
P. chizhovi sp. n.	California, Sutter County, Sutter Buttes State Park; 39°14.683′, −121°48.877′, unknown plants.	BC557	PX657227	PX657237	PX662744
P. gracilis sp. n.	California, Mendocino County, Van Damme State Park; 39°16.528′, −123°47.319′, unknown plants.	CD3998	PX657226	PX657236	PX662746
P. hippeastri	California, Kern County, grapevine	CD1390	-	PX657233	-
P. pinguicaudatus	California, Marin County, Dillon Beach; 38°13.932′, −122°54.792′, unknown plants.	BC374	-	-	PX662745
P. scribneri	California, Fresno County, tomato	CD594	-	PX657232	-
P. thornei	California, Colusa County, Calvin's Creek; 39°08.282′, −122°20.695′, unknown plants.	BC707	-	PX657231	-
P. vulnus	California, Stanislaus County, walnut	CD3481	-	PX657235	-
Pratylenchus sp.	Mexico, State of Mexico, Amecameca, San Diego Huehuecalco, *Solanum* sp.; 19°.0.0′, −98°44.734′, unknown plants.	CD2793	-	PX657234	-

2.2. Morphological Study

Nematodes were relaxed and killed by heat, fixed in 4% formaldehyde, and processed to anhydrous glycerin following Siddiqi's [32] method. Specimens were mounted on permanent glass slides for light microscopic (LM) observation. Specimens were examined and measured using a Nikon Eclipse NiU light microscope (Nikon, Tokyo, Japan) equipped with differential interference contrast (DIC). Morphometrics included de Man's indices and standard measurements. Several preserved specimens were photographed with an Olympus DP23 digital camera (Olympus, Tokyo, Japan). The images were edited using Adobe® Photoshop® CS (Adobe Systems, San Jose, CA, USA). Line drawings were prepared with the aid of a camera lucida attached to the microscope.

2.3. Molecular Study

DNA was extracted from several specimens of the new species and single individuals from other *Pratylenchus* species using a standard protocol with proteinase K as described by Subbotin [33]. Nematodes were placed in drops of distilled water and dissected using a stainless-steel dental needle under a stereomicroscope. The resulting nematode fragments, suspended in 25 µL of water, were transferred to a 0.2 mL Eppendorf tube containing 3 µL of proteinase K (600 µg/mL; Promega, Madison, WI, USA) and 2 µL of 10× PCR buffer (Taq PCR Core Kit, Qiagen, Venlo, The Netherlands). The samples were incubated at 65 °C for 1 h, followed by 95 °C for 15 min. After incubation, the tubes were centrifuged and stored at −20 °C until further use. PCR amplification and sequencing were carried out as described by Subbotin [33]. Several primer sets were used in the present study: (i) D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) primers for amplifying the D2–D3 expansion segments of 28S rRNA gene; (ii) G18SU (5′-GCT TGT CTC AAA GAT TAA GCC-3′) and R18Tyl1 (5′-GGT CCA AGA ATT TCA CCT CTC-3′) primers for amplifying the partial 18S rRNA gene; (iii) JB3 (5′-TTT

TTT GGG CAT CCT GAG GTT TAT-3′) and JB5 (5′-AGC ACC TAA ACT TAA AAC ATA ATG AAA ATG-3′) primers for amplifying the partial *COI* gene. Amplicons were purified and directly sequenced using forward and reverse primers by Azenta (La Jolla, CA, USA). The newly obtained sequences were deposited in GenBank under the accession numbers listed in Table 2 and shown in the phylogenetic trees.

New sequences of the 18S rRNA, the D2–D3 of 28S rRNA, the ITS rRNA, and *COI* genes were aligned with corresponding published sequences of representatives of *Pratylenchus* [5–7,9,12–14,21,34–38] using ClustalX 1.83 [39] with default parameters for 18S rRNA, ITS, and *COI* gene datasets and with modified parameters (gap opening—5, gap extension—3) for the 28S rRNA gene dataset. Alignments were analyzed with Bayesian inference (BI) using MrBayes 3.1.2 [40]. BI analyses for each gene were initiated from a random starting tree and conducted using four Metropolis-coupled Markov chain Monte Carlo (MCMC) chains for 1.0×10^6 generations. Trees were sampled every 100 generations, and two independent runs were performed for each analysis. After discarding burn-in samples and assessing convergence, the remaining samples were retained for further analyses. The resulting topologies were used to construct a 50% majority-rule consensus tree as described by Subbotin [41]. Posterior probabilities (PPs) are provided for the appropriate clades. Pairwise divergence between sequences was calculated using PAUP*4a [42].

3. Results

3.1. Species Identification and Delimitation

Across the examined materials (Table 2), the combined use of traditional morphological taxonomic characters and complementary molecular evidence enabled us to reliably delimit and identify six known species present in the sampled localities in California: *P. bolivianus* Corbett, 1983, *P. hippeastri*, *P. pinguicaudatus* Corbett, 1969, *P. scribneri*, *P. thornei*, *P. vulnus*, and two species recognized here as new to science: *P. chizhovi* sp. n. and *P. gracilis* sp. n. Additionally, one unidentified *Pratylenchus* sp. was found in a sample from Mexico.

3.2. Taxonomic Studies

The morphological characteristics and morphometric data of the two new root-lesion nematode species are provided herein.

3.2.1. *Pratylenchus chizhovi* sp. n.

(Figures 2 and 3, and Table 3). http://zoobank.org/urn:lsid:zoobank.org:act:4AA351A5-9315-4CDA-B571-B8D6678DC76D.

Nineteen specimens collected in one location (Table 2) were available for morphological study.

Table 3. Morphometrics of female *Pratylenchus chizhovi* sp. n. from Sutter Buttes State Park, California. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Holotype	Paratypes
n	1	18
L	469	474 ± 30 (428–532)
a	28.7	27.3 ± 1.8 (24.3–30.3)
b	6.3	5.7 ± 0.4 (5.1–6.4)
b′	3.9	3.6 ± 0.3 (3.1–4.3)
c	21.7	18.8 ± 2.0 (15.8–22.5)
c′	2.0	2.3 ± 0.2 (1.9–2.7)
V (%)	80	80 ± 1.4 (76–83)
G (%)	35.0	34.5 ± 3.8 (28–44)
o (%)	18.3	23.0 ± 3.7 (16–31)

Table 3. *Cont.*

Character	Holotype	Paratypes
m (%)	48.3	48.0 ± 1.7 (45–51)
MB (%)	76	67 ± 4.9 (61–76)
Lip region diameter	8.0	7.7 ± 0.2 (7.5–8.0)
Lip region height	2.0	2.2 ± 0.2 (2.0–3.0)
Stylet length (St)	15.5	15.7 ± 0.7 (14.5–17.0)
St/L (%)	3.3	3.3 ± 0.1 (3.0–3.5)
Conus length	7.5	7.5 ± 0.5 (6.5–8.5)
Stylet shaft + knob height	8.0	8.1 ± 0.4 (7.5–8.5)
Knob width	4.5	4.5 ± 0.2 (4.0–5.0)
Knob height	2.0	2.4 ± 0.2 (2.0–2.5)
Stylet base to DGO	3.0	3.6 ± 0.5 (3.0–4.5)
Anterior end to center of metacorpus	57	55 ± 2.6 (51–60)
Metacorpus length	11.5	12.2 ± 0.5 (11.5–13.5)
Metacorpus width	9.5	10.1 ± 0.6 (9.5–11.0)
Body width at metacorpus	17.0	16.9 ± 0.8 (15.0–18.5)
Anterior end to excretory pore (Ep)	86	86 ± 3.7 (80–94)
Ep/L (%)	18.3	18.2 ± 0.8 (16.4–19.4)
Pharynx length	74	83 ± 4.7 (74–92)
Pharyngeal overlap length	48	47 ± 6.4 (37–60)
Anterior end to end of pharyngeal lobe	120	132 ± 13 (113–172)
Max. body width	16.5	17.4 ± 1.1 (15.5–19.5)
Lateral field width	4.0	5.0 ± 0.5 (4.0–5.5)
Genital tract length	164	165 ± 24 (124–220)
Spermatheca length	10.0	10.6 ± 1.0 (9.0–12.5)
Post-vulval uterine sac (PUS)	18.0	19.9 ± 5.1 (13.5–31.5)
PUS/VA (%)	25.5	29.0 ± 8.0 (18–44)
Distance anterior end to vulva	375	378 ± 26 (338–437)
Distance from vulva to anus (VA)	71	69 ± 5.2 (61–82)
Distance from vulva to tail terminus	93	94 ± 5.2 (84–106)
Body width at anus	10.5	11.0 ± 0.8 (9.5–12.5)
Number of tail annuli	17.0	19 ± 3 (16–24)
Tail length	21.5	25.6 ± 2.2 (20.5–29.0)

