Phylogeography and Molecular Species Delimitation of *Pratylenchus capsici* n. sp., a New Root-Lesion Nematode in Israel on Pepper (*Capsicum annuum*)

Xue Qing,¹ Wim Bert,² Abraham Gamliel,³ Patricia Bucki,¹ Svetlana Duvrinin,⁴ Tamar Alon,⁴ and Sigal Braun Miyara^{1,†}

¹Department of Entomology, Nematology and Chemistry Units, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan 50250, Israel; ²Nematology Research Unit, Department of Biology, Ghent University, K. L. Ledeganckstraat 35, 9000 Ghent, Belgium;

³Laboratory for Pest Management Research, Institute of Agricultural Engineering, ARO, The Volcani Center; and ⁴Extension Service (Shaham); Israel Ministry of Agriculture & Rural Development, Israel

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ABSTRACT

Root-lesion nematodes of the genus *Pratylenchus* parasitize the roots of numerous plants and can cause severe damage and yield loss. Here, we report on a new species, *Pratylenchus capsici* n. sp., from the Arava rift, Israel, which was characterized by integrative methods, including detailed morphology, molecular phylogeny, population genetics, and phylogeography. This species is widely spread across the Arava rift, causing significant infection in pepper (*Capsicum annuum*) roots and inhibiting plant growth. Both morphological and molecular species delimitation support the recovered species as a new species. We found high cytochrome oxidase subunit I haplotype diversity, and phylogeography

analysis suggests that contemporary gene flow is prevented among different agricultural farms, while population dispersal from weeds (*Chenopodium album* and *Sonchus oleraceus*) to pepper occurs on a relatively small scale. Our results suggest that weeds are an important reservoir for the dispersal of *P. capsici* n. sp., either as the original nematode source or at least in maintaining the population between growing seasons.

Keywords: Arava rift, *Capsicum annuum*, Nematoda, new species, pepper, *Pratylenchus*, Tylenchomorpha

Root-lesion nematodes of the genus *Pratylenchus* are migratory endoparasites belonging to the family Pratylenchidae (Nematoda, Tylenchomorpha), with 100 species currently recognized (Geraert 2013; Hodda et al. 2014; Palomares-Rius et al. 2014; Wang et al. 2015). *Pratylenchus* spp. are considered to be the third most economically important plant-parasitic nematodes (Castillo and Vovlas 2007), causing yield losses of up to 85% of expected production (Nicol et al. 2011). Such losses can be even higher when nematodes interact synergistically with certain soilborne plant pathogens (Jones and Fosu-Nyarko 2014). Hence, detection and identification of *Pratylenchus* spp. is important. However, the taxonomical status and diagnosis of *Pratylenchus* spp. is often problematic. Past studies have been limited by their reliance on a small set of morphological traits or a few molecular barcoding genes (Hodda et al. 2014; Palomares-Rius et al. 2014; Singh et al. 2018; Wang et al. 2015), with no incorporation of historical phylogeography or underlying genetic variation. Although these types of data may be useful diagnostic implements, they can be deficient in species boundary delimitation given their high intraspecific variability, including variation of molecular barcodes (Castillo and Vovlas 2007; De Luca et al. 2011; Janssen et al. 2017; Roman and Hirschmann 1969; Tarte and Mai 1976). Consequently, an integrative approach, incorporating unbiased molecular delimitation methods, is needed for an accurate description of *Pratylenchus* spp.

The Arava rift is part of the Syrian African rift, located in the southeastern part of Israel and stretching from the Dead Sea to the Red Sea. It is an arid region characterized by an extremely harsh

environment, with a hot climate and high water salinity (Pen-Mouratov et al. 2010). Agriculture in this region consists of intensive indoor production in net houses supported by drip irrigation and fertilization to provide adequate conditions for crop production. Over the past 50 years, the Arava rift has evolved into a unique agroecosystem with advanced agricultural technologies, producing highly profitable fresh vegetables, herbs, and cut flowers, mainly for export. However, in recent years, severe root infections caused by a root-lesion nematode have been detected in pepper from several agricultural farms in the Arava rift, suggesting a highly specialized nematode–plant interaction. Moreover, management of this nematode has not been successful. Hence, questions have been raised as to the identity and origin of this nematode, considering two major aspects: first, plant-parasitic nematodes are generally well managed in other regions in Israel; second, the Arava region is remote and isolated from other agricultural areas. Therefore, the objectives of the present study were to (i) characterize and formally describe this species by integrative approaches, (ii) employ different sequence-based delimitation methods to objectively interpret species boundaries, and (iii) trace the possible origin and transmission pathway to facilitate further management.

