Original article

First report with morphometrics and molecular characterization of phtyonematodes associating mango trees in the tropics of Saudi Arabia

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A R T I C L E   I N F O

A B S T R A C T

A sum of 218 composite rhizosphere soil samples were collected from around the feeder roots of mango, Mangifera indica growing in Jazan region, the tropical south west corner of Saudi Arabia. Samples were rendered for nematodes extraction using the centrifugal floatation method, and the stylet-bearing nematodes were morphologically identified according to the standardized taxonomical keys. A list of 14 stylet-bearing nematode genera and/or species were found to be associating the roots of mango in this study. Species identification of the most important parasitic nematodes, in this list, was carried-out, based on morphometrics and morphological features. Identification of these species was then molecularly confirmed using the D3 expansion region of 28S ribosomal RNA (rRNA) gene. These nematodes included; Tylenchorhynchus mediterraneus, Hoplolaimus seinhorsti, Hemicriconemoides strictathecatus, Longidorus latocephalus and Xiphinema elongatum. Some new local nematode-host records in Saudi Arabia were recorded including; Aphelenchus sp., H. strictathecatus, L. latocephalus, and T. mediterraneus. Some new world nematode-host records were also reported including; L. latocephalus and T. mediterraneus.

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1. Introduction

Jazan region is a very distinguished agricultural area located in the tropical south west corner of Saudi Arabia. This region has been noted for its high-quality production of mango, mangifera indica L. (Family: Anacardiaceae), many years ago. Unlike, many species and genera of plant-parasitic nematodes were reported associating the roots of mango trees in many countries all over the world (Castillo and Gomez-Barcina, 1993; Quijada et al., 2002; Van den Berg and Mekete, 2010), and in Saudi Arabia as well (Al-Hazmi et al., 1995; Al-Yahya, 1999, 2005; Mokbel, 2014). However, these nematode records in Saudi Arabia were mostly identified to genera, and no further studies were carried-out to identify them to the species level, either on morphometrics or molecular bases.

In fact, species characterization of plant nematodes is not so easy as it could be assumed due to their microscopic sizes, mor-phological analogy, overlapping morphometric characters and the minority of the definite taxonomic criteria (Handoo and Palomares-Rius, 2014). The use of modern molecular tools and the phylogenetic analysis offer a good solution for the misleading problems of nematode identification by the classical methods. The large subunit of the nuclear ribosomal RNA (28S rRNA), also known as LSU-rRNA has conserved domains and certain expansion segments such as D2-D3 expansion region which is very useful for the molecular characterization of many nematode species (Bae et al., 2010; Susulovska et al., 2017). However, D2 segment is more variable, compared to D3 segment (Douda et al., 2013). Thus, various studies have adopted the D3 segment of the 28S rRNA for identifying nematodes (Bae et al., 2010, Carta et al., 2001). Accordingly, the integration of morphometrics, morphological and molecular tools is being the most effective in the nematode characterization (De Ley et al., 2005; Handoo and Palomares-Rius, 2014). Many workers have successfully identified many different nematode species following the classical methods and confirmed their identification using 28S rRNA sequence data (Handoo and Palomares-Rius, 2014; Carta et al., 2016).

The purpose of this study was to identify and analyze the communities of the stylet-bearing nematodes associating mango trees in the tropical south west corner of Saudi Arabia, and to clearly

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characterize the most important nematode species associating these trees on morphometrics, morphological and molecular bases.

2. Materials and methods

2.1. Sampling and nematode extraction

A survey of stylet-bearing nematodes associated with mango, *Mangifera indica* L., was carried-out in Jazan region, the tropical south west corner of Saudi Arabia. A sum of 218 composite rhizosphere soil samples were collected randomly from around the roots of mango trees. The composite samples (5–7 cores/tree) were collected from 3 to 4 sites surrounding the feeder roots of each tree, then sealed in transparent plastic bags, labeled and air-transported to our university campus. Once samples reached the lab., they were processed for nematode extraction immediately, or kept in a refrigerator (<10 °C) until they were handled within a week.

Whenever handled, samples were thoroughly mixed, sieved to remove stones and debris. Representative sub-samples (200 cm^3 soil) were taken from the whole samples to be subjected for nematode extraction using a modified centrifugal floatation procedure, with nested 20, 150 and 400 mesh sieves (Hooper, 1986). Stylet-bearing nematodes were preliminary identified and counted using a specific counting slide under a compound microscope. Nematode communities were analyzed using the absolute frequency of occurrence (FO%), mean population density (PD) and the prominence value (PV) (Norton, 1978).

2.2. Morphological identification

Nematode individuals were collected in Syracuse dishes containing water, then the dishes were kept in an electrical oven (50–70C) for a few minutes to kill nematodes. Nematodes were then mounted in a fresh formalin solution (2%) on microscopic glass slides for examination (Shurtleff and Averre III, 2000). Stylet-bearing nematodes were morphologically identified either to genera and/or species according to the standardized taxonomical keys (Goodey, 1963; Mai and Lyon, 1975; Siddiqi, 1977; Handoo and Palomares-Rius, 2014; Kumari, 2014).

