Tylenchulus semipenetrans (Nematoda: Tylenchulidae) on pomegranate in Iran

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Received: 4 March 2014 / Accepted: 29 December 2014 / Published online: 23 January 2015
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Abstract *Tylenchulus semipenetrans* was identified morphologically from root samples collected from a pomegranate plant in a garden in the Shahdad region of southeastern Iran. Molecular analysis based on the D2-D3 segment of 28S rDNA confirmed this population as *T. semipenetrans*. Phylogenetic analysis using the Maximum Likelihood (ML) method, places this population close to the same species from the USA, Egypt and Korea. This is the first record of *T. semipenetrans* on pomegranate in Iran and worldwide.

Keywords Iran · Pomegranate · *Tylenchulus semipenetrans* · 28S rDNA

The pomegranate, *Punica granatum*, is native to Iran, the largest producer of this horticultural product in the world. Although pomegranate is one of the largest export goods of Iran, there is little information about nematode parasites of pomegranate in the country. During a diagnostic visit to a pomegranate orchard in the south of Iran, we observed stunting, yellowing, wilting and slow decline in the trees. Citrus nematodes were recovered from soil and root samples.

During April–June 2013, root samples were collected from pomegranate in an orchard in Shahdad, south-eastern

Iran (N. 30°25' 27.2"; E. 057°41' 50.6"). Roots were washed, blotted dry, cut in pieces and processed for nematode extraction by blender followed by centrifugal flotation (Coolen and D'Herde 1972). The roots were stained (Southey 1970) and immature and mature females observed directly on the root surfaces. Nematodes were identified and counted using an Olympus microscope CH-2 and a Peters slide counting chamber. Specimens were fixed with hot 4 % formaldehyde solution and processed to anhydrous glycerine by the method of De Grisse (1969). Measurements were taken directly using an ocular micrometer and/or a curvimeter upon drawing the corresponding organ or structure. Morphometrical characteristics and the De Man ratios (V%, a, b, b', c, and c') were obtained from 5 second stage juveniles (J2), six adult males and 15 adult females. The molecular characterisation was based on Mehdizadeh and Shokoohi (2013). The original partial 28S (D2-D3 expansion) sequence of *Tylenchulus semipenetrans* is deposited in GenBank under accession number KJ577615.

Characters measured were consistent with *T. semipenetrans* (Inserra et al. 1988). Adult females (Figs. 1 and 2; Table 1): body swollen, 0.24–0.30 mm long, conical behind the vulva. Cuticle smooth and without annulation. Lateral field not observed. Cuticle 4–9 µm at mid-body. Lip region continuous with body contour, labial framework weakly sclerotised. Stylet 10–22 µm with rounded knobs. Stylet knob width 2.8 µm; stylet knob length 1.9 µm. DEGO (Dorsal Esophageal Gland Orifice) 3–8 µm from the stylet knob. PVSC (Post-Vulval Section Cavity) 4–8 µm; PVSL (Post-Vulval Section Length) 14–31 µm; PVSW (Post-Vulval Section Width) 9–18 µm. Pharyngeal procarpus cylindrical, about 2.4 times metacarpus length. Metacarpus swollen, with valve about 11 µm long. Isthmus 12 µm long. Basal bulb ovoid, 15–24 µm long and 14–25 µm in width. Cardia conoid, surrounded by

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Fig. 1 *Tylenchulus semipenetrans* Cobb, 1913. **a** Entire male. **b** Entire female [Anus, Vulva and Secretory Excretory pore (SE pore) indicated by arrow]. **c** Anterior end of Juvenile. **d** Juvenile (J2). **e, f, g, i** posterior end of J2 [in **e** and **f**: arrow indicates genital primordium]. **h** Male posterior end

intestinal tissue. Nerve ring at isthmus level. Excretory pore at 67–78 % of body length at 10–23 μm to vulva. Deirid not visible. Intestine without distinct specialisations. Reproductive system monodelphic prodelphic with reflexed ovaries. Ovary long with oocytes arranged in one to two distinct rows in germinal zone. Oviducts short, not well distinguished from the adjacent uterus. Vagina with narrow lumen. Vulval opening postequatorial (around 80 % from

anterior end). Vulva lips weakly developed, not protruding. Anus slightly visible, but rectum not visible. Tail conical without mucro. Phasmid not visible. Male (Table 2): body “J” shaped after fixation. Cuticle smooth, lateral field not observed. Body width 10–13 μm at mid-body. Lip region continuous with body contour, labial framework weakly sclerotised. Stylet 8–11 μm with rounded knobs. DEGO not visible. Pharynx tylenchoid, having pharyngeal corpus

Fig. 2 *Tylenchulus semipenetrans* Cobb, 1913. On the root surface of pomegranate. **a, b, d** immature female. **c** mature female