MATERIALS AND METHODS

Sampling and isolation of nematode specimens. Nine samples were collected between November 2017 and April 2018 from Israel's Arava rift region. Seven samples were taken from the root zone soil of pepper (*Capsicum annuum*) at the following farms: Ein Tamar (ET), Hatzeva (HZ), Ein Yahav (Ein Yahav pepper, EYP), Tsafar-B (TB), Tsafar-A (TA), Tsafar-East (TE), and Paran (PR). Two root-zone soil samples from weeds (*Chenopodium album* and *Sonchus oleraceus*) in Ein Yahav (Ein Yahav weeds [EYW]) were taken from the same nethouse as EYP. Additional samples of *Pratylenchus mediterraneus* were collected from alfalfa in Shefaa, Israel. Nematodes were extracted using a Baermann funnel.

[†]Corresponding author: S. Braun Miyara; E-mail: sigalhor@volcani.agri.gov.il

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Morphological analyses. Extracted fresh nematodes were fixed in 4% formalin solution at 65°C. Fixed specimens were rinsed several times with deionized water and gradually transferred to anhydrous glycerine for permanent slides (Sohlenius and Sandor

1987). Morphological characteristics of nematodes were observed with a Nikon Eclipse Ni light microscope and photographed using Nikon DS-Ri2 (Nikon Corporation, Tokyo, Japan) equipped with differential interference contrast. To visualize the nematode

Fig. 1. Scanning electron microscopy photographs of *Pratylenchus capsici* n. sp. **A, B, D, and E**, female en face view; **C**, female anterior end; **F, G, and H**, female tail; **I**, vulva region; **J and K**, male tail region; and **L to N**, lateral field.

infection, root tissues were rinsed with water and then stained with acid fuchsin following the protocol of Bybd et al. (1983). Illustrations were prepared based on light microscopy drawings using Adobe Illustrator CS3.

For scanning electron microscopy (SEM) observation, samples of live specimens were heated in a microwave in Trump's fixative (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M Sorensen's

buffer) for a few seconds. The nematodes were then rinsed three times in double-distilled water and dehydrated through a series of gradient ethanol solutions (30, 50, 75, and 95%, 20 min each) and, finally, three times in pure ethanol (10 min each). Thereafter, the rinsed nematodes were critical point dried with liquid CO₂, mounted on stubs with carbon discs, and coated with gold (25 nm). The fixed nematodes were then observed through a

Fig. 2. Line drawing of *Pratylenchus capsici* sp. n. **A** and **B**, female anterior body region; **C**, vulva; **D**, male reproductive system showing the spicule, gubernaculum, and bursa; **E** and **F**, spermatheca; **G** and **H**, tail; **I**, female lateral region showing lateral lines; **J**, lip region; **K**, female body habitus; and **L**, male body habitus.

microscope before observation in a JSM-840 electron microscope (Jeol, Tokyo, Japan) at 15 kV.

Molecular analyses. DNA was extracted from living nematode samples. Briefly, single individuals were transferred separately to a

polymerase chain reaction (PCR) tube containing 10 μ l of 0.05 N NaOH solution and 1 μ l of 4.5% (wt/vol) Tween 20. The tubes were incubated for 15 min at 95°C; then, 40 μ l of double-distilled water was added to each sample. The 18S ribosomal RNA (rRNA) gene

Fig. 3. Photomicrographs of *Pratylenchus capsici* n. sp. **A** and **B**, female anterior body; **C** to **E**, male tail; **F**, vulva region of nongravid female; **G**, vulva region of gravid female, showing the egg; **H**, spermatheca (sp.) and uterus (ut.); **I**, oocytes (oc.) and spermatheca (sp.); **J** to **L**, female body habitus; **M** to **P**, female tail; and **Q**, lateral field.

(18S) was amplified using the primers G18S4 (5'-GCT TGT CTC AAA GAT TAA GCC-3') and 18P (5'-TGATCC WKC YGC AGG TTC AC-3') (Blaxter et al. 1998). The D2D3 domain of 28S rDNA (28S) was amplified using D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (Nadler et al. 1999). The internal transcribed rRNA gene spacer (ITS) was amplified using VRAIN2 F (5'-CTT TGT ACA CAC CGC CCG TCG CT-3') and VRAIN2 R (5'-TTT CAC TCG CCG TTA CTA AGG GAA TC-3') (Vrain et al. 1992). The cytochrome c oxidase subunit 1 (COI) gene fragment was amplified using JB3 (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and JB4.5 (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3') (Bowles et al. 1992). The PCR products were cloned (except for COI) using pGEM-T easy vector systems (Promega, Madison, WI) and subsequently sequenced in two primer directions at Hy-labs (Rehovot, Israel). Contigs were assembled using Geneious R6.1.8. The resulting sequences were deposited in the GenBank database under accession numbers MH796905 to MH796906, MH796969 to MH796980, and MH798799 to MH798802.