2.3. Morphometric studies

Adult stages of the most important parasitic-nematodes were heat-killed and fixed using the following fixatives (De Grisse, 1969): fixative # (1) 4% formalin: glycerin (99: 1), fixative # (2) 96% ethyl alcohol: glycerin (95:5,), fixative # (3) 96% ethyl alcohol:glycerin (50:50).

A few drops of the fixative # 1 were heated up to 50–60 °C and added to labeled staining blocks containing the live nematode specimens. The staining blocks were placed uncovered in a glass desiccator containing 1/10th (v/v) 96% ethyl alcohol. After 24 h, the staining blocks were pulled out from the desiccator, and the excess liquid volume inside was sucked out using a fine pipette, then the volume was topped up again with a few drops of the fixative # 2. Staining blocks were covered partially to allow slow evaporation of alcohol, then placed inside the incubator. A few drops of fixative # 2 were added every 2–3 h (3–4 times). Finally, a few drops of the fixative # 3 were added. After 48 h, the staining blocks were transferred from the incubator and tightly covered. Whenever needed, a few nematode specimens were mounted in a glycerin droplet on microscopic glass slides and measurements of the different important characters of the examined nematodes were recorded.

2.4. Molecular characterization

Adult females of the most important parasitic nematodes were hand-picked, put singly in PCR tubes and crushed using the tip of <10 µl pipette tip. Extraction of DNA was accomplished in 10 µl nematode lysis buffer [10X PCR buffer (1 µl), double distilled water (8 µl), proteinase K (0.06 µl)] (Subbotin et al., 2000). PCR reaction and program are shown in (Tables 1 and 2). The reaction was run-out for enzyme inactivation, firstly @ 60 ℃ for 1 h, then @ 95 °C for 15 min. Finally, the analyst was subjected to centrifugation @ 12,000 rpm for 1 min.

The D3 expansion regions of 28S ribosomal RNA (rRNA) gene of nematode samples was amplified using the forward D3A (5′_ GACCCGTCTTGAAACACGGA -3′) and the reverse D3B (5′_ TCGGAAGGAACCAGCTACTA -3′) primers (Courtright et al., 2000; Douda et al., 2013). PCR-DNA products were visualized by the aid of ethidium bromide stain and the UV gel documentation system, then were cut-off from the gels and purified using QIAquick Gel Extraction Kit. The purified PCR-DNA products were sent to Macrogen company, south Korea (info@macrogen.com) for sequencing using D3A and D3B primers. BLASTN was operated to analyze the nematode sequences, and those sequences with the highest similarity e-values along with the nearst nematode species that had GenBank sequences were aligned in ClustalW as described by Thompson et al. (1994).

Phylogenetic trees were reconstructed by the maximum likelihood (ML) method using MEGA6 software (Tamura et al., 2013). The bootstrap consensus tree inferred from 100 replicates was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) were shown next to the branches (Felsenstein, 1985). The analysis involved 9, 9, 11, 7 and 7 nucleotide sequences for *Tylenchorhynchus mediterraneus*, *Hoplolaimus seinhorsti*, *Hemicriconemoides strictathecatus*, *Longidorus latocephalus* and *Xiphinema elongatum*, respectively. There were a total of 513, 163, 164, 222 and 289 clean positions (without gaps and missing data) in the final dataset for *T. mediterraneus*, *H. seinhorsti*, *H. strictathecatus*, *L. latocephalus* and *X. elongatum*, respectively.

3. Results and discussion

3.1. Community analysis of stylet-bearing nematodes associated with mango, M. indica in Jazan region, south west corner of Saudi Arabia

Fourteen species and/or genera of stylet-bearing nematodes were found to be associating mango, *M. indica*, trees in Jazan region, south west corner of Saudi Arabia (Table 3). These nematodes, according to their frequency of occurrence (FO%), were: *Hoplolaimus seinhorsti* (33.49%), *Tylenchorhynchus mediterraneus* (29.36%), *Aphelenchus* sp. (16.51%), *Trichodorus* sp. (14.68%), *Xiphinema elongatum* (11.01%), *Hemicriconemoides strictathecatus* (10.55%), *Pratylenchus* sp. (8.26%), *Ditylenchus* sp. (2.75%), *Helicotylenchus* sp. and *Longidorus latocephalus* (2.29%, both), *Tylenchus* sp. (1.38%), *Aphelenchoides* sp., *Rotylenchus* sp. and *Tetylenchus* sp. (0.46% each). The highest nematode population density (PD) was

Table 1
PCR reaction.

	1×	33×
2× PCR Master Mix - Promega	12.5	412.5
10 picomol D3A primer	1	33
10 picomol D3B primer	1	33
DNA	4	–
H$_2$O	6.5	214.5

Table 2
PCR program.

