exeley
JOURNAL OF NEMATOLOGY
Article | DOI: 10.21307/jofnem-2018-020
Issue 3 | Vol. 50 (2018)

First Report of *Bitylenchus hispaniensis*, *Pratylenchoides alkani*, and *Helicotylenchus vulgaris* in Association with Cultivated and Wild Olives in Crete, Greece and Molecular Identification of *Helicotylenchus microlobus* and *Merlinius brevidens*

Emmanuel A. Tzortzakakis,[1] Carolina Cantalapiedra-Navarrete,[2] Maria Kormpi,[3] Maria S. Lazanaki,[1] Pablo Castillo,[2] and Antonio Archidona-Yuste[2]*

[1]Department of Viticulture, Vegetable Crops, Floriculture and Plant Protection, Institute of Olive Tree, Subtropical Crops and Viticulture, N.AG.RE.F., Hellenic Agricultural Organization-DEMETER, Crete, Greece.

[2]Institute for Sustainable Agriculture (IAS), CSIC, Córdoba, Spain.

[3]Benaki Phytopathological Institute, Athens, Greece.

*E-mail: aarchidona@ias.csic.es.

This paper was edited by Erik J. Ragsdale.

Received for publication January 30, 2018.

Abstract

Nematode samplings in cultivated and wild olive in Crete, Greece, yielded the presence of *Bitylenchus hispaniensis*, *Helicotylenchus microlobus*, *Helicotylenchus vulgaris*, *Merlinius brevidens*, and *Pratylenchoides alkani*. With the exception of *H. microlobus* and *M. brevidens*, reports of these plant-parasitic nematode species constitute new records for Greece. *Bitylenchus hispaniensis* is also reported for first time in a country outside of Spain, where it was originally described. *Pratylenchoides alkani* is herein reported for the second time in the Mediterranean area and for the first time in association with olive. Two further populations of *H. microlobus* and *H. vulgaris*, from walnut and goji berry from Greece, were identified. Molecular data for all of these nematode species are provided, resulting in the first integrative identification of these Greek populations.

Key words

28S rRNA, Detection, New geographic record, ITS.

Olive (*Olea europaea* subsp. *europaea* L.) is the most common crop on the island of Crete, Greece, while clusters of wild olives (*Olea europaea* subsp. *sylvestris* L.) are located also in several non-agricultural areas (Tzortzakakis et al., 2014). Surveys for detection of plant-parasitic nematodes were conducted on both cultivated and wild olives during the period 2013 to 2015. Soil samples were collected with a hoe, discarding the upper 5-cm top soil profile, from 5 to 40 cm depth in the close vicinity of active roots from 2 to 5 olive trees randomly chosen in each from 146 orchards. Similarly, soil samples were also collected from the rhizosphere of individual 36 wild olives distributed in various areas. Furthermore, three additional soil samples, where populations of *Helicotylenchus* were detected, were included in this study. One sample was from pots where cultivated olive stock plants were maintained for research purposes (Institute of Olive Tree, Subtropical Crops, and Viticulture, Chania, Crete), the second from the roots of walnut (*Juglans regia* L.) in Evia, Greece, and the third from the roots of goji berry (*Lycium barbarum* L.) in Thessaly, Greece. Nematodes were extracted from several soil samples of 500 g by the wet-sieving and decanting method (Cobb, 1918). Additional samples were collected later from the same sampling sites to obtain sufficient specimens for morphological and molecular identification. Specimens to be observed under light microscopy (LM) were heat killed by adding hot 4% formaldehyde solution and were processed to pure glycerin using De Grisse's (1969) method. Microscopical observations were carried out using a Zeiss

Table 1. Sequenced genes pf plant-parasitic nematodes sampled from cultivated and wild olive in Crete and two other hosts in Evia and Thessaly, Greece.