DNA sequences representing *P. capsici* n. sp. were analyzed together with other *Pratylenchus* sequences from GenBank. Multiple alignments for rRNA genes were built using the G-INS-i algorithm of MAFFT v. 7.205 (Katoh and Standley 2013) and the COI gene was aligned using TranslatorX (Abascal et al. 2010) under the invertebrate mitochondrial genetic code. For phylogeny reconstruction, the best-fitting substitution model was estimated

using the Akaike Information Criterion in jModelTest v. 2.1.2 (Darriba et al. 2012). Maximum-likelihood (ML) and Bayesian inference (BI) analyses were performed on the CIPRES Science Gateway (Miller et al. 2010) using RAXML 8.1.11 (Stamatakis et al. 2008) and MrBayes 3.2.3 (Ronquist et al. 2012), respectively. ML analysis included 1,000 bootstrap (BS) replicates under the GTRCAT model whereas BI used the GTR+I+G model with four independent chains for 1×10^7 generations in two runs. For BI, Markov chains were sampled every 100 generations and 25% of the converged runs were discarded as burn-ins.

Species delimitation. Molecular species-delimitation analyses were performed using the generalized mixed-yule coalescent (GMYC) method (Pons et al. 2006), a Bayesian implementation of the Poisson tree processes (bPTP) method (Zhang et al. 2013), and the automatic barcode gap discovery (ABGD) method (Puillandre et al. 2012). Duplicated haplotypes and outgroup sequences were pruned from the dataset prior to analysis. The GMYC species-delimitation method was performed in the R package *Splits* (Ezard et al. 2009) with a single threshold. An ultrametric tree was constructed using BEAST v1.8.4 (Drummond et al. 2012). Default prior distributions were used except for the uncorrelated lognormal relaxed clock, and a constant size coalescent prior and uniform prior for ucl.d.mean parameter. Analyses were run for 1×10^8 generations, saving trees every 1×10^4 generations. The final output was examined visually in Tracer 1.7 (Rambaut et al. 2018) to determine whether the run had reached convergence (ESS values > 200). The

Fig. 4. Symptoms caused by *Pratylenchus capsici* n. sp. **A**, Fuchsin staining of nematodes infected root; **B**, infected root showing lesions; and **C**, scanning electron microscopy photograph of root-lesion. Scale bar: A = 100 μ m, B = 8 mm, and C = 600 μ m.

TABLE 1. Morphometrics of population of *Pratylenchus capsici* n. sp. from the type location Tsfar, Israel^a