Name of primer	Amplification conditions	Cycles
D3A/D3B	94 °C for 4 min	1 cycle
	94 °C for 30 s	35 cycles
	55 °C for 30 s	
	72 °C for 1.30 s	
	72 °C for 10 min	1 cycle

Table 3
Frequency of occurrence (FO%), mean population density (PD)/200 cm³ soil and prominence value (PV) of stylet-bearing nematodes associated with mango, *Mangifera indica* L., in Jazan region, south west Saudi Arabia.

Nematode genus/species	FO%[1]	PD[2]	PV[3]
Aphelenchoides sp.	0.46	60.00	4.07
Aphelenchus sp.	16.51	61.64	25.05
Ditylenchus sp.	2.75	28.00	4.64
Helicotylenchus sp.	2.29	223.40	33.81
Hemicriconemoides strictathecatus	10.55	1041.38	338.25
Hoplolaimus seinhorsti	33.49	85.86	49.69
Longidorus latocephalus	2.29	88.40	13.38
Pratylenchus sp.	8.26	145.67	41.87
Rotylenchus sp.	0.46	16.00	1.09
Tetylenchus sp.	0.46	56.00	3.80
Trichodorus sp.	14.68	82.22	31.50
Tylenchorhynchus mediterraneus	29.36	99.91	54.14
Tylenchus sp.	1.38	36.00	4.23
Xiphinema elongatum	11.01	50.79	16.85

No. collected samples = 218.
[1] Frequency of occurrence (FO%) = (number of samples containing a genus or species ÷ number of collected samples) × 100.
[2] Mean population density (PD) = total number of individuals in a genus or species ÷ number of samples containing this genus/species.
[3] Prominence value (PV) = Density $\sqrt{frequency}$, based on absolute frequency and absolute density.

recorded for *H. strictathecatus* (1041.38 individuals/200 cm³ soil), whereas *Rotylenchus* sp. had the least PD (16 individuals/200 cm³ soil). According to the prominence value (PV), *H. strictathecatus* was the most prominent (PV = 338.25), followed by *T. mediterraneus* (PV = 54.14). However, *Rotylenchus* sp. and *Tetylenchus* sp. were the least prominent (PV = 1.09 and 3.80, respectively) (Table 3).

The fourteen nematode species and/or genera which have been found to be associated with mango in Jazan region, in this study, have also been reported to be associated with mango in different countries worldwide except for *T. mediterraneus* and *L. latocephalus* (McSorley et al., 1981; McSorley, 1992; Castillo and Gomez-Barcina, 1993; Quijada et al., 2002; Anwar et al., 2012). In Saudi Arabia, all of these species and/or genera have previously listed among the nematodes associating mango trees in Jazan region (Al-Yahya, 2005; Mokbel, 2014) except for *Aphelenchus* sp., *H. strictathecatus*, *L. latocephalus* and *T. mediterraneus*. However, *Paratylenchus* sp. and *Subanguina* sp. were previously reported on mango trees in Jazan region (Al-Yahya, 2005) but not in the present study. Consequently, *Aphelenchus* sp., *H. strictathecatus*, *L. latocephalus*, and *T. mediterraneus* are recorded in the rhizosphere of mango trees for the first time in Saudi Arabia in this study. Moreover, *L. latocephalus* and *T. meditteraneus* are considered to be a new world record on mango herein.

3.2. Morphometric studies of the most important parasitic nematode species associating mango in Jazan region, south west corner of Saudi Arabia

3.2.1. Tylenchorhynchus mediterraneus
(Table 4) shows the morphometrics of the adult stages (females and males) of *T. mediterraneus*. Lip region is hemispherical in

Table 4
Morphometrics (μm) of *Tylenchorhynchus mediterraneus* adult stages collected from the rhizosphere of guava trees growing in Abu Arish governorate.

Character	n	Mean ± SD	Range
Body length	27	708.67 ± 50.33	552.76–754.73
Stylet length	27	18.10 ± 1.02	15.60–19.50
Greatest body width	27	20.17 ± 0.91	18.20–20.80
Pharynx length	27	107.23 ± 9.16	91.00–137.80
Anterior end to excretory pore	27	104.67 ± 6.12	93.60–117.00
Anal body width	27	13.91 ± 1.24	11.70–15.60
Spicule length	5	22.36 ± 1.09	20.80–23.40
Gubernaculum length	5	8.86 ± 2.37	7.80–9.10
a	27	35.21 ± 2.30	28.35–37.96

shape, and is also offset by a constriction. The stylet is moderately strong (18.1 μm), with a laterally to posteriorly directed rounded basal knobs. Female has two ovaries, and a cylindrical tail. These characters are greatly similar to that population of *T. mediterraneus* which was firstly reported by Handoo and Palomares-Rius (2014) except, that the body length of the Saudi populations of *T. mediterraneus* is slightly shorter.

3.2.2. Hoplolaimus seinhorsti
Morphometrics of *H. seinhorsti* females are listed in (Table 5). Body stout, about 1.4 mm long, and slightly ventrally arcuate when relaxed. Head has a strong distinguished cephalic framework sclerotization. Stylet strong, 42.52 μm long, with tulip-shaped basal knobs. Tail rounded and the reproductive system having two amphidelphic outstretched ovaries. Vulva opens near the median of the body (V = 54.1%). This Saudi population of *Hoplolaimus* sp. was identified as *H. seinhorsti* according to their morphometrics and morphological features which are greatly supported by those of *H. seinhorsti* population described by Handoo and Golden (1992).

