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TWO NEW NEMATODE SPECIES FROM PISTACHIO FAUNA IN KHORASAN RAZAVI PROVINCE OF IRAN

^aReyhane Hadadfar*, ^aEsmat M. Moghadam, ^aSareh Baghaee, ^{a,b}Majid S. Bajestani

^aDepartment of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

^bEducational management office, Ferdowsi University of Mashhad, Iran.

ABSTRACT

Pistachio is an important nut crop in Iranian horticulture which is a strategic and valuable export product and holds the first position in non-oil export of Iran. So that Iran is the largest producer of pistachio that is the first exporter in world and Khorasan Razavi province as the second largest producer in the country has a special position in this regard. Considering that nematodes are one of the most important soil pathogenic agents and can cause considerable damage to pistachio in high populations, since there are no studies on biodiversity of plant parasite nematodes in pistachio gardens of Khorasan Razavi province, so identification of Pistachio plant parasite nematodes is very important. To identify plant parasitic nematodes in pistachio fields of Khorasan Razavi province northeast of Iran, in the years 2016 and 2017 collected 70 root and soils samples from pistachio root rhizosphere (depth 30-50 cm). Samples transferred to laboratory on ice and nematode were extracted by centrifugal and Whithead methods. After obtaining permanent microscopic slides from extracted nematodes, samples considered by BH2 Olympus microscope equipped with drawing tube. In this study 19 species belongs to 8 genus described as: *Ditylenchus medicaginis*, *Pratylenchus neglectus*, *Pratylenchus coffeae*, *Pratylenchus wescolagricus*, *Psilenchus iranicus*, *Psilenchus hilarus*, *Psilenchus aestuarius*, *Psilenchus bilineatus*, *Psilenchus curcumerus*, *Psilenchus pratensis*, *Psilenchus bahiablancea* *Merlinius graminicola*, *Merlinius planitierus*, *Merlinius gatevi*, *Nagelus leptus*, *Geocenamus tessellatus*, *Geocenamus rugosus*, *Geocenamus koreanus*, *Meloidogyne javanica* and *Helichotelenchus exallus*. Between this species *Geocenamus koreanus* and *Merlinius gatevi* described for first time from Iran nematode fauna and five species have been reported for the first time in Iran. In this study, *Pratylenchus* (root lesion nematodes) among the other species has the most abundance and dispersion, so they are important.

Keywords: Plant parasitic nematodes, *Geocenamus koreanus*, *Merlinius gatevi*, *Pistachio*

INTRODUCTION

Pistachio is one of the agricultural products that its name mixed with the name of Iran, according to the historical records for native land of Pistachio showed there was in northeast of Great Khorasan Provinces and Khaje Sarakhs forests in Khorasan Razavi province (Tavallali and Rahemi, 2007). *Pistacia vera* is a semi-tropical plant with two vegetative bases,

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* Corresponding Author:

Email: r.hadadfar@ymail.com

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which are found in the form of massive forests in northeastern Iran (Nakhaee nejad, 2007). Pistachio planting moved from Iran to other parts, especially to the Mediterranean coast (southern Europe and North Africa). Pistachio is a strategic product and has a special place in Iran's agricultural production, because of economic significance it known as a green gold. This product accounted about 55% of non-oil exports and more than 60% of global exports in Iran. The income from pistachio exports in Iran is over \$ 400 million for one year (Amuzegar Jahangir, 2003). The most important cultivars of this product in Iran are Akbari, Kaleh Qouchi, Ahmad Aghaei, Ohadi, Badami, Zarand Momtaz, Khanjari Damghan, Shahpasand, Sefid Qazvin (Amuzegar Jahangir, 2003).

Currently, Iran's pistachio cultivation areas are more than 440 thousand hectares. Khorasan Razavi province is one of the important areas for pistachio planting in Iran and according to information released by the statistical Agriculture in 2015; more than 20% of the total country's pistachio production was in this province (Iranian statistical agriculture reports 2015). This plant has several diseases including, plant parasitic nematodes (Neshat, 2011). In Iran, only Susan Neshat et al. in 2011 surveyed pistachio nematode fauna in Rafsanjan Province of Iran and described 23 species belongs to 16 genus. They reported that genus *Meloidogyne* with a frequency of 40%, genus *Geocenamus* with 33% frequency, *Pratylenchus* genus with 31% frequency and *Longidorus* genus with 20% frequency had the highest percentage of distribution among other genus.