Characteristic	Holotype		Paratypes	
	Female	Male	Male	Female
n	1 (fixed)	5 (fixed)	15 (fixed)	15 (fixed)
a	26.7	30.6 ± 3.97 (25.2–34.9)	26.7 ± 2.21 (22.3–30.1)	26.7 ± 2.21 (22.3–30.1)
b	6.53	6.65 ± 0.54 (6.12–7.47)	6.89 ± 0.66 (5.26–8.11)	6.89 ± 0.66 (5.26–8.11)
b'	4.96	4.90 ± 0.19 (4.56–5.07)	5.26 ± 0.42 (4.19–5.78)	5.26 ± 0.42 (4.19–5.78)
c	18.8	21.5 ± 3.07 (18.5–26.1)	20.5 ± 2.31 (17.3–24.3)	20.5 ± 2.31 (17.3–24.3)
c'	2.58	2.42 ± 0.62 (1.90–3.30)	2.45 ± 0.33 (1.92–3.11)	2.45 ± 0.33 (1.92–3.11)
V	78.2	...	78.3 ± 3.04 (70.1–81.4)	78.3 ± 3.04 (70.1–81.4)
O	20.8	18.6 ± 2.36 (15.2–20.4)	20.8 ± 3.19 (15.3–27.1)	20.8 ± 3.19 (15.3–27.1)
L	620	567 ± 28.7 (530–598)	606 ± 22.1 (559–642)	606 ± 22.1 (559–642)
Anterior end to vulva	485	...	474 ± 18.7 (446–498)	474 ± 18.7 (446–498)
Tail length	33.0	26.9 ± 4.53 (20.3–32.3)	29.9 ± 4.29 (24.4–37.0)	29.9 ± 4.29 (24.4–37.0)
Pharyngeal overlap	30.1	30.3 ± 4.68 (26.5–38.0)	27.1 ± 4.86 (18.8–33.0)	27.1 ± 4.86 (18.8–33.0)
Pharynx	125	116 ± 6.96 (106–123)	115 ± 9.71 (108–142)	115 ± 9.71 (108–142)
Cone of stylet	6.85	6.86 ± 0.88 (5.93–7.2)	6.95 ± 0.36 (6.57–7.31)	6.95 ± 0.36 (6.57–7.31)
Stylet length	14.4	14.3 ± 0.94 (12.9–15.1)	14.5 ± 0.47 (13.7–15.3)	14.5 ± 0.47 (13.7–15.3)
DGO from stylet base	3.00	2.72 ± 0.29 (2.30–2.98)	3.02 ± 0.44 (2.1–3.8)	3.02 ± 0.44 (2.1–3.8)
Anterior end to central of metacarpus	58	56.1 ± 3.49 (52.2–61.0)	54.4 ± 3.81 (49.1–63.8)	54.4 ± 3.81 (49.1–63.8)
Postuterine sac	26.2	...	30.2 ± 4.50 (21.8–37.9)	30.2 ± 4.50 (21.8–37.9)
Anus/cloacal width	12.8	11.5 ± 1.76 (9.80–13.9)	12.2 ± 0.70 (11.2–13.8)	12.2 ± 0.70 (11.2–13.8)
Lip width	7.85	7.44 ± 0.53 (6.9–8.1)	8.25 ± 0.53 (7.5–9.49)	8.25 ± 0.53 (7.5–9.49)
Maximum body diameter	23.2	18.8 ± 2.65 (15.2–21.9)	22.8 ± 2.16 (20.1–27.2)	22.8 ± 2.16 (20.1–27.2)
Anterior end to nerve ring	73.5	76.5 ± 5.56 (69.0–82.0)	75.3 ± 6.73 (65.3–89.2)	75.3 ± 6.73 (65.3–89.2)
Anterior end to excretory pore	70.6	77.7 ± 10.3 (70.4–85.5)	86.9 ± 8.31 (72.8–97.4)	86.9 ± 8.31 (72.8–97.4)
Spicule length	...	16.2 ± 0.89 (15.1–17.3)	...	...
Gubernaculum length	...	4.45 ± 0.55 (3.80–5.26)	...	...

^a All measurements are in micrometers and are presented as mean ± standard deviation (range). DGO = dorsal pharyngeal gland orifice.

Fig. 5. Bayesian 50% majority-rule consensus tree inferred on 18S ribosomal RNA (18S, left) and internal transcribed spacer (ITS, right) aligned with G-INS-i implemented in MAFFT. Section's order of support values: posterior probability/bootstrap. New species are indicated by arrows and new sequences original to this study are indicated in bold.

final tree was produced after removing 2,000 samples (20%) as burn-ins, and the maximum clade credibility tree was calculated using TreeAnnotator 1.8 (Drummond et al. 2012). For the bPTP method, an unrooted Bayesian 50% majority-rule consensus tree was used containing only ingroups and unique haplotypes; 1×10^6 Markov chain Monte Carlo (MCMC) generations were performed using an online server (<https://species.h-its.org/>). For the ABGD method, the alignment without outgroups and only unique haplotypes was used. ABGD analyses were performed using the online version of the program (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>) with the default program settings, and distances were calculated using the Jukes-Cantor (JC69) substitution model.

Population structure. To study the relationships between populations and their geographical distributions, we estimated COI haplotype diversity and population genetic structure using the PopART program (Leigh and Bryant 2015) by means of the TCS networks (Clement et al. 2002).

We applied the Bayesian clustering program STRUCTURE v2.3.4 (Falush et al. 2003, 2007; Pritchard et al. 2000) to further estimate population structure, possible migrants, and individuals with admixed population ancestry. In the absence of prior knowledge of relationships, and given the capacity for dispersal across geographical barriers by agricultural practice, we used the 'admixture' model for analyses. We used the a priori constraint of number of populations, from a single, panmictic population throughout the entire Arava rift ($K = 1$) to a series of populations including all sampling sites ($K = 8$). We ran five independent analyses, each for 5 million MCMC iterations, discarding 500,000 burn-ins. We selected the preferred number of populations (K value) based on the

ΔK method (Evanno et al. 2005) and maximum posterior probability method (Pritchard et al. 2000) using the program CLUMPAK (Kopelman et al. 2015).