Description

Female: Body showing various postures when heat-killed, from almost straight, slightly curved ventrally, C-shaped to irregular. The lip region is flattened, offset from the body contour, and bears three annuli, which narrow in diameter towards the anterior end. Stylet is relatively small and robust, with a conus about 48.0 ± 1.7% (45–51) of the entire stylet length. The stylet shaft is slender and terminates in rounded basal knobs. The pharyngeal procorpus narrows slightly just anterior to the small, oval metacorpus, which bears a conspicuous median bulb valve. The isthmus is short and surrounded by the nerve ring, expanding posteriorly into a pharyngeal lobe. This lobe contains a dorsal nucleus located just posterior to the cardia and ventro-sublateral nuclei positioned near its tip, overlapping the intestine ventro-laterally. The secretory–excretory pore is located at the level of the cardia, just posterior to the hemizonid. Body annulation is distinct. Lateral field occupying about 28.4 ± 2.2% (24–32) of body diameter, usually with four smooth incisures (Figure 3L,O), sometimes with a few oblique striae in the middle of the central band (Figure 3L). Outer ridges are broader than inner ridges, and some specimens have several finer ridges close to the vulval region (Figure 2K). The outline of the outer bands becomes indented towards the tail end. The genital tract is well-developed with oocytes arranged in a single row. Spermatheca is small and spherical, empty of sperm. Vulval lips are slightly prominent. The post-uterine sac (PUS) is about 1.1 ± 0.3 (0.8–1.8) vulval body

diameter long. Phasmids are located in the mid-tail, 13.6 ± 1.2 (12–16) µm from the tail tip. The tail has 16–24 annuli, typically subcylindrical, tapering towards the tip with a rounded or truncated terminus.

Male: Not found.

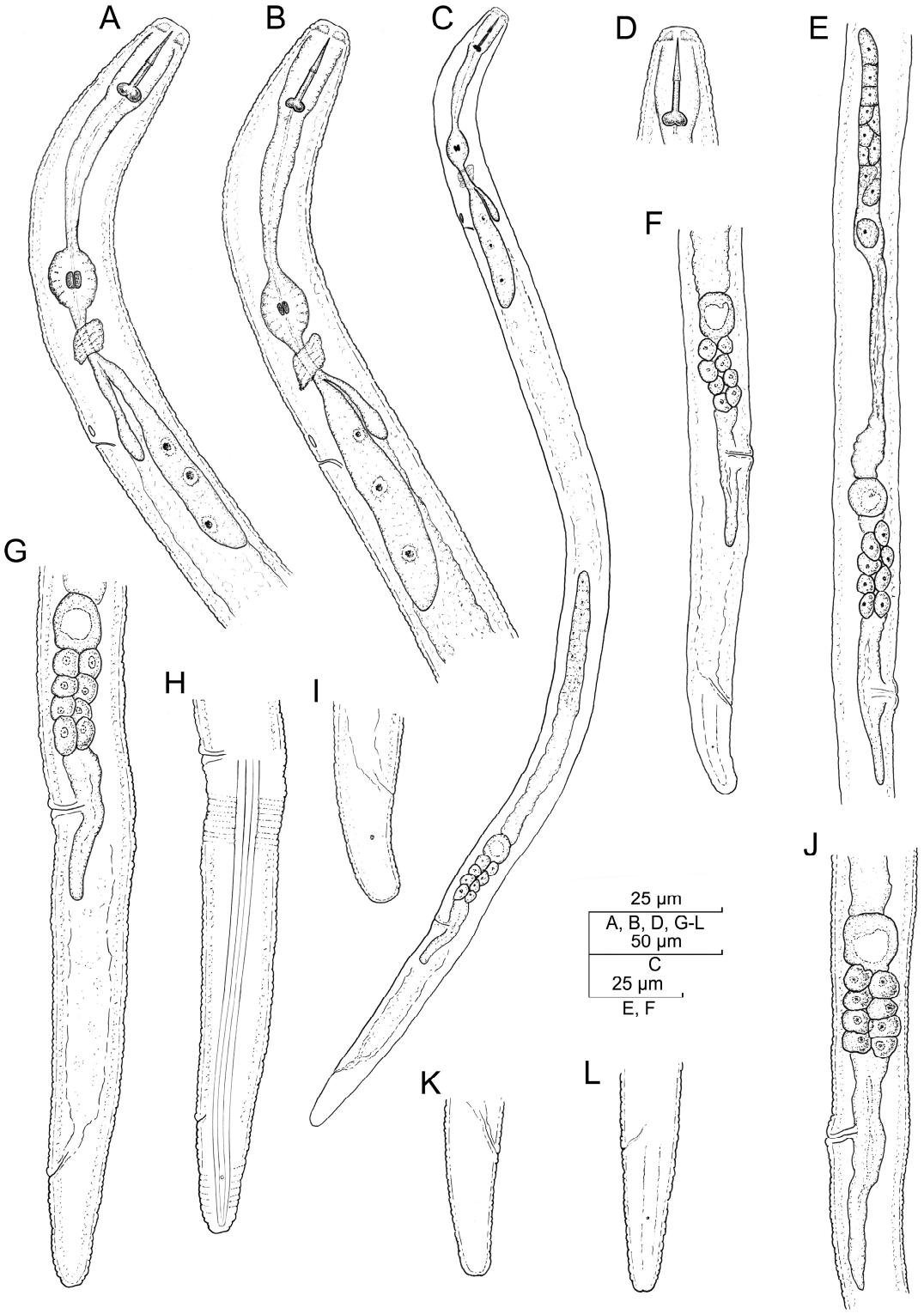


Figure 2. Camera lucida drawings of *Pratylenchus chizhovi* sp. n. female from Sutter Buttes State Park, California (BC557). (**A**,**B**): Anterior body region. (**C**): Entire body. (**D**): Lip region and stylet. (**E**): Genital system. (**F**,**G**): Posterior body region. (**H**): Posterior body region, showing the lateral field marked by four incisures. (**I**,**K**,**L**): Tail. (**J**): Vulval region, with detail of spermatheca and PUS.



Figure 3. Light microscopic photos of *Pratylenchus chizhovi* sp. n. female from Sutter Buttes State Park, California (BC557). (**A**): Anterior body region. (**B**,**C**): Lip region. (**G**,**H**): Lip region and stylet. (**D**–**F**,**I**): Entire body, showing different body shapes. (**J**): Vulval region and PUS. (**K**,**L**): Lateral field marked by four incisures. (**M**,**P**): Genital system. (**N**): Posterior body region. (**O**): Posterior body region showing lateral field. (**Q**): Vulval region. (**R**–**T**): Tail. (Scale bars: (**A**,**G**,**H**,**J**–**T**) = 10 µm; (**B**,**C**) = 5 µm; (**D**,**E**) = 50 µm; (**F**,**I**) = 100 µm).