Table 1 Measurements of *Tylenchulus semipenetrans* isolated from pomegranate

Locality	Shahdad
Province	Kerman
Habitat	Pomegranate
n	15♀♀
L	276.9±28.2 (245–300)
a	5.6±2.2 (3.6–8)
Stylet	14.7±6.1 (9.8–21.5)
DEGO	5±2.4 (3.4–7.8)
Anterior end to median bulb	28.1±3.4 (24.5–31.3)
Median bulb length	19.1±6.2 (14.7–23.5)
Median bulb width	14.9±5.2 (11.2–18.6)
Pharynx length	135.3
Basal bulb length	19.4±3.1 (14.7–23)
Basal bulb width	18.7±3.8 (13.7–24.5)
Anterior end to excretory pore	200.8±35.5 (163.4–234.1)
Excretory pore from anterior end as % of body length	72±5.7 (66.5–78)
Vulva-excretory pore distance	17.4±3.6 (10.2–22.5)
Post-vulva section width (PVSW)	12.9±3.1 (9–18)
Post-vulva section length (PVSL)	20.9±5.9 (13.7–31.3)
Post-vulval section cavity (PVSC)	5.4±0.3 (5.1–5.7)
Swollen posterior body length	175.2±21.2 (153.6–209.7)
Swollen posterior body as % of total body length	58.2±4.1 (55.2–62.9)
Body width at vulva	45.3±8.6 (35.2–64.6)
Body width at mid-body	81±14.2 (54.9–100)
Cuticle thickness at mid-body	5.2±0.8 (3.9–6.8)

All measurements are in µm and in the form: mean ± s.d. (range)

0.6–0.8 times longer than postcorpus (isthmus + basal bulb). Pharyngeal procorpus cylindrical, 2.8–3.3 times metacarpus length. Metacarpus swollen, with valve 9.3–10 μm long. Isthmus 25–30 μm long. Basal bulb ovoid, 20–26 μm long and 13–14 μm width. Cardia conoid, surrounded by intestinal tissue. Nerve ring at 68–71 % of neck length, at isthmus level. Excretory pore at 51–53 % of body length, 12 μm to vulva. Deirid not visible. Intestine without distinct specialisations. Anus and rectum visible. Tail first conical, tapering, rounded terminus and curved ventrally. Phasmid not visible. Reproductive system monorchic, testis straight. Spicules free, curved ventrally: manubrium rounded, calamus without hump, and lamina ventrally curved. Gubernaculum simple, 4–5 μm long or about 25–27 % of the spicule length. Second stage juveniles (Table 2): body almost straight after fixation. Cuticle not annulated. Lateral field not observed. Cuticle 0.6–0.8 μm at mid-body. Lip region continuous with body contour, labial framework weakly sclerotised. Stylet 12–14 μm with rounded knobs. Stylet knob width 2.4–2.6 μm , stylet knob length 1.5–1.8 μm . DEGO not visible. Pharynx tylenchoid, having pharyngeal corpus 0.4–0.7 times longer than postcorpus (isthmus + basal bulb). Pharyngeal procorpus cylindrical, 1–2.1 times metacarpus length.

Metacarpus swollen, with valve, 10.6–11 μm long. Isthmus 28–30 μm long. Basal bulb ovoid, 15–18 μm long and 10–13 μm width. Cardia conoid, surrounded by intestinal tissue. Nerve ring at 65–84 % of neck length, at isthmus level. Excretory pore at 57–58 % of body length, 20–49 μm to vulva. Deirid not visible. Intestine without distinct specialisations. Genital primordium with three cells, 68–71 % from the anterior end. Anus and rectum not visible. Hyaline portion of posterior body having few small fat globules (<2 μm width). Tail with rounded terminus. The De Man ratios obtained were: Female: $a=5.6\pm 2.2$; male: $a=30.6\pm 3.3$, $b=4.7\pm 0.7$, $c=8.2\pm 0.3$; second stage juvenile: $a=23.9\pm 1.1$, $b=3.1\pm 0.1$, $c=6.9\pm 0.7$.

The sequence lengths flanked by the two primers D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and the reverse primer D3B (5'-TCGGAAGGAACCAGCTACTA-3') (according to Subbotin et al. 2006) of the 28S region of *T. semipenetrans* isolate are 747 base pairs long. The Blast test revealed that this population is 9, 11 and 13 base pairs different to the closest populations from Korea (FJ969712, 99 % identity; FJ969715, 98 % identity; FJ969710, 98 % identity, respectively). In comparison, the nearest population studied from Egypt showed a difference of four base pairs