RESULTS

Description of *Pratylenchus capsici* n. sp. The measurements are listed in Table 1. *Female.* Body slender, vermiform, straight or slightly curved ventrally. The lip region is slightly offset from the body contour, with three annuli distinctly higher than the adjoining body annuli. In a single specimen, four distinct annuli on one side of the lip region and three on the other side were observed (Fig. 1D). En face view characterized by a divided face with rectangular subdorsal and subventral lips fused to an oral disc in a dumbbell pattern which is separated from the lateral lip sectors by two almost straight incisures forming an obtuse angle (Figs. 1A and B and 2J), in accordance with group 2 in the classification scheme of Corbett and Clark (1983). Lateral fields with four equidistant completely areolated lines, occupying almost one-third of the body width at midbody (Figs. 1L to N, 2I, and 3Q). Stylet well developed, conus half of entire length, basal knobs strongly developed and rounded to slightly anteriorly concave. Pharyngeal procorpus narrowing just anterior to rounded-oval, muscular median bulb. Valve of median bulb conspicuous. Secretory-excretory pore at the level of or slightly posterior to the pharyngo-intestinal junction. Hemizonid located immediately anterior to the secretory-excretory pore to approximately 3 annuli anterior. Pharyngeal glands overlapping intestine ventrally. Reproductive system monoprodelfic, oocytes arranged in a single row. Spermatheca large, round to slightly oval, full of sperm, 11 to 18 μ m in length (Figs. 2E and F and 3H and I). Vulva protruding, lips

Fig. 6. Bayesian 50% majority-rule consensus tree inferred on cytochrome c oxidase subunit 1 (COI, left) and D2/D3 of 28S ribosomal RNA (28S, right) aligned with G-INS-i implemented in MAFFT. Section's order of support values: posterior probability/bootstrap. New species are indicated by arrows and new sequences original to this study are indicated in bold.

well developed, no lateral flaps or epiptygma. Postuterine sac (PUS) well developed, 1.1 to 1.7 times the vulval body diameter in length. Tail terminus smooth, conoid-rounded to subcylindrical. Phasmid at or slightly posterior to midtail level.

Male. Similar to female except in reproductive system, and with a slightly smaller body length. Testis outstretched, short. Spicules paired, weakly cephalated, ventrally arcuate. Gubernaculum slightly curved. Tail conical, elongate, characteristically bent on ventral side,

Fig. 7. Molecular species-delimitation analysis of the *Pratylenchus* spp. by three methods: generalized mixed yule coalescent model (GMYC), a Bayesian implementation of the Poisson tree processes (bPTP), and automatic barcode gap discovery (ABGD). Delimitation results are visualized as bars on an ultrametric Bayesian maximum clade credibility tree of the cytochrome c oxidase subunit 1 gene. For the ABGD analysis, columns a to d correspond to the 23, 24, 26, and 27 species grouping, respectively, as recovered for different prior intraspecific divergence assumptions. Bayesian posterior probabilities are indicated on the branches of the phylogenetic tree. New sequences original to this study are indicated in bold.

enveloped by a poorly protruding, crenate bursa (Figs. 1J and K, 2D, and 3C and D).

Type host and symptoms. *P. capsici* n. sp. was found in roots of sweet pepper (*Capsicum annuum*) at the nethouse in Tsofar, Arava rift, Israel (GPS coordinates 30°33'11.6"N, 35°11'30.3"E). Two additional populations were found from *Chenopodium album* and *S. oleraceus* in the nethouse at Ein Yahav but at much lower density. The infected sweet pepper had necrotic lesions on the surface and throughout the cortex of the infected root (Fig. 4). Aboveground parts of plant showed stunted growth, yellowing, and marginal and tip curving of the leaves.

Type material. Holotype female, two female and three juvenile paratypes are deposited on one slide at the Ghent University Museum, Zoology Collections, collection number UGMD104364. Additional paratypes are deposited in the Nematology Research Unit of the Volcani Center, Israel. The new species name has been registered at ZooBank (identifier: F0EA56A6-BEDA-4378-893D-BAEFE980E9AA).

Etymology. The species epithet is derived from the name of its host, *Capsicum annuum*.

Diagnosis and relationships. *P. capsici* n. sp. is characterized by a combination of the following traits: relatively long body (559 to 642 µm), lip region slightly offset from body contour, bearing three annuli. A divided SEM face, with rectangular subdorsal and subventral lips fused with oral disc in a dumbbell pattern which is separated from lateral lip sectors by two incisures forming an obtuse angle (group 2 according to Corbett and Clark [1983]); a stylet 14 to 15 µm long; lateral fields with four areolated incisures, a functional spermatheca, and numerous males.