Nematode species	Sample code	Host and Locality	D2-D3	ITS
Bitylenchus hispaniensis	OLI106	Cultivated olive, Episkopi, Crete	MG770479	–
B. hispaniensis	OLI013	Cultivated olive, Roufas, Crete	*	–
B. hispaniensis	OLE011	Wild olive, Trypiti, Crete	*	
B. hispaniensis	OLE015	Wild olive, Lentas, Crete	*	–
B. hispaniensis	OLE023	Wild olive, Ag. Ioannis, Crete	*	–
B. hispaniensis	OLE029	Wild olive, Ag. Georgios, Crete	*	–
B. hispaniensis	OLE032	Wild olive, Tsoutsouros, Crete	*	–
Helicotylenchus microlobus	OLI066	Cultivated olive, Vori, Crete	MG770480	MG770406
H. microlobus	OLI002	Cultivated olive, Vori, Crete	*	–
H. microlobus	OLI074	Cultivated olive, Vori, Crete	*	–
H. microlobus	POT017	Potted cultivated olive, Chania, Crete	MG770481	MG770407
H. microlobus	WAL17	Walnut, Evia, Greece	MG770482	MG770408
Helicotylenchus vulgaris	OLI004	Cultivated olive, Petrokefali, Crete	MG770483	–
H. vulgaris	OLI005	Cultivated olive, Petrokefali, Crete	*	–
H. vulgaris	OLI046	Cultivated olive, Gonies, Crete	*	–
H. vulgaris	OLI055	Cultivated olive, Voutes, Crete	*	–
H. vulgaris	OLI071	Cultivated olive, Pentamodi, Crete	*	–
H. vulgaris	OLI129	Cultivated olive, Perama, Crete	*	–
H. vulgaris	OLE026	Wild olive, Agiofarago, Crete	*	–
H. vulgaris	GOBERR	Goji berry, Thessaly, Greece	MG770484	–
Merlinius brevidens[a]	OLI106	Cultivated olive, Episkopi, Crete	MG770485	–
M. brevidens	OLE009	Wild olive, Kefali, Crete	*	–
Pratylenchoides alkani[b]	OLI060	Cultivated olive, Sivas, Crete	MG770486	–
P. alkani	OLI111	Cultivated olive, Amygdali, Crete	*	–
P. alkani	OLE007	Wild olive, Vathy, Crete	*	–
P. alkani	OLE017	Wild olive, Agiofarago, Crete	*	–
P. alkani	OLE018	Wild olive, Agiofarago, Crete	*	–
P. alkani	OLE031	Wild olive, Listis, Crete	*	–
P. alkani	OLE032	Wild olive, Tsoutsouros, Crete	*	–
P. alkani	OLE036	Wild olive, Agiofarago, Crete	*	–

Notes: (*) Sequenced population but not deposited in GenBank database because of its high similarity with previously deposited sequences.
(-) Not obtained.
[a]Found in 29 further cultivated olive samples (OLI007, OLI010, OLI030, OLI031, OLI033, OLI050, OLI051, OLI066, OLI070, OLI071, OLI080, OLI084, OLI086, OLI097, OLI100, OLI102, OLI103, OLI104, OLI105, OLI108, OLI110, OLI111,

OLI114, OLI129, OLI131, OLI134, OLI135, OLI142, and OLI146) and in nine further wild olive samples (OLE011, OLE013, OLE015, OLE021, OLE027, OLE028, OLE029, OLE030, and OLE032).

[b]May be a junior synonym of *P. ritteri*, as supported by phylogenetic analyses by Azizi et al. (2016) and noted by some other researchers (Brzeski, 1998; Ghaderi and Karegar, 2014; Ghaderi et al., 2014).

III compound microscope with Nomarski differential interference contrast at up to ×1,000 magnification. Diagnostic measurements were made using a drawing tube attached to the microscope. Specimens for molecular analysis were preserved in DESS (Yoder et al., 2006). Here, we report five species as identified by morphological and molecular analyses of females: *Bitylenchus hispaniensis* (Handoo et al., 2014); *Helicotylenchus microlobus* (Perry et al., 1959); *Helicotylenchus vulgaris* (Yuen, 1964); *Merlinius brevidens* (Allen, 1955; Siddiqi, 1970); and *Pratylenchoides alkani* (Yüksel, 1977; Table 1, Fig. 1). These plant-parasitic nematode species, except *H. microlobus* and *M. brevidens*, are new records for Greece. The morphology and morphometry of the isolated nematode species (Table 2) agreed with original and previous descriptions of them, except for minor intraspecific differences (Koliopanos and Vovlas, 1977; Castillo and Gómez Barcina, 1988; Ghaderi et al., 2014;