Table 2 Measurements of *Tylenchulus semipenetrans* isolated from pomegranate

Locality	Shahdad	
Province	Kerman	
Habitat	Pomegranate	
N	5 J2	6 ♂♂
Body length	304.5 $\pm$ 13.4 (282–318)	325 $\pm$ 18.5 (300–345)
a	23.9 $\pm$ 1.1 (22.5–25.2)	30.6 $\pm$ 3.3 (24.4–34.4)
b	3.1 $\pm$ 0.1 (2.9–3.3)	4.7 $\pm$ 0.7 (4.1–6)
c	6.9 $\pm$ 0.7 (5.8–7.7)	8.2 $\pm$ 0.3 (7.7–8.8)
Stylet	13.3 $\pm$ 0.6 (12.2–13.7)	9.4 $\pm$ 1 (7.8–10.7)
Anterior end to median bulb	42.9 $\pm$ 7 (34.1–51.2)	37.6 $\pm$ 1.7 (35.2–40.2)
Pharynx length	96.7 $\pm$ 7.1 (86.5–103.6)	69.8 $\pm$ 9.4 (55.8–82.3)
Anterior end to hemizonid	71.3 $\pm$ 5.1 (65.8–78)	67.1 $\pm$ 1.7 (65.2–68.6)
Anterior end to excretory pore	176.7 $\pm$ 9.4 (164.2–180.9)	167.6 $\pm$ 5.8 (160.1–175.6)
Anterior end to nerve ring	68.9 $\pm$ 12.5 (56.1–87.2)	50.4 $\pm$ 5.4 (40.2–55.8)
Excretory pore to genital primordium	30.7 $\pm$ 13.7 (19.5–48.7)	–
Anterior end to genital primordium	210.9 $\pm$ 7.8 (200–217)	–
Genital primordium to posterior end	120.7 $\pm$ 4.6 (117–126.8)	–
Max. body width	12.7 $\pm$ 0.7 (12.2–13.7)	10.7 $\pm$ 1.4 (9.7–13.4)
Excretory pore from anterior end as % of body length	59.7 $\pm$ 4.1 (52.8–63.1)	20.61 $\pm$ 2.3 (17.3–23.3)
Genital primordium (%)	68.9 $\pm$ 1.2 (67.8–70.9)	–
Spicules	–	17.8 $\pm$ 1.9 (14.7–19.6)
Gubernaculum	–	4.7 $\pm$ 0.6 (3.9–5.4)
Tail	–	38.9 $\pm$ 2.4 (35.2–42.1)

All measurements are in μm and in the form: mean $\pm$ s.d. (range)

Fig. 3 The Maximum Likelihood tree inferred from known and newly sequenced *Tylenchulus* spp. from Iran based on 28S rDNA region

(AY780972; 99 % identity). Compared with the populations from the USA (JN112249; JN112250; JN112252) 9 (99 % identity), 14 (98 % identity) and 17 (97 % identity) base pair, respectively, was shown. A ten base pair difference was shown when it was compared to a South African population (JN112251; 98 % identity).

Our phylogenetic analysis using 28S rDNA, placed the Iranian *T. semipenetrans* population in a clade together with other *T. semipenetrans* populations (Fig. 3). Molecularly characterised species of *T. semipenetrans* form a monophyletic group. This result is in agreeance with previous results obtained (Van Megen et al. 2009 inferred from 18S rRNA). Three permanent microscope slides of *T. semipenetrans*

(slide accession number: ES-TS1 to ES-TS3) were deposited in the Nematology laboratory collection of Shahid Bahonar University of Kerman, Iran. According to the literature, this is the first record of citrus nematode on pomegranate worldwide. Pomegranate seems to be affected by *T. semipenetrans*.

References

- Coolen WA, D'Herde CJ (1972) A method for the quantitative extraction of nematodes from plant tissue. State Agricultural Entomology Research Station, Ghent, Belgium

- De Grisse A (1969) Redescription ou modifications de quelques techniques utilisés dans l'étude des nématodes phytoparasitaires. Meded Rijksfaculteit Landbouwetenschappen Gent 34:351–369
- Inserra RN, Vovlas N, O'Bannon JH, Esser RP (1988) *Tylenchulus graminis* n. sp. and *T. palustris* n. sp. (Tylenchulidae) from native flora of Florida, with notes on *T. semipenetrans* and *T. furcus*. J Nematol 20:266–287
- Mehdizadeh S, Shokoohi E (2013) The genera *Nothacrobeles* Allen & Noffsinger, 1971 and *Zeldia* Thorne, 1937 (Nematoda: Rhabditida: Cephalobidae) from southern Iran, with description of *N. abolafiai* sp. n. Zootaxa 3637:325–340
- Southey JF (1970) Laboratory methods for work with plant and soil nematodes. Ministry of agriculture, fisheries and food, technical bulletin 2. Her Majesty's Stationery Office, London
- Subbotin SA, Sturhan D, Chizhov VN, Vovlas N, Baldwin JG (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology 8:455–474
- Van Megen H, Van Den Elsen S, Holterman M, Karssen G, Mooyman P, Bongers T, Holovachov O, Bakker J, Helder J (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11:927–950