Morphologically, *P. capsici* n. sp. can be distinguished from all other known species by a number of specific characteristics resulting from its specific matrix code Castillo and Vovlas (2007): A2, B2, C2, D2, E2, F5(4), G3(2), H2, I1(2), J1, and K2 (variation in characteristics is given in parentheses). For a detailed comparison of morphologically and molecularly related species, see Table 2. *P. capsici* n. sp. is most similar to *P. oleae* (with 10 identical traits out of 11, according to the matrix code) but differs in the presence of males (versus their absence), a functional spermatheca (versus nonfunctional and empty), a larger body (559 to 642 versus 412 to 511 µm), and a shorter stylet (14 to 15 versus 15 to 17 µm). With *P. bhattii*, *P. pseudofallax*, and *P. unzenensis*, *P. capsici* n. sp. shares the combination of three lip annuli, a round functional spermatheca, common presence of males, and a stylet of 13 to 16 µm. It differs from *P. bhattii* in having an areolated (versus smooth) lateral region, striated (versus smooth) female tail tip, and a longer PUS (22 to 38 versus 11 to 22 µm). It differs from *P. pseudofallax* in its areolated (versus smooth) lateral region, larger body (559 to 642 versus 415 to 525 µm), and most of its morphometric ratios. It differs from *P. unzenensis* in its round (versus oval) spermatheca, striated (versus smooth) female tail tip, and larger body (559 to 642 versus 368 to 470 µm).

Phylogenetic relationships. The amplification of 28S, ITS, 18S, and COI regions yielded fragments of approximately 748, 778,

1,797, and 441 bp, respectively. The pairwise comparison of the sequence of *P. capsici* n. sp. with other *Pratylenchus* sequences from GenBank gave the highest similarity to *P. oleae* for all genes (nucleotide differences: 18S, 22 to 23 bp, 1.19 to 1.24%; 28S, 33 to 45 bp, 3.83 to 5.23%; ITS, 59 to 148 bp, 4.25 to 10.67%; and COI, 59 to 62 bp, 13.1 to 13.78%).

Phylogenetic relationships among *Pratylenchus* spp. inferred from 18S, 28S, ITS, and COI sequences were congruent across analyses and, in general, our results supported those of previous analyses (Janssen et al. 2017; Palomares-Rius et al. 2014; Subbotin et al. 2008). In all analyses, *P. capsici* n. sp. was well supported as a sister group of *P. oleae* (18S: posterior probability [PP] = 1, BS = 100; 28S: PP = 1, BS = 100; ITS: PP = 1, BS = 97; and COI: PP = 0.82, BS = 70) (Figs. 5 and 6). In the 28S phylogeny, *P. capsici* n. sp. is nested in the monophyletic *Penetrans* group (Janssen et al. 2017), including *P. rwandae*, *P. convallariae*, *P. dunensis*, *P. fallax*, *P. oleae*, *P. penetrans*, *P. pinguicaudatus*, and three undescribed species (*Pratylenchus* sp. 1, 2, and 4), or clade IV (Palomares-Rius et al. 2014), including the aforementioned species plus *P. brachyurus*. Similar placements were also seen in 18S and ITS (except for a divergent placement of *P. brachyurus*) but the interspecific relationship was not resolved for COI. In addition, the newly obtained 18S and 28S sequences from *P. mediterraneus* were fully supported (PP = 1, BS = 100) as sister to *P. thornei*, concurring with De Luca et al. (2004).

Molecular species delimitation. In an attempt to compare *P. capsici* n. sp. with other similar species and identify populations that may merit species rank, we employed species delimitation using GMYC, bPTP, and ABGD methods based on COI sequences (Fig. 7). We included all recovered haplotypes of the new species, as well as of other *Pratylenchus* spp. that are available in GenBank, and the resultant alignment contained 63 different 444-bp-long haplotypes. Molecular species delimitation appointed the highest species number using the single-threshold GMYC model prediction ($n = 30$), followed by 28 species according to bPTP, while ABGD revealed variation in the number of species—from 23 to 27—according to the models and priors used. In general, the applied methods were largely congruent among themselves and with traditional methods, and the *P. capsici* n. sp. populations were well separated from all known species. Surprisingly, in two cases (GMYC and 27 species of ABGD), one haplotype (at the PR site) was separated from the other populations and, thus, according to these species-delimitation methods, can be separated into two cryptic species which are morphologically and morphometrically identical. This variable number of estimated species is not unexpected given that molecular species-delimitation analyses are known to generate a variety of different species hypotheses (Kekkonen and Hebert 2014; Prévot et al. 2013). We opted to follow the most stringent criterion, by which a species is only recognized when it is supported by morphological and molecular species delimitation. Consequently, the population from PR is considered to represent intraspecific variation.