Type Host and Locality

Pratylenchus chizhovi sp. n. was found in a sample collected from rhizospheres of unknown plants growing at Sutter Buttes State Park, Sutter County, CA, USA. GPS: 39°14.683′, −121°48.877′.

Type material

Holotype female and 11 female paratypes (16.904–16.906), mounted on glass slides, were deposited in the nematode collection of the National Museum of Natural Sciences, Madrid, Spain. An additional four female paratypes (T-8306p) were deposited in the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA.

Etymology

The specific epithet commemorates the late Dr. Vladimir N. Chizhov (Russia) for his distinguished scientific contributions to nematology.

Diagnosis and Relationships

The new species is characterized by a lip region offset with three annuli narrowing towards the anterior end; a small stylet (14.5–17.0 µm long) with rounded knobs; a short pharyngeal overlap; a lateral field with four incisures in most specimens; a spherical spermatheca without sperm; a vulva with somewhat prominent lips; a subcylindrical tail with a truncate-rounded and smooth terminus; and males unknown. The matrix code of the new species according to Castillo and Vovlas [1] is A2, B1, C2(3), D2, E3(2), F2(1,3,4,5), G2, H1, I3(2,4), J1, K1 (variation in characters is given in brackets).

In having a lip region with three annuli, a stylet longer than 13 µm, an empty spermatheca, and male absent, the new species resembles *P. andinus* Lordello, Zamith and Boock, 1961, *P. arlingtoni* Handoo, Carta and Skantar, 2001, *P. elamini* Zeidan and Geraert, 1991, *P. rwandae* Singh, Nyiragatare, Janssen, Couvreur, Decraemer and Bert, 2018, and *P. yassini* Zeidan and Geraert, 1991. *Pratylenchus chizhovi* sp. n. can be distinguished from *P. andinus* in its shorter (0.43–0.53 vs. 0.56 0.54–0.56 mm) and more slender body (a = 24.3–30.3 vs. a = 21.5–22.0), shorter stylet (14.5–17.0 vs. 17.0–18.0 µm), and lower b and c ratios (b = 5.1–6.4 vs. b = 7.0–7.4 and c = 15.8–22.5 vs. c = 27.0–28.3). From *P. arlingtoni* in its lateral fields (4 vs. 6–8 lines in the lateral fields from the pharyngeal to the vulval region, tail tip (smooth vs. crenate), spermatheca (rounded vs. oval), and molecular data of 28S rRNA gene. From *P. elamini* in its shorter stylet (15.7 (14.5–17.0) vs. 14.0 (13.0–14.5) µm), spermatheca (rounded vs. oval), more posterior vulva position (V = 80 (76–83) vs. V = 75 (72–77)), and tail shape (subcylindrical vs. conical). From *P. rwandae* in its narrower (7.7 (7.5–8.0) vs. 9.4 (8.5–10.4) µm wide) and more flattened (vs. rounded) lip region, longer stylet (15.7 (14.5–17.0) vs. 13.6 (13.0–14.6) µm), and molecular data of 18S, 28S rRNA, and *COI* genes. Finally, from *P. yassini* in its more posterior vulva position (V = 80 (76–83) vs. V = 74 (71–76), tail shape (subcylindrical vs. conoid), and tail tip (smooth vs. crenate).

The new species, in having a lip region with three annuli and males absent, also resembles *P. bolivianus*, *P. delattrei* Luc, 1958, *P. kumaoensis* Lal and Khan, 1990, and *P. japonicus* Ryss, 1988. It is easy to distinguish from *P. bolivianus* and *P. japonicus* in its shorter stylet (15.7 (14.5–17.0) vs. 19.0 (17.0–20.0) and 19.8 (18.5–21.5) µm, respectively), and molecular data of 28S rRNA and *COI* genes. From *P. delattrei* in the tail shape (subcylindrical with a truncate-rounded tip vs. subcylindrical with conical to rounded tip), and molecular data of 28S rRNA genes. Finally, from *P. kumaoensis* in its lip region offset (vs. almost continuous), spermatheca present (vs. inconspicuous), tail shape (subcylindrical vs. conoid-rounded), and tail tip (smooth vs. crenate).

3.2.2. *Pratylenchus gracilis* sp. n.

(Figures 4 and 5, and Table 4). http://zoobank.org/urn:lsid:zoobank.org:act:13AF158A-2FE5-4173-A3C5-6A34D5F15EA9.

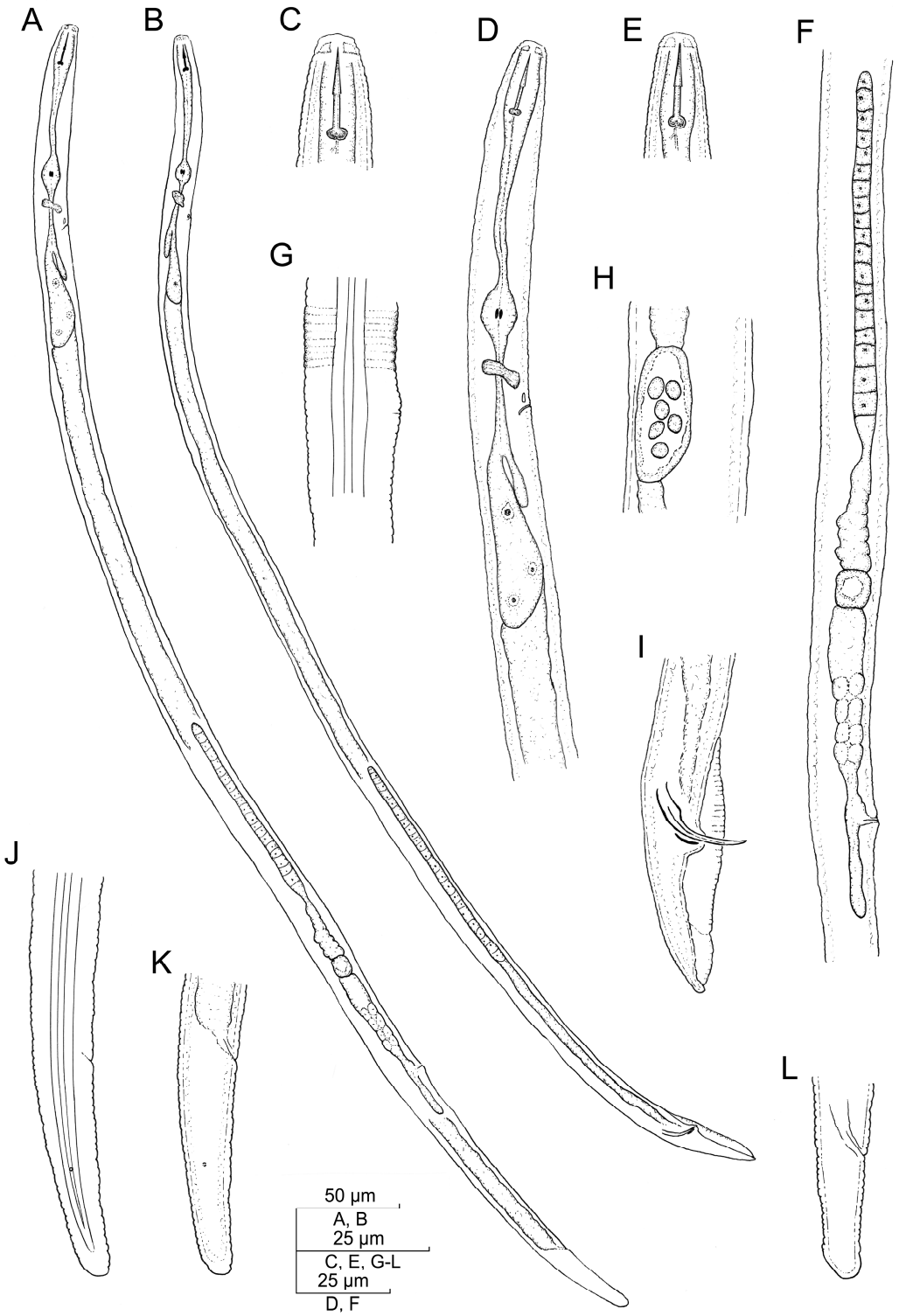


Figure 4. Camera lucida drawings of *Pratylenchus gracilis* sp. n. female and male from Van Damme State Park, California (CD3998). (Female: (**A**,**C**,**D**,**F**–**H**,**J**–**L**). Male: (**B**,**E**,**I**)). (**A**,**B**): Entire body. (**C**,**E**): Lip region and stylet. (**D**): Anterior body region. (**F**): Genital system. (**G**): Vulval region, showing the lateral field marked by four incisures. (**H**): Detail of spermatheca with sperm cells. (**I**): Posterior body region, with detail of the spicule and bursa. (**J**): Posterior body region, showing the lateral field marked by four incisures. (**K**,**L**): Tail.