3.2.3. Hemicriconemoides strictathecatus
(Table 6) shows the morphometrics of *H. strictathecatus* adult females. Body is curved ventrally, 613.95 μm long. Lip region set off. Stylet knobs anchor shaped and anteriorly directed. The female has a single ovary, and the vulva slit at about 90.46% of the anterior end of the body. Tail conoid, with a pointed terminus. The morpho-

Table 5
Morphometrics (μm) of *Hoploilaimus seinhorsti* adult females collected from the rhizosphere of fig trees growing in Abu Arish governorate.

Character	n	Mean ± SD	Range
Body length	17	1400.64 ± 126.13	1162.16–1648.65
Stylet length	17	42.52 ± 1.88	37.21–47.84
Conus length	17	21.48 ± 0.95	18.81–24.17
Base of stylet to DGO	17	5.12 ± 0.32	3.90–5.20
Lip region height	17	10.40	
Lip region width	17	15.83 ± 0.69	15.6–18.20
Excretory pore	17	120.06 ± 10.33	101.40–132.60
Pharynx length	17	208.61 ± 8.79	182.00–221.00
Head tip to pharynx gland end	17	216.30 ± 29.28	170.08–265.75
Head tip to median bulb valve	17	108.36 ± 6.52	91.00–117.00
Head tip to oesophageal glands	17	195.92 ± 19.43	161.20–218.40
Head tip to metacorpus	17	111.49 ± 5.11	104.00–119.60
Head tip to vulva	17	755.04 ± 56.28	659.06–882.29
Body width	17	44.71 ± 3.29	42.52–53.15
Anal body width	17	28.37 ± 3.13	26.00–33.80
Tail length	17	29.67 ± 2.44	26.00–33.80
a	17	31.33 ± 1.73	27.33–33.05
b	17	12.94 ± 0.98	10.63–14.10
b′	17	7.21 ± 0.95	6.31–9.56
C	17	47.34 ± 3.95	39.98–54.05
c′	17	1.06 ± 0.13	0.85–1.33
V	17	54.10 ± 3.95	48.99–62.78
O	17	12.07 ± 0.93	9.17–13.98
MB%	17	52.26 ± 6.33	48.99–62.78

Table 6
Morphometrics (μm) of *Hemicriconemoides strictathecatus* adult females collected from the rhizosphere of mango trees growing in Abu Arish governorate.

Character	n	Mean ± SD	Range
Body length	20	613.95 ± 20.34	585.00–638.00
Stylet length	20	79.71 ± 2.41	74.50–85.00
Basal knobs height	20	4.49 ± 0.67	3.90–5.20
Basal knobs width	20	7.61 ± 0.48	6.50–7.80
Base of stylet to DGO	20	4.55 ± 0.67	3.90–5.20
Excretory pore	20	142.0 ± 12.57	117.00–159.00
Body width	20	40.17 ± 4.88	31.20–46.80
Distance from anterior end to vulva (L-VL)	20	555.36 ± 18.71	523.00–577.80
Distance from vulva to tail (VL)	20	58.57 ± 6.30	44.20–67.60
Distance from anterior end to oesophago-intestinal valve	20	146.55 ± 9.83	130.00–159.00
Tail length	20	39.52 ± 4.96	36.40–52.00
Total annules (R)	20	132.05 ± 5.28	124.00–144.00
Annules between anterior to excretory pore (Rex)	20	32.60 ± 2.48	27.00–37.00
Annules between posterior to valve pore (Rv)	20	12.85 ± 1.31	11.00–15.00
Annules of tail (Ran)	20	9.80 ± 1.11	8.00–12.00
Ovary length	20	142.61 ± 14.20	114.40–169.00
Anal body width	20	24.44 ± 2.29	20.80–28.60
a	20	15.47 ± 1.70	13.40–19.07
b	20	4.21 ± 0.32	3.74–4.78
c	20	15.76 ± 1.99	12.71–19.07
c′	20	1.62 ± 0.23	1.30–2.00
V	20	90.46 ± 0.94	88.98–92.89
O	20	5.65 ± 0.83	4.76–6.52

metrics of the Saudi population of *H. strictathecatus* agree to a large extent with those of a population from Florida, USA (Van den Berg et al., 2015) and another world populations as well (Geraert, 2010).

3.2.4. Longidorus latocephalus

Morphometrics of *L. latocephalus* females are shown in (Table 7). Body long (6.6 mm in average) and thin. When relaxed, it forms an open C-shape. Head region rounded. Odontostylet 127.66 μm long.

Table 7
Morphometrics (μm) of *Longidorus latocephalus* adult females collected from the rhizosphere of guava trees growing in Abu Arish governorate.