Population structure. In total, 68 COI sequences obtained from nine samples were used for the population analysis. The most

TABLE 2. Comparison of matrix codes of tabular key for *Pratylenchus* spp. identification, proposed by Castillo and Vovlas (2007), between *Pratylenchus capsici* n. sp. (in bold) and its morphologically and molecularly related species

Species	A	B	C	D	E	F	G	H	I	J	K
<i>P. capsici</i> n. sp.	2	2	2	2	2,1,3	5,4	3,2	2	1,2	1	2
<i>P. brachyurus</i>	1	2	4	1	4	3	3	1	4	2,3	1
<i>P. convallariae</i>	2	2	3	2	2	6	2	2	3	1	1
<i>P. crenatus</i>	2	2	3	1	3	3	2	2	1	3	1
<i>P. dunensis</i>	1	2	3	2	2	4	1	1	4	1	2
<i>P. fallax</i>	2	2	3	2	2	3	3	2	2	1	1
<i>P. mediterraneus</i>	2	2	2	2	2	3	2	1	3	1	1
<i>P. oleae</i>	2	1	2,3	2	2,3	3,4,5	2,3	2	1,2	1	2
<i>P. penetrans</i>	2	2	3	2	3	4	2	1	3	1	1
<i>P. pinguicaudatus</i>	2	1	3	1	3	1	2	1	4	1	2
<i>P. rwandae</i>	2	1	2	2,3	2	3,4	2,3	1	1,2	3	1

interesting general result was the presence of multiple haplotypes occurring in a relatively small geographical region (approximately 90 km). In total, 14 unique COI haplotypes were identified (Fig. 8A and B). Haplotype diversity was highest in TA (five haplotypes), followed by EYW (three haplotypes), and TE (three haplotypes), whereas only one haplotype was found in EYP and TB. Most haplotypes were closely related and separated by a single mutation, while the most common haplotype in PR (H14) was the exception, diverging by 10 mutational steps. Most of the sampling sites were

characterized by their own endemic haplotype (12 haplotypes were only present once), no ubiquitous haplotype could be found, and only three sampling locations shared 2 haplotypes (EYW, TA, and TE shared haplotype H5 and ET, TE, and TA shared haplotype H12). Haplotype H2 was found in two adjacent sites (EYP for pepper and EYW for weeds). Interestingly, it was the only haplotype in EYP but one of three in EYW, suggesting possible dispersal from weeds to pepper. Conversely, H10 was the only haplotype found in TB but it was not present in the adjacent TA or in any other sites,

Fig. 8. Geographical distribution of the sampling sites, cytochrome c oxidase subunit 1 haplotype network, and the inferred population structure of *Pratylenchus capsici* n. sp. in Arava rift, Israel. **A**, Geographic location of Arava rift region and the distribution of sampling sites. Pie chart indicates the composition and proportion of haplotypes in each location. **B**, TCS haplotype network inferred from PopART. Each circle corresponds to one haplotype and its size is proportional to its frequency. Each line connecting the haplotypes refers to a mutational step. Marks on the lines indicate the number of steps. Shades correspond to different sampling locations and names are given in abbreviations. **C**, Population structure inferred from STRUCTURE runs with the best probability values measured by ΔK ($K = 4$) and maximum posterior probability method ($K = 7$). Sampling regions are separated by black lines. Clusters with similar shades are more closely related. Within each cluster, an individual sequenced nematode corresponds to a vertical segment that reflects its ancestry. Mixed ancestries are indicated by differently shaded sectors, corresponding to inferred genetic percentages of the corresponding clusters.

suggesting that this population may have been recently introduced from an unknown source.

According to the maximum posterior probability method (Pritchard et al. 2000), the most likely number of clusters (K) is 7, whereas the ΔK method (Evanno et al. 2005) suggested $K = 4$ ($\Delta K = 60.6$). However, the ΔK values for $K = 7$ show a pronounced second peak ($\Delta K = 25.8$), giving at least some support to the maximum posterior probability approach. Based on this evidence, the inferred population structure for both $K = 4$ and $K = 7$ is presented (Fig. 8C). Under both K values, the recognized clusters did not correspond to their geographical distributions. For example, ET is geographically adjacent to HZ but lumped together with TB (Fig. 8A and C). Regarding each sampling site, more ancestry admixture appeared when $K = 7$ compared with $K = 4$, except for HZ and PR, where only very minor admixtures were detected. Taken together, the network and population structure analyses suggest the existence of historical admixture across the range; however, all sampling sites are currently well separated by unique haplotype compositions.