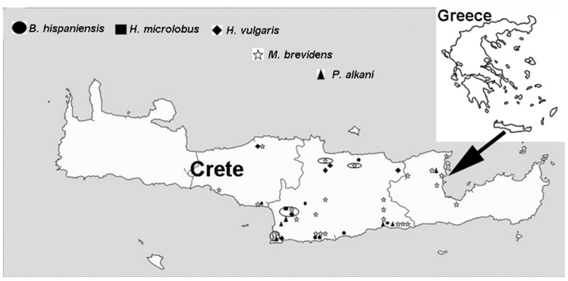

Figure 1: Geographic distribution of plant-parasitic nematodes of the genera *Bitylenchus*, *Helicotylenchus*, *Merlinius* and *Pratylenchoides* in field survey of cultivated and wild olive in Crete, Greece. Numbers of samples for each nematode species are as follows: *Bitylenchus hispaniensis* (seven samples), *Helicotylenchus microlobus* (three samples), *Helicotylenchus vulgaris* (seven samples), *Merlinius brevidens* (40 samples) and *Pratylenchoides alkani* (eight samples). 44 sites are presented, as some samples were derived from spatially close (encircled) areas and may therefore not be independent populations.

Subbotin et al., 2015; Azizi et al., 2016). Nematode DNA was extracted from single individuals and PCR assays were conducted as described by Castillo et al. (2003). Primers and PCR conditions used in this research were specified in Cantalapiedra-Navarrete et al. (2013), and single amplicons of 800 bp and 1,100 bp in length was obtained by sequencing of the D2-D3 expansion segments of 28S and of ITS rRNA genes, respectively. Sequence alignments for D2-D3 from the Cretan *B. hispaniensis*, which showed 99 to 100% similarity (differing in 0 to 1 bp, 0 indels) to the Spanish type population, were deposited in NCBI GenBank (accession numbers MG770479, KJ461547, and KJ461545, respectively). The *H. microlobus* populations from cultivated olive from Crete and walnut from Evia (MG770480–MG770482) showed 99% similarity (differing in 1 to 6 bp, 0 indels) with *H. microlobus* (KM506793–KM506800) and *Helicotylenchus pseudorobustus* populations (HM014264, KU722387) deposited in the NCBI. ITS from populations of *H. microlobus* (MG770406–MG770408) showed 98 to 99% similarity (differing in 13 to 18 bp, 9 indels) to *H. microlobus* populations (KM506864–KM506867) and only 93% similarity (differing in 68 to 69 bp, 26 indels) to *H. pseudorobustus* populations (KM506875 and KM506880), which agree with previous data by Subbotin et al. (2015) and which confirmed the integrative diagnosis of our specimens. Similarly, the *H. vulgaris* populations from wild and cultivated olive, Crete and goji berry, Thessaly (MG770483–MG770484) showed 99 to 100% similarity (differing in 0 to 4 bp, 0 indel) with *H. vulgaris* populations (FJ485650, KU722388, DQ328759–DQ328761) deposited in NCBI. Intraspecific variability for D2-D3 was low for *H. microlobus* and *H. vulgaris* (differing in 5 to 7 bp, 1 indels, and 1 bp, 0 indels, respectively). The D2-D3 sequence of the Cretan population of *M. brevidens* (MG770485) showed 99% similarity (differing in 4 to 9 bp, 0 to 1 indel) to *M. brevidens* populations (KJ585416, KP313844, and KP313847). Finally, the D2-D3 28 S expansion segment from the Cretan populations of *P. alkani* (MG770486) showed 99% similarity to *P. alkani* (JX261953 and JX261962) and *Pratylenchoides ritteri* populations (KP313850 and KU855004) (differing in 2–4 bp, 1 to 2 indels, and 4–9 bp, 1–2 indels, respectively). These data support previous suggestions for synonymizing *P. alkani* and

Table 2. Morphometrics of *Bitylenchus hispaniensis* (Handoo, et al., 2014); *Helicotylenchus microlobus* (Perry et al., 1959); *Helicotylenchus vulgaris* (Yuen, 1964); *Merlinius brevidens* (Allen, 1955; Siddiqi, 1970); and *Pratylenchoides alkani* (Yüksel, 1977) from olive in Crete, Greece[a].