Character	n	Mean ± SD	Range
Body length	10	6605.40 ± 665.11	5405.4–7621.61
Lip region width	10	18.85 ± 1.53	16.90–20.80
Odontostlet length	10	127.66 ± 17.95	101.40–150.80
Odontophore length	10	70.72 ± 7.73	54.60–80.60
Total stylet length	10	195.52 ± 19.14	156.00–215.00
Oral aperture to guding ring	10	36.27 ± 2.70	32.50–39.00
Oesophagous length	10	469.56 ± 21.66	449.80–507.00
Pharyngeal bulbus length	10	135.72 ± 29.33	104.00–210.60
Pharyngeal bulbus width	10	27.95 ± 7.07	23.40–46.80
Anterior end to end of oesophagous	10	488.18 ± 60.68	468.00–522.60
Anterior end to vulva	10	2898.80 ± 168.00	2604.35–3135.85
Anterior ovary length	10	73.84 ± 17.04	44.20–91.00
Posterior ovary length	10	79.30 ± 17.26	54.60–104.00
Tail length	10	38.74 ± 1.48	36.40–41.60
Mid body width	10	53.17 ± 7.31	45.50–62.40
Anal width	10	37.44 ± 5.51	28.60–44.20
Hyaline tail length	10	12.22 ± 2.14	7.80–15.60
Hyaline tail width	10	20.93 ± 2.84	15.60–23.40
a	10	127.05 ± 25.33	86.63–162.855
b	10	13.12 ± 1.08	9.82–15.43
c	10	170.58 ± 16.79	138.60–195.43
c′	10	1.06 ± 0.18	0.82–1.36
V	10	44.12 ± 2.94	38.35–48.18

Vulva located near the mid of the body (44.12%), and the female has two ovaries. Tail hemispherical or bluntly conoid. The morphology of Saudi *L. latocephalus* population resembles to a great extent that of a Bulgharian population (Choleva et al., 1991) with only minor differences especially that the females of the Saudi population were somewhat longer (5405.4–7621.61 μm).

3.2.5. Xiphinema elongatum

Morphometrics of *X. elongatum* females are shown in (Table 8). Female has a ″J″ shaped cylindrical body, Odontostyle 98.99 ± 13.21 μm. Odontophore 48.75 ± 7.61 μm, with prominent basal flanges. Female has two amphidelphic equal ovaries, and vulva located at 884.95 ± 99.63 μm from the head tip. Tail elongated with a rounded end. The morphometrics and morphological features of the Saudi *X. elongatum* population fits perfectly with that of *X. elongatum* from Ethiopia (Getaneh et al., 2015). They also fit well with those of *X. elongatum* populations reported from different countries (Heyns and Coomans, 1991; Luc and Coomans, 1992; Chen et al., 2004).

3.3. Molecular characterization of the most important nematode species associating mango, M. indica in Jazan region, south west corner of Saudi Arabia

(Fig. 1) shows the 345 bp PCR products that were amplified using D3A/D3B primer, and were taken from *T. mediterraneus*, *H. seinhorsti*, *H. strictathecatus*, *L. latocephalus* and *X. elongatum*. All the amplified products were obtained from single nematode samples.

3.3.1. Tylenchorhynchus mediterraneus

The expansion region of 28S rRNA sequence of *T. mediterraneus* (Jazan isolate) and other *T. mediterraneus* sequences deposited in the GenBank were used for phylogenetic analysis. Outgroup taxa included two *Pratylenchus bolivianus* sequences from GenBank. The maximum likelihood (ML) method (Fig. 2) revealed that all the used isolates of *T. mediterraneus* clustered together in one clade, whereas the *P. Bolivianus* isolates (out group) located in another separate clade. Within the clade of *T. mediterraneus* isolates, the six GenBank isolates were clustered in one subclade, while Jazan isolate of *T. mediterraneus* was clustered separately in another subclade. This indicate that the Jazan population of *Tylenchorhynchus* strongly belongs to *T. mediterraneus* (Handoo and Palomares-Rius, 2014). However, this Saudi population of *T. mediterraneus* might has some minor genetic differences, compared to that described by Handoo and Palomares-Rius (2014)

Table 8
Morphometrics (μm) of *Xiphinema elongatum* adult females collected from the rhizosphere of fig trees growing in Abu Arish governorate.