The genus *Geocenamus* was established by Thorne and Malek (1968) in the subfamily Tylenchoryhynchinae. The name of the genus is derived from a contraction of Geographic center of North America, reflecting the region from which it was first described. As of October 2003, the Zoological Record indicates 51 species names associated with this genus. The genus is distinguished from other genera within the subfamily by a labial disc. The cephalic framework is weak and the stylet slender.

In the genus *Geocenamus*, body has six lateral fields that in some species, in addition to the lateral fields, existence other longitudinal grooves witch along the entire surface of the skin that interrupts the transverse grooves and lattice appear to the skin surface. The stylet, elegant and slim to strong, length varies from 25 to 130 micrometers; stylet cone length is larger than the cylindrical portion. Tohren and Malik placed this genus in *Tylenchorhynchidae* family in 1968. Siddiqi placed this genus in *Merlininae* family and considered with 14 species.

The aim of this study was to complete investigate of pistachio parasitic nematodes in Khorasan Razavi province, because after the comprehensive identification of pathogenic nematodes, appropriate decisions can be made to control the damage caused by these pathogens. Since only, one study has been done in the Rafsanjan region on this product (Neshat et al., 2011), this study would be an appropriate

option for research on Iranian nematode fauna.

MATERIALS AND METHODS

Soil sampling: Soil and root samples collected from the rhizospheres of Pistachio gardens of Khorasan Razavi provinces in East of Iran. Soil samples (70) were taken from the depth of 30-50 cm put in polyethylene bags with necessary labeled and brought to the laboratory and processed.

Processing of samples: Nematodes were extracted from soil samples by using the Jenkins (1964) and *Whitehead* and Hemming (1965) methods, killed and fixed according to De Grisse (1969). Genera and species were identified based on morphological and morphometric characters (Siddiqi, 2000; Geraert, 2008). Specimens were heat-killed by adding boiling 4% formalin solution, and were then transferred to anhydrous glycerin according to De Grisse (1969). Measurements and drawings were performed using a drawing tube attached to an Olympus BH2 light microscope (LM). Morphometric abbreviations and ratios were used in the present study (Siddiqi, 2000). Nematodes were identified based on morphological and morphometric characteristics, using identification keys (Raski, 1975; Huang and Raski, 1987; Geraert, 2008).

Measurements and drawings: Measurements were taken with an ocular micrometer of "Olympus BH2" model microscope. Drawings were made with drawing tube attached to the compound microscope.

RESULTS AND DISCUSSION

In this study 19 species from 8 genus were identified viz. *Ditylenchus medicaginis*, *Pratylenchus neglectus*, *Pratylenchus coffeae*, *Pratylenchus wescolagricus*, *Psilenchus iranicus*, *Psilenchus hilarus*, *Psilenchus aestuarius*, *Psilenchus bilineatus*, *Psilenchus curcumerus*, *Psilenchus pratensis*, *Psilenchus bahiablancea* *Merlinius graminicola*, *Merlinius planitierus*, *Merlinius gatevi*, *Nagelus leptus*, *Geocenamus tessellatus*, *Geocenamus rugosus*, *Geocenamus koreanus*, *Meloidogyne javanica* and *Helichotelenchus exallus* (Table 1). *Merlinius gatevi* (Sturhan, 2012) (Figure 1, Table 2)

According to Sturhan's studies in 2012 *Geocenamus gatevi* was renamed to *Merlinius gatevi*. This renaming was based on Sturhan's observations, including presence of dirids, 4 to 6 lateral fields, presence of inner cuticle layer on tail, semi-cylindrical tail with sharp tip end, stylet with more than 30 μm long and lack of longitudinal lines. This species was similar to those described by Budurova in the 1988 and Garrett in 2008.

Table 1. List of nematode species from Pistachio garden in Khorasan Razavi province east of Iran with GPS pots, name of sampling areas, diagnostic key and first description in Iran.