	B. hispaniensis	*H. microlobus*	*H. vulgaris*	*M. brevidens*	*P. alkani*
	Episkopi	Vori	Petrokefali	Episkopi	Sivas
Characters/ ratios[b]	Females	Females	Females	Females	Females
n	3	4	3	3	3
L	722 ± 47 (670–760)	652 ± 31 (609–680)	672 ± 35 (634–702)	564 ± 116 (490–698)	907 ± 69.1 (840–978)
a	32.3 ± 0.6 (31.9–33.0)	25.4 ± 1.6 (24.2–27.7)	30.6 ± 0.4 (30.2–31.0)	26.3 ± 3.8 (22.9–30.3)	35.3 ± 0.8 (34.7–36.2)
B	6.3 ± 0.4 (6.1–6.8)	4.5 ± 0.4 (4.1–4.9)	5.1 ± 0.02 (5.1–5.2)	4.4 ± 0.6 (4.0–5.1)	6.7 ± 0.2 (6.4–6.8)
C	14.9 ± 0.6 (14.4–15.5)	43.7 ± 8.4 (35.8–55.4)	48.1 ± 1.1 (46.8–48.8)	14.3 ± 2.4 (12.9–17.0)	16.7 ± 0.2 (16.5–16.9)
c′	3.0 ± 0.2 (2.7–3.2)	1.1 ± 0.2 (0.9–1.4)	1.1 ± 0.0 (1.1–1.1)	3.0 ± 0.1 (2.9–3.2)	3.1 ± 0.2 (2.8–3.2)
V	55.0 ± 1.0 (54.0–56.0)	61.2 ± 1.6 (59.3–63.2)	64.0 ± 2.0 (62.0–66.0)	54.7 ± 3.1 (52.0–58.0)	52.7 ± 1.5 (51.0–54.0)
Stylet length	17.3 ± 0.3 (17.0–17.5)	26.4 ± 1.2 (25.0–28.0)	24.0 ± 1.0 (23.0–25.0)	14.3 ± 1.5 (13.0–16.0)	23.7 ± 0.6 (23.0–24.0)
Pharynx length	114.0 ± 5.3 (110–120)	145.5 ± 7.0 (139–153)	130.7 ± 6.8 (123–136)	127.7 ± 7.2 (123–136)	135.7 ± 11.2 (123–144)
Max. body diam.	22.3 ± 1.2 (21.0–23.0)	25.7 ± 1.5 (24.0–27.0)	22.0 ± 1.0 (21.0–23.0)	21.3 ± 2.1 (19.0–23.0)	25.7 ± 1.5 (24.0–27.0)
Anal body diam.	16.5 ± 2.2 (14.0–18.0)	14.1 ± 1.4 (12.5–16.0)	13.0 ± 1.0 (12.0–14.0)	13.0 ± 1.0 (12.0–14.0)	17.7 ± 0.6 (17.0–18.0)
Tail	48.3 ± 3.1 (45.0–51.0)	15.3 ± 2.4 (12.0–17.0)	14.0 ± 1.0 (13.0–15.0)	39.3 ± 1.5 (38.0–41.0)	54.3 ± 3.5 (51.0–58.0)

[a]Measurements are in µm and in the form of mean ± SD (range).
[b]Abbreviations are defined in Siddiqi (2000).

P. ritteri, as suggested by some other researchers (Brzeski, 1998; Karegar, 2006; Ghaderi and Karegar, 2014; Ghaderi et al. 2014; Azizi et al., 2016). Nevertheless, additional studies with other ribosomal and mitochondrial markers on topotype populations of both species are needed to clarify whether the two taxa are conspecific or cryptic species.