Character	n	Mean ± SD	Range
a	12	68.44 ± 3.02	63.56–72.63
b	12	7.25 ± 0.43	6.75–7.95
c	12	37.52 ± 1.93	35.34–39.98
c′	12	2.55 ± 0.21	2.16–2.78
Body length (L)	12	2113.03 ± 344.18	1837.84–2473.00
Odontostyle	12	98.99 ± 13.21	78.00–122.20
Odontophore	12	48.75 ± 7.61	39.00–65.00
Onchiostyle	12	147.77 ± 16.70	117.00–174.20
Head to vulva	12	884.95 ± 99.63	744.1–1063.00
Tail length	12	53.08 ± 2.06	49.40–57.20
Midbody width	12	42.52 ± 0.00	42.52–42.52
Lips width	12	10.62 ± 0.75	10.40–13.00
Width at anus	12	25.53 ± 2.80	20.80–31.20
Pharynx length	12	352.15 ± 56.69	265.75–452.20
Oral to guide ring	12	73.23 ± 12.87	54.60–104.00
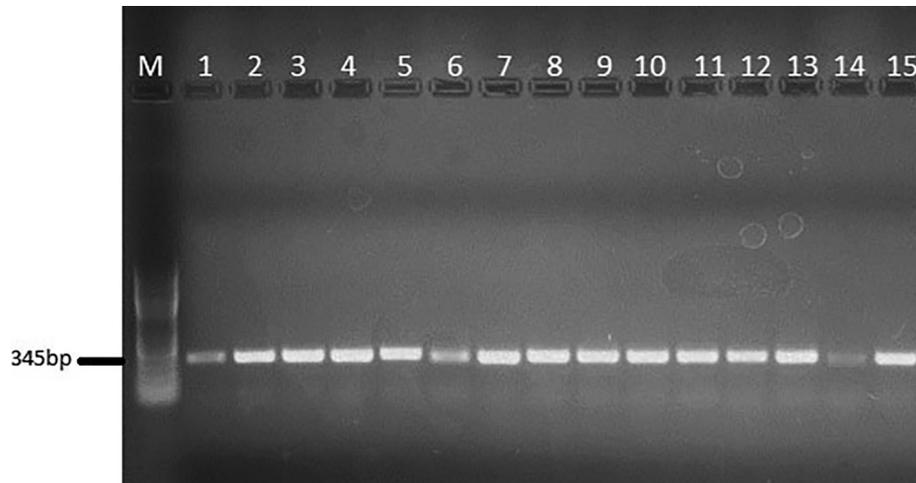
Fig. 1. 345 bp PCR products amplified using D3A/D3B primer (a universal primer for amplifying the D3 expansion region of the 28S gene). The amplified products are obtained from single nematode samples. M) lader, 1–3) *Longidorus latocephalus*, 4–6) *Xiphinema elongatum*, 7–9) *Hoplolaimus seinhorsti*, 10–12) *Hemicriconemoides strictathecatus*, 13–15) *Tylenchorhynchus mediterraneus*.
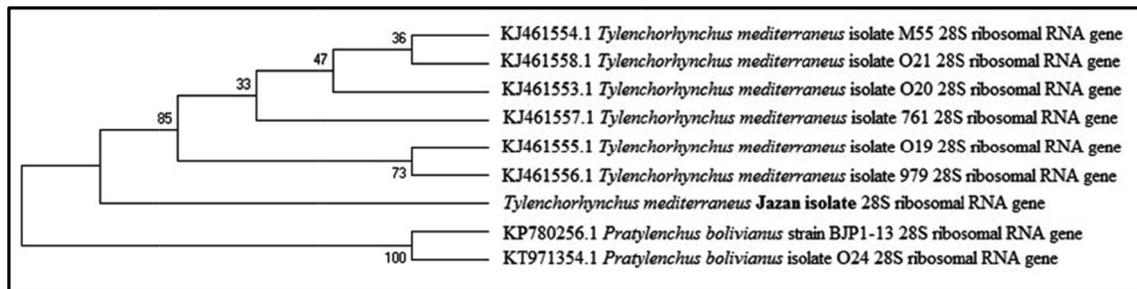
Fig. 2. Phylogenetic relationships of the Jazan isolate of *Tylenchorhynchus mediterraneus* with other *T. mediterraneus* isolates based on 28S rRNA, reconstructed using maximum likelihood (ML) method. Nodes show the percentage bootstrap values (out of 100).

due to certain environmental local changes (Dawabah et al., 2012) (see Figs. 3–6).