Number	species	Areas	UTM	Diagnostic key	Firs description in Iran
1	<i>Ditylenchus medicaginis</i>	Feiz abad city	665020-3878188	Wasilewska, 1965	-
2	<i>Merlinius gatevi</i> **	Feiz Abad City	665031-3878147	(Budurova, 1988) Brzeski, 1991	-
3	<i>Geocenamus koreanus</i> **	Kheir Abad city	656790-3873902	(Choi and Geraert, 1971) Brzeski, 1991	-
4	<i>Geocenamus rugosus</i>	Feiz Abad City	665010-3878142	(Siddiqi,1963) Brzeski, 1991	Mojtahedi et al, 1983
5	<i>Geocenamus tessellatus</i>	Taybad city	288946-3848257	(Goodey,1952) Brzeski 1991	Baadl et al., 2012
6	<i>Helichotylenchus insignis</i>	Bardaskan City	594560-3890373	Sher, 1966	Jabberi et al., 2008
7	<i>Merlinius graminicola</i>	Kheir Abad city	656883-3874124	(kirjanova,1951) siddiqi, 1976	Mojtahedi et al, 1983
8	<i>Meloidogyne javanica</i>	Feiz Abad city	665010-3878142	(Treub, 1885) Chitwood, 1949	Kheiri, 1972
9	<i>Nagelus leptus</i>	Rokn Abad village	593414-3891102	(Allen, 1955) Siddiqi, 1979	Mojtahedi et al, 1983
10	<i>Pratylenchus coffeae</i>	Shams Abad village	642708-3875236	(zimmermann, 1898) filipjev and Schuurmans Stekhoven, 1941	Kheiri, 1972
11	<i>Pratylenchus neglectus</i>	Shams Abad village	642006-3878430	(Rensch, 1924) Filipjev, Schurmans Stekhoven, 1941	Kheiri, 1972
12	<i>Pratylenchus wescolagricus</i>	Dogh Abad village	665031-3878144	Corbet, 1983	Alvani et al., 2016
13	<i>Psilenchus aestuarius</i>	Feiz Abad City	665031-3878147	Andrassy, 1962	Mokarram Hesar et al., 2010
14	<i>Psilenchus curcumerus</i>	Bardaskan City	594560-3890373	Rahaman Ahmad and Jairajpuri, 1994	-
15	<i>Psilenchus iranicus</i>	Rokn Abad village	593414-3891102	Kheiri, 1970	Kheiri, 1970
16	<i>Psilenchus hilarus</i>	Shams Abad village	642024-3878403	Siddiqi,1963	Ebrahimi et al., 2003