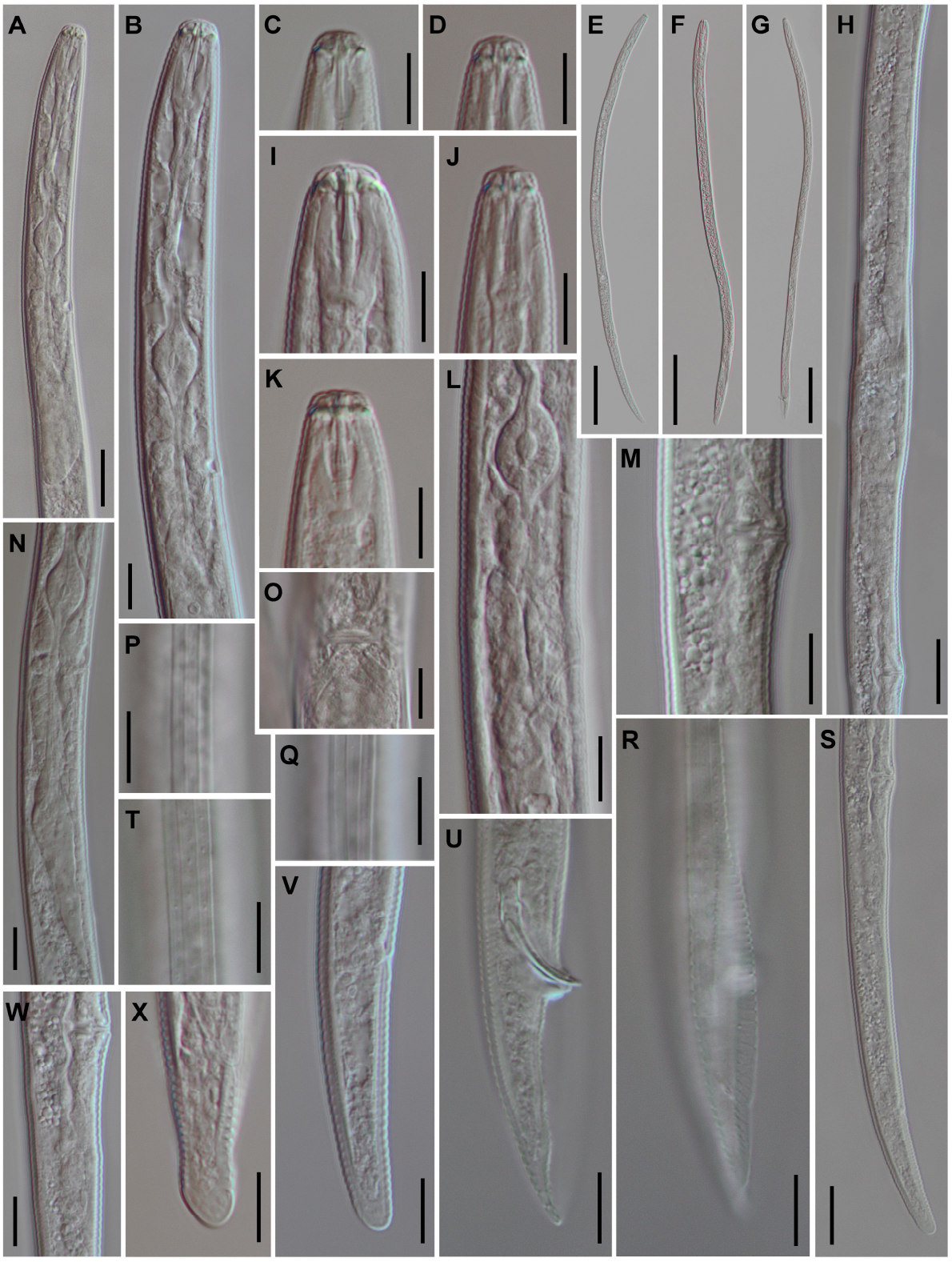


Figure 5. Light microscopic photos of *Pratylenchus gracilis* sp. n. female and male from Van Damme State Park, California (CD3998). (Female: (**A**,**B**,**E**,**F**,**H**,**I**,**K**–**M**,**O**,**S**,**V**–**X**). Male: (**C**,**D**,**G**,**J**,**N**,**P**–**R**,**T**,**U**)). (**A**,**B**): Anterior body region. (**C**,**D**): Lip region. (**E**–**G**): Entire body. (**H**): Genital system. (**I**–**K**): Lip region and stylet. (**L**): Details of the metacorpus bulb and excretory pore. (**M**): Vulval region. (**N**): Details of the metacorpus and pharyngeal lobe. (**O**): Vulva in frontal view. (**P**,**Q**,**T**): Lateral field marked by four incisures. (**R**): Posterior body region showing the bursa. (**S**): Posterior body region. (**U**): Tail and spicule. (**V**,**X**): Tail. (**W**): Vulval region and PUS. (Scale bars: (**A**,**S**) = 20 µm; (**B**–**D**,**H**–**R**,**T**–**X**) = 10 µm; (**E**–**G**) = 100 µm).