3.3.2. Hoplolaimus seinhorsti

The expansion region of 28S rRNA sequence of *H. seinhorsti* (Jazan isolate) and other *H. seinhorsti* sequences deposited in the GenBank were subjected for phylogenetics. Outgroup taxa included the sequences of two related species (*H. galeatus* and *H. stephanus*) and another non-related one (*Globodera rostochiensis*) selected from GenBank. The maximum likelihood (ML) method showed that the related (*H. galeatus* and *H. stephanus*) and the outgroup (*Globodera rostochiensis*) species were clustered nearby each other. However,
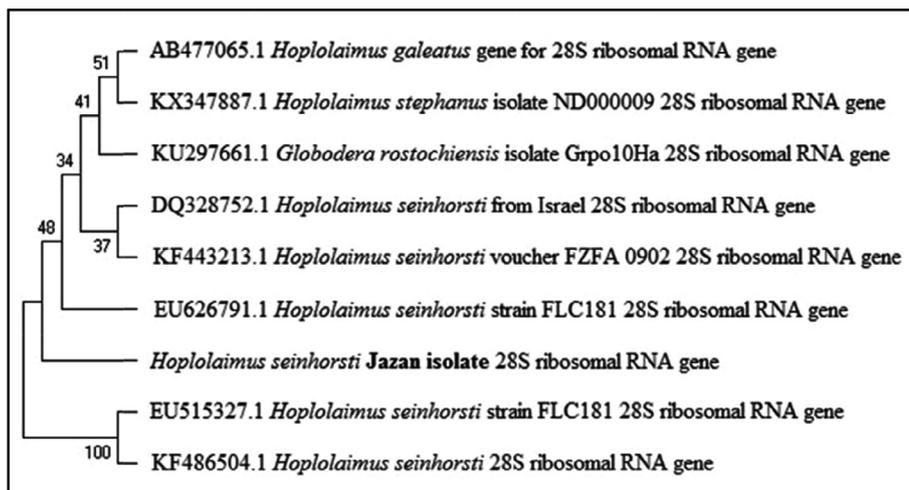
Fig. 3. Phylogenetic relationships of the Jazan isolate of *Hoplolaimus seinhorsti* with other *H. seinhorsti* isolates based on 28S rRNA, reconstructed using maximum likelihood (ML) method. Nodes show the percentage bootstrap values (out of 100).
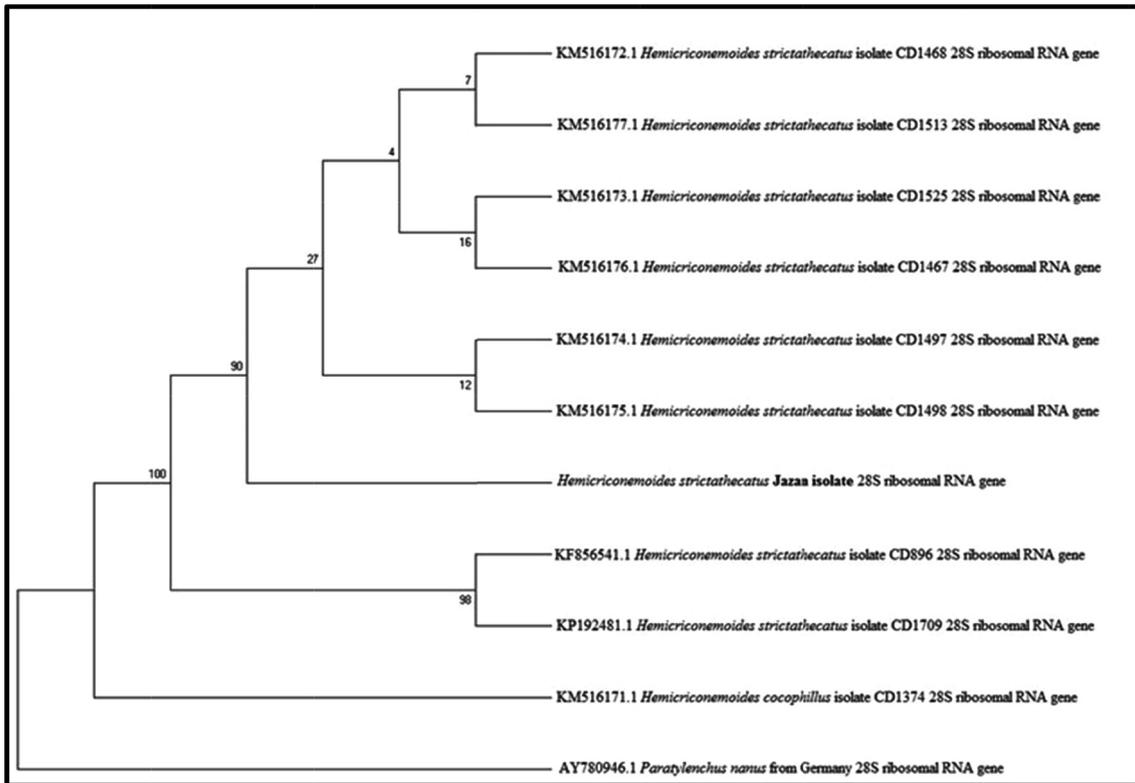
Fig. 4. Phylogenetic relationships of the Jazan isolate of *Hemicriconemoides strictathecatus* with other *H. strictathecatus* isolates based on 28S rRNA, reconstructed using maximum likelihood (ML) method. Nodes show the percentage bootstrap values (out of 100).
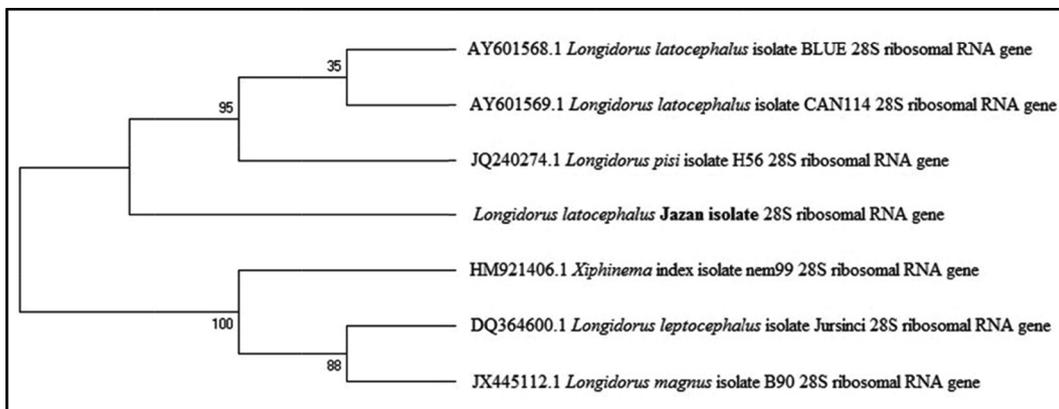
Fig. 5. Phylogenetic relationships of the Jazan isolate of *Longidorus latocephaalus* with other *L. latocephalus* isolates based on 28S rRNA, reconstructed using maximum likelihood (ML) method. Nodes show the percentage bootstrap values (out of 100).
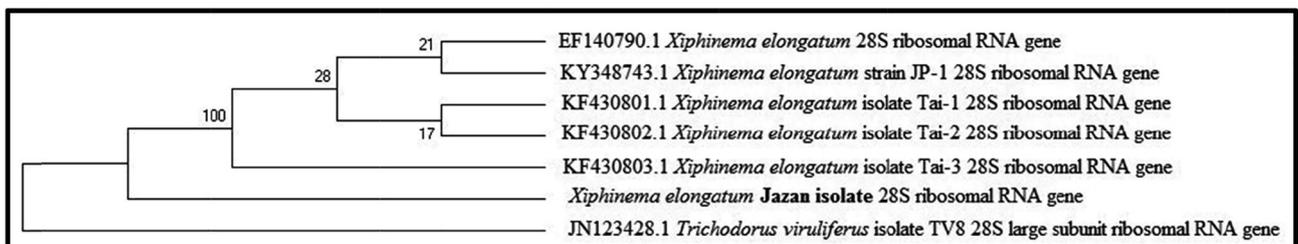
Fig. 6. Phylogenetic relationships of the Jazan isolate of *Xiphinema elongatum* with other *X. elongatum* isolates based on 28S rRNA, reconstructed using maximum likelihood (ML) method. Nodes show the percentage bootstrap values (out of 100).