DISCUSSION

Nematodes of the genus *Pratylenchus* is notorious for being difficult to identify at the species level. Despite a few well-defined diagnostic characteristics (e.g., presence or absence of males and nature of the lateral field), most widely used characteristics are continuous and overlapping (e.g., stylet size and PUS length) or subject to interpretation (e.g., shape of spermatheca and tail) (Castillo and Vovlas 2007; Roman and Hirschmann 1969; Tarte and Mai 1976). Several biochemical and molecular methods have been used for accurate diagnosis, including isozyme electrophoresis (Andrés et al. 2000; Ibrahim et al. 1995), restriction fragment analysis of ITS (Waeyenberge et al. 2000), species-specific primers (Al-Banna et al. 2004; Palomares-Rius et al. 2014; Subbotin et al. 2008; Troccoli et al. 2016), duplex and quantitative PCR methods (De Luca et al. 2012; Waeyenberge et al. 2009), and ribosomal and mitochondrial gene-based barcoding and species delimitation (De Luca et al. 2011; Janssen et al. 2017; Subbotin et al. 2008). Today, sequence-based molecular barcodes are most often used; however, they are also often intraspecifically variable (Janssen et al. 2017). In this study, we described *P. capsici* n. sp. using integrative approaches and the results support recognition of *P. capsici* n. sp. as a new species. The inclusion of phylogeography and population genetics analysis underscores the great potential of such methods as tools in plant-parasitic nematology to understand the nematodes' life history and transmission. In most cases, novel and distinct molecular lineages may be recognized by morphological data. However, in this case, two cryptic species within *P. capsici* n. sp. were appointed by some molecular species-delimitation methods, whereas no morphological differences (Tables 1 and 2) were observed. Given that single-locus data represent the history of a single gene that might not be representative of the organism's history (Fujita et al. 2012), we consider traditional morphology-based taxonomy to be a reasonable and, in fact, indispensable basis for species hypotheses. Therefore, we followed the consensus protocol proposed by Padial et al. (2010), whereby the molecularly delimited lineages are not recognized as new species unless they are morphologically diagnosable and supported by independent posthoc analysis.

In the present study, sampling throughout the Arava rift range allowed for robust genetic analyses of the populations. The results of the mitochondrial haplotype network analyses revealed high COI haplotype diversity in *P. capsici* n. sp. (14 unique haplotypes). Such high diversity within this limited geographical region is surprising because, for example, only 16 haplotypes were provided from five countries with highly variable *P. penetrans* populations (Janssen et al. 2017), and *P. mediterraneus* samples from four locations in Israel shared a single haplotype (unpublished data). While not providing support for a straightforward genetic-geographical pattern, our STRUCTURE analyses did reveal admixtures among

the sampling sites (except for HZ, with a less admixed composition), suggesting either retention of ancestral polymorphisms or contemporary gene flow. However, the latter is unlikely to be the main reason because (i) most of the haplotypes were only present in a specific location (12 of 14 haplotypes), and those locations sharing haplotypes were not necessarily geographically proximate (EYW, TA, and TE for haplotype H5 and ET, TE, and TA for haplotype H12); (ii) the hyperaridity and hypersalinity in this area are extremely unfavorable, posing a strong habitat barrier, and the nematodes are entirely absent in the open area below sea level (Pen-Mouratov et al. 2010); and (iii) man-mediated dispersal is limited because the spread of pathogens is well controlled in highly developed and commercialized sampling regions. Conversely, on a small scale (e.g., within one nethouse), contemporary gene flow is likely to contribute to the observed genetic patterns. EYW were collected from weed (*Chenopodium album*, a widespread species in the nethouse of Arava rift) roots, a few meters away from sweet pepper root (EYP). The geographical proximity gives rise to the possibility of population migration or man-mediated transport. Indeed, EYW has three haplotypes (H2, H5, and H9) whereas EYP bears only one of them (H2), suggesting plausible dispersal from EYW to EYP. Interestingly, the sister species *P. oleae* was also recovered in both wild and cultivated olive (Palomares-Rius et al. 2014), and an unidentified *Pratylenchus* population was recovered from a natural environment in a closely related area (Shukurov et al. 2005). Several *Pratylenchus* spp. (e.g., *P. brachyurus*, *P. coffeae*, and *P. vulnus*) have a wide range of host plants (Castillo and Vovlas 2007), and the ability to parasitize a wide range of weed species is also known for *P. penetrans* (Bélair et al. 2007). This suggests that *P. capsici* n. sp. may be native and parasitize wild plants, or that it was introduced by another, unknown, source but coincidentally survived in the weeds. Taken together, our results indicate that weeds are important either as the original nematode source or in maintaining the population. Further analyses with geographically broader sampling and more plant species are needed to clarify this.

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