https://doi.org/10.3390/d18010045

Table 4. Morphometrics of females of *Pratylenchus gracilis* sp. n. from Van Damme State Park, California. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Holotype	Paratypes	
	Female	Female	Male
n	1	3	6
L	728	590–691 (n = 2)	653.0 ± 71.1 (566–739)
a	39.1	34.5–38.0 (n = 2)	43.2 ± 5.4 (35.2–49.3)
b	6.3	5.4–6.4 (n = 2)	6.4 ± 0.7 (5.7–7.2)
b′	4.5	3.5–4.5 (n = 2)	4.3 ± 0.4 (3.8–4.8)
c	17.3	25.2–39.8 (n = 2)	19.8 ± 2.1 (17.5–23.2)
c′	3.8	1.5–2.2 (n = 2)	2.5 ± 0.4 (1.8–2.7)
V (%)	78	81–82 (n = 2)	-
G	27.9	26.8–30.5 (n = 2)	43.3 ± 2.8 (41–48)
m (%)	53	49.2 ± 1.3 (48–50)	53.2 ± 2.8 (49–56)
o (%)	18.2	19.3 ± 1.4 (18.3–20.9)	18.8 ± 1.5 (17–21)
MB (%)	65	59–68 (n = 2)	71.0 ± 4.3 (66–76)
Lip region diameter	8.5	8.4 ± 0.5 (8.0–9.0)	7.5 ± 0.3 (7.0–8.0)
Lip region height	2.5	2.7 ± 0.0 (2.5–2.5)	2.3 ± 0.1 (2.0–2.5)
Stylet length	17.5	17.0 ± 0.8 (16.5–18.0)	16.5 ± 0.9 (15.0–17.0)
St/L (%)	2.4	2.4–3.0 (n = 2)	2.6 ± 0.3 (2.3–3.0)
Conus length	9.5	8.4 ± 0.5 (8.0–9.0)	8.8 ± 0.8 (7.5–9.5)
Stylet shaft + knob height	8.5	8.8 ± 0.2 (8.5–9.0)	7.9 ± 0.3 (7.5–8.5)
Knob width	4.5	4.6 ± 0.6 (4.0–5.0)	3.5 ± 0.3 (3.0–3.5)
Knob height	2.0	2.2 ± 0.1 (2.0–2.5)	2.0 ± 0.0 (2.0–2.0)
Stylet base to DGO	3.0	3.3 ± 0.2 (3.0–3.5)	3.1 ± 0.2 (3.0–3.5)
Anterior end to center of metacorpus	75	64–74 (n = 2)	68.8 ± 6.0 (64–79)
Metacorpus length	14.0	12.2 ± 0.7 (12.0–13.0)	11.6 ± 0.8 (10.5–13.0)
Metacorpus width	10.0	10.0 ± 0.4 (9.5–10.5)	9.1 ± 1.0 (8.0–10.5)
Body width at metacorpus	17.5	17.9 ± 0.6 (17.5–18.5)	15.5 ± 1.1 (14.0–17.5)
Anterior end to excretory pore (Ep)	100	100.0 ± 2.8 (97–102)	90.5 ± 4.6 (85–98)
Ep/L (%)	13.7	14.8–16.4 (n = 2)	13.9 ± 1.0 (13.2–15.5)
Pharynx length	115	109 (n = 2)	97.9 ± 13.1 (84–115)
Pharyngeal overlap length	54	46–62 (n = 2)	45.9 ± 3.0 (42–48)
Anterior end to end of pharyngeal lobe	161	154–167 (n = 2)	147.6 ± 12.0 (129–162)
Max. body width	18.5	17.0–18.0 (n = 2)	15.0 ± 1.1 (13.5–16.0)
Lateral field width	5.5	-	5.7 ± 0.6 (5.0–6.5)
Genital tract length	203	216.8 ± 62.1 (158–282)	274.7 ± 25.3 (233–299)
Spermatheca length	14.0	11.7 ± 4.0 (7.0–14.5)	-
Post-vulval uterine sac (PUS)	38	23–43 (n = 2)	-
PUS/VA (%)	33.5	28.3–37.4 (n = 2)	-
Distance anterior end to vulva	567	530.4 ± 40.0 (484–557)	-
Distance from vulva to anus (VA)	114	81–115 (n = 2)	-
Distance from vulva to tail terminus	160	107–131 (n = 2)	-
Body width at anus/cloaca	11.0	10.5–11.5 (n = 2)	13.5 ± 2.1 (12.0–16.5)
Number of tail annuli	25	12 (n = 2)	-
Tail length	42	17.5–23.5 (n = 2)	32.9 ± 1.9 (30–35)
Spicule arc	-	-	17.6 ± 0.6 (16.5–18.0)
Spicule chord	-	-	19.4 ± 0.3 (19.0–19.5)
Gubernaculum length	-	-	5.6 ± 0.1 (5.5–6.0)

Ten specimens collected in one location (Table 2) were available for morphological study.

Description

Female: Body showing various postures when heat-killed, from almost straight, slightly curved ventrally, C-shaped to irregular. The lip region is flattened to slightly rounded,

offset from the body contour, and bears three annuli, which narrow in diameter towards the anterior end. The stylet is relatively small and robust, with the conus comprising about 50.2 ± 2.4 (47.8–53.4)% of the entire stylet length. The stylet shaft is slender, ending with rounded basal knobs. The pharyngeal procorpus narrows just anterior to the small, oval metacorpus. The valve of the median bulb is conspicuous. The isthmus is short, encircled by a nerve ring and widened to a pharyngeal lobe with a dorsal nucleus just posterior to the cardia and ventro-sublateral nuclei near the tip of the pharyngeal lobe and overlapping the intestine ventrolaterally. The secretory–excretory pore is located at the level of the cardia, just posterior to the hemizonid. Body annulation is distinct. The lateral field occupies about one-third of the body diameter, usually with four smooth incisures (Figure 5Q). Outer ridges are broader than inner ridges. The outline of the outer bands becomes indented towards the tail end. The genital tract is well-developed with oocytes arranged in a single row. The spermatheca is oval to rounded, with some sperm cells inside. The vulval lips are slightly prominent. The post-uterine sac (PUS) measures about 1.9 ± 0.5 (1.3–2.4) vulval body diameters in length. The phasmids are located in the mid-tail or anteriorly, 18–21 µm from the tail tip. The tail has 12–25 annuli, typically subcylindrical, tapering towards the tip with a rounded terminus.

Male: Generally similar to the female, differing mainly in the posterior body region and typically exhibiting a slimmer body. The lip region is usually higher and narrower than in females (Figure 5J). The stylet is slightly shorter than that of the female and bears smaller knobs. The metacorpus is small and rounded. The isthmus is slender and relatively short, terminating in a long, narrow glandular lobe. The testis is outstretched and contains round spermatozoa within the vas deferens. Spicules are paired, weakly cephalated, and ventrally arcuate. The gubernaculum is simple and slightly curved. The tail is conical, ventrally bent, and bears a prominent, crenate bursa.

Type Host and Locality

Pratylenchus gracilis sp. n. was found in a sample collected from rhizospheres of unknown plants growing at Van Damme State Park, Mendocino County, CA, USA. GPS: 39°16.528′, −123°47.319′.

Type Material

A holotype female, as well as two female and five male paratypes (16.907–16.910), mounted on glass slides, were deposited in the nematode collection of the National Museum of Natural Sciences, Madrid, Spain. An additional female and one male paratype (T-8307p) were deposited in the United States Department of Agriculture Nematode Collection, Beltsville, MD, USA.

Etymology

The species epithet *gracilis* is derived from the Latin word *gracilis* (slender), referring to the notably slender body of the new species, which distinguishes it from many other members of the genus *Pratylenchus*.

Diagnosis and Relationships

The new species is characterized by a lip region offset with three annuli narrowing towards the anterior end; a small stylet (15–18 µm long) with rounded knobs; a short pharyngeal overlap; a lateral field with four incisures, oval to rounded spermatheca with some sperm cells inside; a vulva with somewhat prominent lips; a subcylindrical tail with a rounded and smooth terminus; and males with spicules 19.0–19.5 µm long and a well-developed bursa. The matrix code of the new species according to Castillo and Vovlas [1] is A2, B2, C3(2), D3(2), E3(2), F5(3,4,6), G2, H1, I4(3), J1, K1 (variation in characters is given in brackets).

In having a lip region with three annuli, a stylet longer than 13 µm, generally common males, and spermatheca filled with sperm, the new species resembles *P. mediterraneus* Cor-

bett, 1983, *P. morettoi*, *P. mulchandi* Nandakumar and Khera, 1970, *P. unzenensis* Mizukubo, 1992, and *P. vulnus*. *Pratylenchus gracilis* sp. n. can be distinguished from *P. goodeyi* in its longer (0.59–0.73 vs. 0.43–0.58 and 0.57–0.74 vs. 0.43–0.54 mm long, in females and males, respectively) and more slender body (a = 34–39 vs. a = 24–31 and a = 35–49 vs. a = 27–35, in females and males, respectively), and longer male spicules (19.0–19.5 vs. 16–18 µm long). From *P. morettoi,* in its lip region offset (vs. not separated from the rest of the body), and tail shape (tail lacking vs. always with a terminal projection and a terminal thickening of the cuticle). From *P. mulchandi* in its slender body (a = 37 (34–39) vs. a = 30 (26–32) and a = 35–49 vs. a = 33, in females and males, respectively), more posterior vulva position (V = 80 (78–82) vs. V = 75 (72–77)), and longer male spicules (19.0–19.5 vs. 14 µm long). From *P. unzenensis* in its longer (0.67 (0.59–0.73) vs. 0.42 (0.37–0.47) and 0.57–0.74 vs. 0.37–0.40 mm long, in females and males, respectively) and more slender body (a = 37.2 (34.5–39.1) vs. a = 25.8 (21.9–28.5) and a = 35.2–49.3 vs. a = 29.2–30.0, in females and males, respectively), longer stylet (17.1 (16.5–18.0) vs. 15.0 (14.5–15.5) µm and 15.0–17.0 vs. 14.0–14.5 µm, in females and males, respectively), and longer male spicules (19.0–19.5 vs. 13.0–13.5 µm long). Finally, from *P. vulnus* in its lip region offset (vs. almost continuous with body contour), female tail with a rounded tip (vs. narrowly rounded to subacute tip), and molecular data of 18S, 28S rRNA, and *COI* genes.