Bitylenchus hispaniensis was found in two fields of cultivated olives and five of wild olives in Crete with low population density (1–10 nematodes per 500-cm^3 soil). The species is widespread in both cultivated and wild olives in Spain (Handoo et al., 2014; Palomares-Rius et al., 2015), and our report provides the second record of this species for the country. *Helicotylenchus microlobus* and *H. vulgaris* are common species in the Mediterranean area (Subbotin et al., 2015; Palomares-Rius et al., 2015). In Crete, low population densities (2–17 nematodes per 500-cm^3 soil) of both species were detected in cultivated olive (three fields for *H. microlobus* and six for *H. vulgaris*) and the former also in walnut (150 nematodes per 500-cm^3 soil, Evia, Greece) and the latter in one wild olive sample (Crete) and one goji berry sample (50 nematodes per 500-cm^3 soil, Thessaly, Greece). *Helicotylenchus microlobus* was previously found in olive in Greece (Hirschmann et al., 1966), but *H. vulgaris*, despite being a quite common species in Mediterranean area, was not previously reported from Greece. According to our sampling, *Merlinius brevidens* has a wide distribution in both cultivated (30 fields) and wild (10 plants) olives in Crete with low population density (2–12 nematodes per 500-cm^3 soil). It is a cosmopolitan species and has been already reported in olive and pear in Greece (Hirschmann et al., 1966; Koliopanos and Vovlas, 1977) as well as from olive in Cyprus and Spain (Philis and Siddiqi, 1976; Palomares-Rius et al., 2015). *Pratylenchoides alkani* was found in two fields with cultivated olive and from six wild olives in Crete with low population density (1–22 nematodes per 500-cm^3 soil). It was originally described from snap bean in Turkey (Yüksel, 1977), with wheat and almond tree in Iran (Taheri et al., 2013; Ghaderi et al., 2014), and in the only Mediterranean record of it, *P. alkani* was associated with Aleppo pine (*Pinus halepensis* L.) in Spain (Castillo and Gómez Barcina, 1988). Thus, the occurrence of this nematode in Crete is the second report for the Mediterranean area and the first one indicating an association of the nematode with cultivated and wild olives.

Acknowledgments

This research was partially funded by grant KBBE 219262 ArimNET-ERANET FP7 2012 to 2015 Project PESTOLIVE “Contribution of olive history for the management of soilborne parasites in the Mediterranean basin” from the Hellenic Agricultural Organization-DEMETER and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), grant AGR-136 from “Consejería de Economía, Innvovación y Ciencia” from Junta de Andalucía, and Union Europea, Fondo Europeo de Desarrollo regional, “Una manera de hacer Europa.” Maria S. Lazanaki was employed by the ARIMNET-PESTOLIVE project. The authors thank J. Martín Barbarroja and G. León Ropero from IAS-CSIC for the excellent technical assistance, and anonymous reviewers and editor for their helpful suggestions.

References

Allen, M.W. 1955. A review of the genus *Tylenchorhynchus*. *University of California Publications in Zoology* 61: 129–66.

Azizi, K., Eskandari, A., Karegar, A., Ghaderi, R., Van Den Elsen, S., Holterman, M., and Helder, J. 2016. Morphological and molecular data support the monophyletic nature of the genus *Pratylenchoides* Winslow, 1958 (Nematoda: Merliniidae) and reveal its intrageneric structuring. *Nematology* 18: 1165–83.

Brzeski, M.W. 1998. Nematodes of Tylenchina in Poland and temperate Europe. Warsaw, Poland, Muzeum i Instytut Zoologii PAN.

Cantalapiedra-Navarrete, C., Navas-Cortés, J.A., Liébanas, G., Vovlas, N., Subbotin, S.A., Palomares-Rius, J.E., and Castillo, P. 2013. Comparative molecular and morphological characterisations in the nematode genus *Rotylenchus*: *Rotylenchus paravitis* n. sp., an example of cryptic speciation. *Zoologischer Anzeiger* 252: 246–68.

Castillo, P., and Gómez Barcina. 1988. Some species of Tylenchida from natural habitats in southeastern Spain. *Nematologia Mediterranea* 16: 75–86.

Castillo, P., Vovlas, N., Subbotin, S., and Troccoli, A. 2003. A new root-knot nematode, *Meloidogyne baetica* n. Sp. (Nematoda: Heteroderidae), parasitizing wild olive in southern Spain. *Phytopathology* 93: 1093–102.

Cobb, N.A. 1918. Estimating the nema population of soil. Agricultural Technology Circular, Bureau of Plant Industry, United States Department of Agriculture. No 1, p. 48.

De Grisse, A.T. 1969. Redescription ou modifications de quelques techniques utilisées dans l’étude des nématodes phytoparasitaires. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 34: 351–69.

Ghaderi, R., and Karegar, A. 2014. Contribution to a revision of the genus *Pratylenchoides* Winslow, 1958 (Nematoda: Merliniidae), with redescription of *P. erzurumensis* Yüksel, 1977 from Iran. *Zootaxa* 3900: 339–69.