H. seinhorsti (Jazan isolate) clustered within the other *H. seinhorsti* from GenBank confirming that Jazan population of *Hoplolaimus* strongly belongs to *H. seinhorsti* (Mansourabad et al., 2016).

3.3.3. Hemicriconemoides strictathecatus

The expansion region of 28S rRNA sequence of *H. strictathecatus* (Jazan isolate) and other eight sequences of *H. strictathecatus* from GenBank were used for phylogenetic analysis. Sequences of a related species, *H. cocophillus* and the species *paratylenchus nanus* (belongs to a systematically nearby genus) from GenBank were also included. The maximum likelihood (ML) method showed that the related species, *H. cocophillus* was located in the clade containing *H. strictathecatus* isolates but in a separate subclade, whereas the species *P. nanus* which belongs to a different genus was located in a separate clade. However, *H. strictathecatus* (Jazan isolate) located within the other *H. strictathecatus* isolates from GenBank proved that Jazan isolate of *Hemicriconemoides* fairly belongs to *H. strictathecatus* according to Van den Berg et al. (2015).

3.3.4. Longidorus latocephalus

The expansion region of 28S rRNA sequence of *L. latocephalus* (Jazan isolate) and other *L. latocephalus* sequences deposited in the GenBank were subjected for phylogenetics. The sequences of another related species; *L. magnus* and *L. pisi* as well as the species *Xiphinema index* (another nearby genus) from GenBank were included in the phylogenetic analysis using maximum likelihood (ML) method. Phylogenetic analysis revealed that Jazan isolate of *L. latoceohalus* along with other two isolates of *L. latocephalus* and another isolate of *L. pisi* which was a previous synonym of *L. latocephalus* (Choleva et al., 1991) were clustered in one clade. However, the other clade included the related species *L. magnus* and the species *X. index* (belong to another genus) with only one isolate of *L. latocephalus* from GenBank. This indicate that the Saudi population of *Longidorus* strongly fits to *L. latocephalus* (Choleva et al., 1991; Navas et al., 1993).

3.3.5. Xiphinema elongatum

The expansion region of 28S rRNA sequence of *X. elongatum* (Jazan isolate) and other *X. elongatum* sequences deposited in the GenBank were subjected for phylogenetics using the maximum likelihood (ML) method. The species *Trichodorus viruliferus* was used as an outgroup species. Results showed that all the used isolates of *X. elongatum* clustered in one clade consisting of two sub clades. The first sub clade included Jazan isolate of *X. elongatum* separately, while the other sub clade included the other five isolates of *X. elongatum* from GenBank. However, the outgroup species *Trichodorus viruliferus* (belongs to another genus) separated in a separate clade. This indicating that the Jazan population of *Xiphinema* strongly fits to *X. elongatum* (Heyns and Coomans, 1991; Luc and Coomans, 1992; Chen et al., 2004; Getaneh et al., 2015).

Acknowledgment

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