The new species, in having a lip region with three annuli and males being generally common also resembles *P. bhattii* Siddiqi, Dabur and Bajaj, 1991, *P. brasilensis* Diniz, Mizubuti, Valadares, Moreira, Santos and Santiago, 2025, *P. capsici* Qing, Bert, Gamliel, Bucki, Duvrinin, Alon and Braun Miyara, 2019, *P. convallariae* Seinhorst, 1959, *P. dakotaensis* Handoo, Yan, Kantor, Huang, Chowdhury, Plaisance, Bauchan and Mowery, 2021, *P. ekrami* Bajaj and Bhatti, 1984, *P. globulicola* Romániko, 1960, *P. kralli* Ryss, 1982, *P. mahindii* Maosa, Karuri, Gitonga, Nguyen, Couvreur, Coyne and Bert, 2025, *P. penetrans*, *P. pseudofallax* Café-Filho and Huang, 1989, and *P. thornei*. *Pratylenchus gracilis* sp. n. can be distinguished from *P. bhattii* in its longer male body (0.65 (0.57–0.74) vs. 0.42 (0.35–0.48) mm long), more slender body (a = 37 (34–39) vs. a = 28 (25–31) and a = 43 (35–49) vs. a = 28 (25–29), in females and males, respectively), and longer stylet (17.1 (16.5–18.0) vs. 13.5 (13–14.0) µm and 16.5 (15.0–17.0) vs. 13.0 (12.0–13.5) µm, in females and males, respectively). From *P. brasilensis* in its longer male body (0.57–074 vs. 0.44–0.56 mm long), more slender female body (a = 34.5–39.1 vs. a = 24.5–30.4), and female tail shape (subcylindrical with rounded terminus vs. conical shape). From *P. capsici* in its slender male body (a = 35–49 vs. a = 22–30, in females and males, respectively), and longer female stylet (16.5–18.0 vs. 13.0–15.0 µm), and longer male spicules (19.0–19.5 vs. 15.1–17.3 µm long). From *P. convallariae* in its slender female body (a = 34–39 vs. a = 23–31), and female tail tip (smooth vs. crenate). From *P. dakotaensis* in its longer (0.67 (0.59–0.73) vs. 0.48 (0.39–0.55) and 0.65 (0.57–0.74) vs. 0.45 (0.37–0.40) mm long, in females and males, respectively) and more slender body (a = 37.2 (34.5–39.1) vs. a = 23.4 (20.8–29.8) and a = 43.2 (35.2–49.3) vs. a = 23.7 (20.8–25.2), in females and males, respectively), and female tail tip (smooth vs. crenate). From *P. ekrami* in its longer (0.57–0.74 vs. 0.40–0.54 mm long) and more slender male body (a = 35–49 vs. a = 29–34), and longer stylet (17.1 (16.5–18.0) vs. 12.0 (11.0–13.0) µm and 15–17 vs. 12 µm, in females and males, respectively). From *P. kralli* in its longer (0.59–0.73 vs. 0.40–0.50 and 0.57–0.74 vs. 0.38–0.45 mm long, in females and males, respectively) and more slender body (a = 34–39 vs. a = 20–33 and a = 35–49 vs. a = 21–34, in females and males, respectively), and longer stylet (16.5–18.0 vs. 14.0–15.0 µm and 15.0–17.0 vs. 12.5–14.0 µm, in females and males, respectively). From *P. globulicola* in its longer (0.59–0.73 vs. 0.29–0.57 and 0.57–0.74 vs. 0.38–0.52 mm long, in females and males, respectively) and more slender body (a = 34.5–39.1 vs. a = 15.9–29.0 and a = 35.2–49.3 vs. a = 16.9–31.9, in females and males, respectively), and longer stylet (16.5–18.0 vs. 12.5–16.3 µm and 15.0–17.0 vs. 12.0–15.0)

µm, in females and males, respectively). From *P. mahindii* in its slender body (a = 34–39 vs. a = 21–30 and a = 35–49 vs. a = 26–28, in females and males, respectively), longer stylet (16.5–18.0 vs. 13.5–16.0 µm and 15–17 vs. 12.5–13.0 µm, in females and males, respectively), more posterior vulva position (V = 78–82 vs. V = 70–77%), and longer male spicules (19.0–19.5 vs. 15.0–16.2 µm long). From *P. penetrans* in its slender body (a = 34–39 vs. a = 19–32 and a = 35–49 vs. a = 23–34, in females and males, respectively), and longer male spicules (19.0–19.5 vs. 14.0–17.0 µm long). *P. pseudofallax* in its longer (0.67 (0.59–0.73) vs. 0.47 (0.42–0.53) and 0.65 (0.57–0.74) vs. 0.42 (0.37–0.48) mm long, in females and males, respectively) and more slender body (a = 37.2 (34.5–39.1) vs. a = 29.0 (24.7–36.3) and a = 43.2 (35.2–49.3) vs. a = 28.3 (24.7–31.5), in females and males, respectively), and longer male stylet (16.5 (15.0–17.0) vs. 14.0 (13.5–15.0) µm). And finally, from *P. thornei* in its lip region offset (vs. not offset from body), spermatheca filled with some sperm cells (vs. not containing spermatozoa), and males as frequent as females (vs. males very rare).

3.3. Molecular Characterization and Phylogenetic Relationships

3.3.1. Partial 18S rRNA Gene

The alignment included 26 sequences of *Pratylenchus* species and two sequences of outgroup taxa and was 885 bp in length. One sequence of *P. chizhovi* sp. n. and one sequence of *P. gracilis* sp. n. were obtained in this study. Phylogenetic relationships of new species with other representatives of *Pratylenchus* as inferred from analysis of the 18S rRNA gene sequence alignment are given in Figure 6. The *Pratylenchus chizhovi* sp. n. sequence clustered with representatives of the *Penetrans* clade. The *P. chizhovi* sp. n. sequence differed from those of *P. fallax* by 4.7% and *P. capsici* by 7.0%. The sequence of *P. gracilis* sp. n. formed a separate lineage, and it differed from that of *P. crenatus* by 5.6%.