Ghaderi, R., Karegar, A., Niknam, G., and Subbotin, S.A. 2014. Phylogenetic relationships of Telotylenchidae Siddiqi, 1960 and Merliniidae Siddiqi, 1971 (Nematoda: Tylenchida) from Iran, as inferred from the analysis of the D2D3 expansion fragments of 28S rRNA gene sequences. *Nematology* 16: 863–77.

Handoo, Z.A., Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., Liébanas, G., Subbotin, S., and Castillo, P. 2014. Integrative taxonomy of the stunt nematodes of the genera *Bitylenchus* and *Tylenchorhynchus* (Nematoda, Telotylenchidae) with description of two new species and a molecular phylogeny. *Zoological Journal of the Linnean Society* 172: 231–64.

Hirschmann, H., Paschalaki-Kourtzi, N., and Triantaphyllou, A.C. 1966. A survey of plant parasitic nematodes in Greece. *Annales de l’Institut Phytopathologique Benaki* 7: 144–56.

Karegar, A. 2006. Identification of plant-parasitic nematodes associated with sugar beet and their distribution in Hamadan province, Iran. *Iranian Journal of Plant Pathology* 42: 159–78.

Koliopanos, C.N., and Vovlas, N. 1977. Records of some plant parasitic nematodes in Greece with morphometrical descriptions. *Nematologia mediterranea* 5: 207–15.

Palomares-Rius, J.E., Castillo, P., Montes-Borrego, M., Navas-Cortés, J.A., and Landa, B.B. 2015. Soil properties and olive cultivar determine the structure and diversity of plant-parasitic nematode communities infesting olive orchard soils in southern Spain. *PLOS ONE* 10: e0116890, doi: 10.1371/journal.pone.0116890.

Perry, V.G., Darling, H.M., and Thorne, G. 1959. Anatomy, taxonomy and control of certain spiral nematodes attacking blue grass in Wisconsin. *Research Bulletin, University of Wisconsin* 207: 1–24.

Philis, J., and Siddiqi, M.R. 1976. A list of plant parasitic nematodes in Cyprus. *Nematologia mediterranea* 4: 171–4.

Siddiqi, M.R. 1970. On the plant-parasitic nematode genera *Merlinius* gen. n. and *Tylenchorhynchus* Cobb and the classification of the families Dolichodoridae and Belonolaimidae n. rank. *Proceedings of the Helminthological Society of Washington* 37: 68–77.

Siddiqi, M.R. 2000. *Tylenchida Parasites of Plants and Insects*, 2nd ed., Wallingford, K: CABI Publishing, p. 833.

Subbotin, S.A., Vovlas, N., Yeates, G.W., Hallmann, J., Kiewnick, S., Chizhov, V.N., Manzanilla-López, R.H., Inserra, R.N., and Castillo, P. 2015. Morphological and molecular characterisation of *Helicotylenchus pseudorobustus* (Steiner, 1914) Golden, 1956 and related species (Tylenchida: Hoplolaimidae) with a phylogeny of the genus. *Nematology* 17: 27–52.

Taheri, Z.M., Maafi, Z.T., Subbotin, S.A., Pourjam, E., and Eskandari, A. 2013. Molecular and phylogenetic studies on Pratylenchidae from Iran with additional data on *Pratylenchus delattrei*, *Pratylenchoides alkani* and two unknown species of Hirschmanniella and Pratylenchus. *Nematology* 15: 633–51.

Tzortzakakis, E.A., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Nasiou, E., Lazanaki, M.S., Kabourakis, E.M., Palomares-Rius, J.E., and Castillo, P. 2014. Integrative diagnosis and molecular phylogeny of dagger and needle nematodes of olives and grapevines in the island of Crete, Greece, with description of *Xiphinema cretense* n. sp. (Nematoda, Longidoridae). *European Journal of Plant Pathology* 140: 563–90.

Yoder, M., Tandingan de Ley, I., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L., and De Ley, P. 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8: 367–76.

Yuen, P.H. 1964. Four new species of *Helicotylenchus* Steiner (Hoplolaiminae: Tylenchida) and a redescription of *H. canadensis* Waseem, 1961. *Nematologica* 10: 373–87.

Yüksel, H.S. 1977. *Pratylenchoides alkani* sp. n. and *P. erzurumensis* sp. n. (Nematoda: Tylenchoidea) from soil in Turkey. *Proceedings of the Helminthological Society of Washington* 44: 185–8.