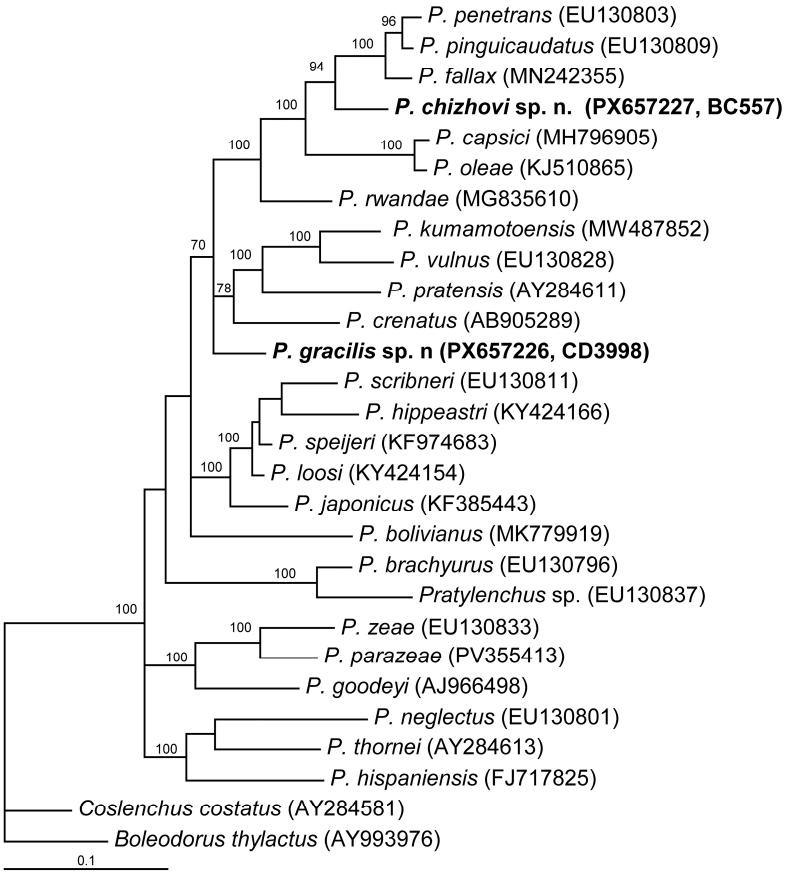


Figure 6. Phylogenetic relationships of *Pratylenchus* from California with other representatives of the genus: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the 18S

rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than 70%, are given for appropriate clades. The new sequences are indicated in bold.

3.3.2. D2–D3 Expansion Segments of 28S rRNA Gene

The alignment included 69 sequences of *Pratylenchus* species and three sequences of outgroup taxa and was 862 bp in length. In this study, we obtained one sequence each of *P. bolivianus*, *P. chizhovi* sp. n., *P. gracilis* sp. n., *P. hippeastri*, *P. scribneri*, *P. thornei,* and *Pratylenchus* sp. from Mexico. Phylogenetic relationships of new species with other representatives of *Pratylenchus* as inferred from analysis of the D2–D3 of 28S rRNA gene sequence alignment are given in Figure 7. *Pratylenchus chizhovi* sp. n. clustered with representatives of the *Penetrans* clade, and it is different from that of *Pratylenchus* sp. (KY828334) by 3.3%. The sequence of *P. gracilis* sp. n. and *Pratylenchus* sp. from Mexico formed separate lineages. The sequence of *P. gracilis* sp. n. was different from that of *P. vulnus* by 12.0%. Sequences of Californian *P. bolivianus*, *P. hippeastri*, *P. scribneri*, *P. thornei,* and *P. vulnus* clustered with corresponding sequences of these species from other regions.

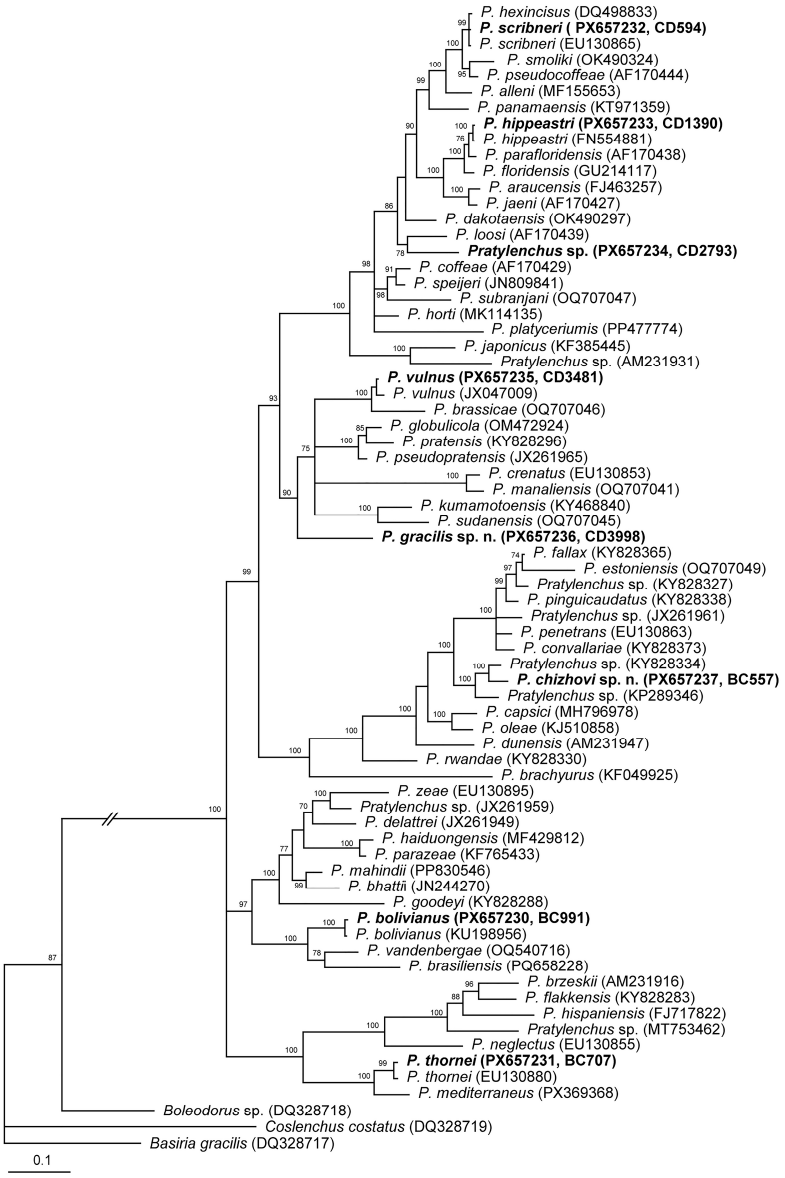


Figure 7. Phylogenetic relationships of *Pratylenchus* from California and Mexico with other representatives of the genus: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the

D2–D3 of 28S rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than 70%, are given for appropriate clades. The new sequences are indicated in bold.

3.3.3. Partial *COI* Gene

The alignment included 48 sequences of *Pratylenchus* species and two sequences of outgroup taxa and was 415 bp in length. In this study, we obtained one sequence each of *P. bolivianus*, *P. chizhovi* sp. n., *P. gracilis* sp. n., and *P. pinguicaudatus*. Phylogenetic relationships of new species with other representatives of *Pratylenchus* as inferred from analysis of the *COI* gene sequence alignment are given in Figure 8. *Pratylenchus chizhovi* sp. n. clustered with representatives of the *Penetrans* clade, and it is different from that of *P. oleae* by 14.3%. The sequence of *P. gracilis* sp. n. formed a separate lineage, and it differed from those of other *Pratylenchus* by more than 21%. Sequences of Californian *P. bolivianus* and *P. pinguicaudatus* clustered with corresponding sequences of these species from other regions, differing by 5.8% and 0.8%, respectively.

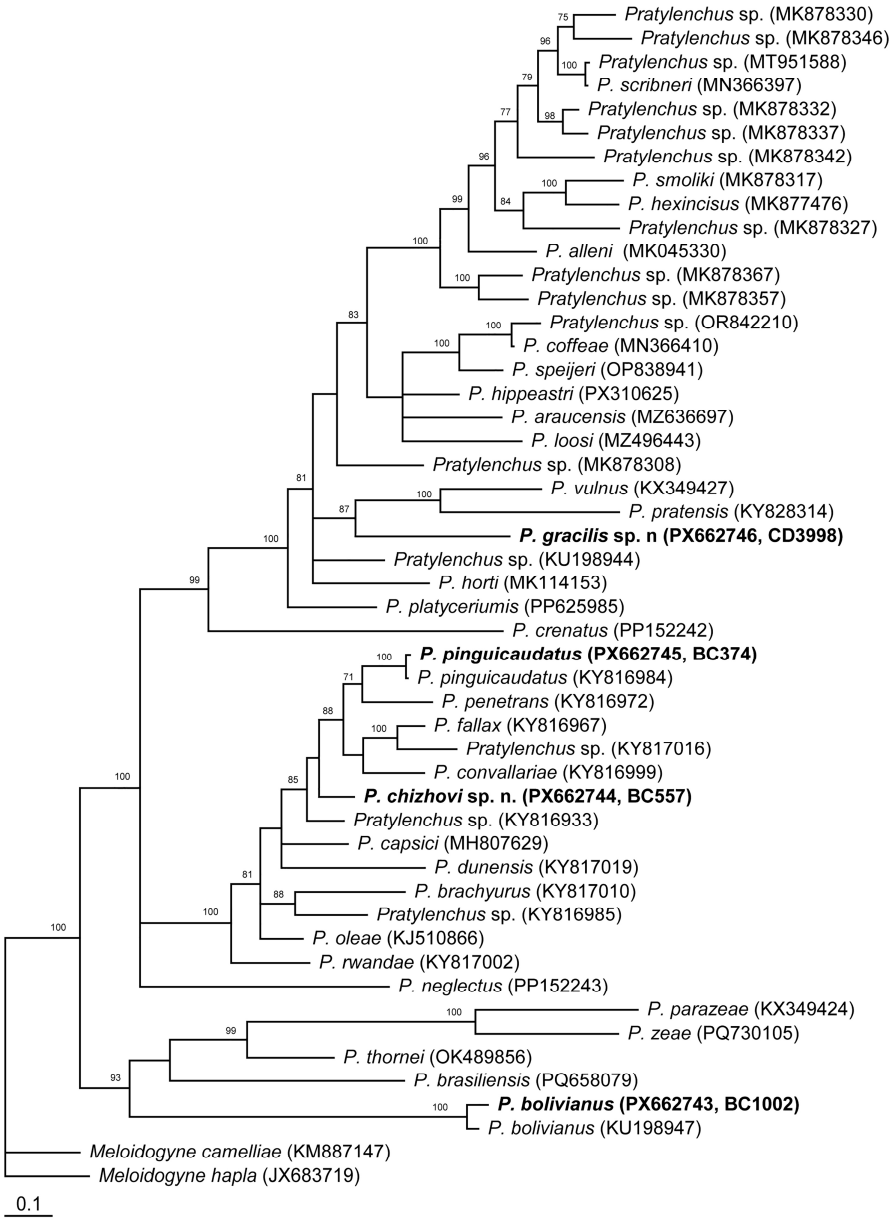


Figure 8. Phylogenetic relationships of *Pratylenchus* from California with other representatives of the genus: Bayesian 50% majority rule consensus tree from two runs as inferred from analysis of the *COI*

gene sequence alignment under the GTR + I + G model. Posterior probabilities equal to, or more than 70%, are given for appropriate clades. The new sequences are indicated in bold.

4. Discussion

Based on the present study and a synthesis of previous records, a total of nineteen valid *Pratylenchus* species could be considered as occurring in California (Table 1). However, molecular confirmation is currently available for only twelve of these species. Several nominal records, including *P. alleni*, *P. crenatus*, *P. hexincisus*, *P. macrostylus*, and *P. zeae,* lack accompanying morphological descriptions or diagnostic details, making their identification impossible to verify. Furthermore, the true status of *P. coffeae sensu stricto* in California agriculture remains uncertain, as early reports were based exclusively on morphology and may represent misidentifications of other *Pratylenchus* species.

Within this context, the results of the present study are particularly significant. We report the detection of two *Pratylenchus* species, *P. bolivianus* and *P. pinguicaudatus*, as noteworthy additions to the nematode fauna of California. Both species are important root-lesion nematodes with expanding global distributions.

The Andean root-lesion nematode *P. bolivianus* was originally described from soil around oat and potato roots in the Bolivian Andes [43]. Since its description, the species has been reported in several regions worldwide, including Chile, China, Colombia, Costa Rica, South Africa, North America, and Europe [1,34,44–47]. Its agricultural importance as a parasite of ornamentals and fruit crops has been demonstrated in Florida, Colombia, and Brazil. To our knowledge, the present finding represents the second report of *P. bolivianus* in the USA and the first confirmed occurrence in California. The detection of this species across multiple continents, a wide range of host plants, and diverse climatic conditions reflects a high degree of ecological adaptability and a significant potential for global dispersal, thereby increasing its invasive risk in non-native regions.

Pratylenchus pinguicaudatus was originally described by Corbett [48] from a population collected in wheat fields at Rothamsted Research, UK. Subsequent reports of this species from Bulgaria, France, Poland, Spain, Tunisia, and Australia [1,49–52] substantially expanded its presumed geographic distribution. However, these records were based exclusively on morphological data and remain in need of molecular confirmation [35]. To date, only the topotype population from Rothamsted has been reliably characterized using molecular markers [21,35]. Recent phylogenetic analyses have suggested that some non-UK populations previously identified as *P. pinguicaudatus* may represent distinct or undescribed species. Our detection, therefore, constitutes the first molecularly confirmed record of *P. pinguicaudatus* in both the USA and California. At present, there is limited evidence that *P. pinguicaudatus* is of major economic importance relative to more widespread root-lesion nematodes such as *P. penetrans*, *P. neglectus*, or *P. thornei*. Its host range, ecological preferences, and potential impact on crops remain poorly understood, partly due to the rarity of confirmed populations and the difficulty of distinguishing it from closely related species without molecular data.

The amaryllis lesion nematode *P. hippeastri* was previously reported from grapevine in Delano, Kern County, California by Handoo et al. [23]. Our finding represents the second report of this species from this county. *Pratylenchus hippeastri* is of considerable agricultural and horticultural concern, particularly for ornamental bulbs (amaryllis), and potentially for grapevine in both California and Florida [23,53–55], apple in China, South Africa, India [56–58], and strawberry in Costa Rica [59]. Additional records include its recovery from the rhizosphere and roots of *Acer palmatum* in Japan [60], *Salix mucronata* in Africa [61], and *Salix chaenomeloides* in Korea [62]. From a taxonomic perspective, *P.*

hippeastri appears to represent a species complex. Sequence and phylogenetic analyses have revealed that a complex of cryptic species genetically similar to *P. hippeastri* occurs in Florida, USA, South Africa, and Russia [54].

Three *Pratylenchus* species, including the two newly described species *P. chizhovi* sp. n. and *P. gracilis* sp. n., as well as *P. morettoi*, are currently known only from California. These species appear to have highly restricted geographic distributions. Their apparent endemism may reflect unique ecological niches or host associations within California's diverse environments. Alternatively, their limited detection outside the state may result from the difficulty of distinguishing closely related *Pratylenchus* species without molecular diagnostic tools.

Overall, the findings of this study underscore the critical need for continued molecular-based surveys of root-lesion nematodes in California. The historical reliance on morphology alone has likely contributed to under-recognition, misidentification, and incomplete documentation of *Pratylenchus* diversity within the state.

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Abbreviations

The following abbreviations of de Man's indices and standard measurements are used in this manuscript:

n	Number of measured specimens
L	Overall body length
a	Body length/greatest body diameter
b	Body length/pharynx length
b′	Body length/distance from anterior to base of pharyngeal gland
c	Body length/tail length
c′	Tail length/body diameter at anus or cloaca
V	% Distance of anterior body end from the vulva/body length
G	% Genital tract length/body length
m	% Conus of stylet/stylet length
o	% Distance of dorsal pharyngeal gland opening from stylet knobs x 100/body length
MB	% Distance from anterior to median bulb relative to the length of the pharynx
St/L	% Stylet length/body length
Ep/L	% Distance from anterior to excretory pore/body length
PUS/VA	% Post-vulval uterine sac/distance from vulva to anus

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