**New species for Iranian nematode fauna

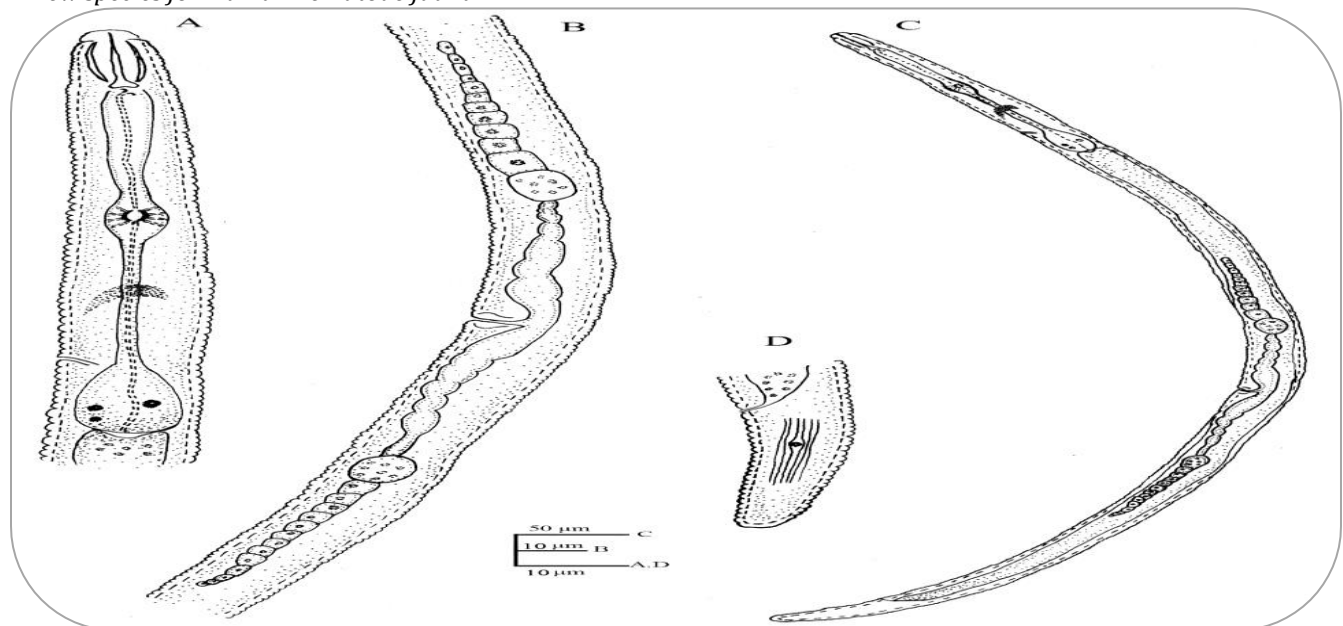


Figure 1. *Merlinius gatevi*, A: Head region with oesophagus; B: Ovary; C: whole of body; D: Tail of female

Body annuli fine, about 1.6 ± 0.1 (1.5-1.8) μm wide. Lateral fields with 6 lines. Head separated from body outline, height 5 μm and width 8 μm ; moderately sclerotized; with 6 annuli. Spear knobs slightly downwards, height 3 μm , width 5 μm . Cardia rounded to semi oval. Spermatheca rounded to oval. Vaginal walls moderately sclerotized. Sub-cylindrical tail with rounded and non-annulated terminus. Tail annuli at the ventral side 21-24. Phasmids situated in the frontal half of the tail.

Male: not founded.

This species is distinguished by the number of tail rings compared to the close species same *Geocenamus dobroticus* and *Geocenamus tenuidens*. In this species, the number of tail rings was 21-24 but in *Geocenamus dobroticus* 27-30 and in *Geocenamus tenuidens* 45-80. This is the first report of this species from Iranian nematode fauna.

Geocenamus koreanus (Choi and Geraert, 1971) (Figure 2, Table 2)

Table 2. Morphometric characters of the Iranian population of *Geocenamus Koreanus* and *Merlinius gatevi* and comparison with original descriptions (Measurements are in μm).

Origin Characters	<i>Merlinius gatevi</i>	Budurova, 1988	<i>Geocenamus koreanus</i>	Choi and Geraert, 1971
	Female/Male	Female/Male	Female/Male	Female/Male
L	871.4±98.9(750-1000)	0.77-0.95	866.3±104.9(690-1000)	0.75-0.92
Body width	29±1(28-30)	29	26±2(24-28)	26
A	30±2.8(25.9-33.3)	25-32	42.6±4.8(38.3-53.5)	28-44
B	5±0.5(4.3-5.6)	-	5.5±0.9(4.9-7.8)	-
C	16.2±2.3(14.1-20.5)	14-18	15.6±1.8(12.5-17.9)	11-16
c'	2.6±0.3(2.2-3.2)	2.3-2.6	3.4±0.4(2.8-3.9)	3.4-3.8
V	54.8±2.9(51-60)	53-57%	55.4%±1.9(52.2-58.1)	53-60%
V'	58.5±3.5(54-64)	-	59.3±2.1(55.7-62.5)	-
G1	30.9±1.6(28.5-33.3)	-	30.6±3(23.9-34.5)	-
G2	25.2±3.4(20.2-28.9)	-	24.8±3(21.5-30)	-
Stylet	23.6±1(22-25)	23-24	23.8±0.7(23-25)	22-25
Pharynx length	174.9±5.7(167-185)	176	158.6±20.5(129-181)	130-160
SE-pore	128.6±8.4(120-144)	-	125.9±18.3(105-150)	-
MB	46.8±2.7(42.6-50)	47%	49±4.5(43.2-63.6)	48-50%
DGO	2.5±0	2.5	2±0(2-2)	2
Tail length	54.4±8.4(43-71)	55	55.4±2.1(52-58)	55-70

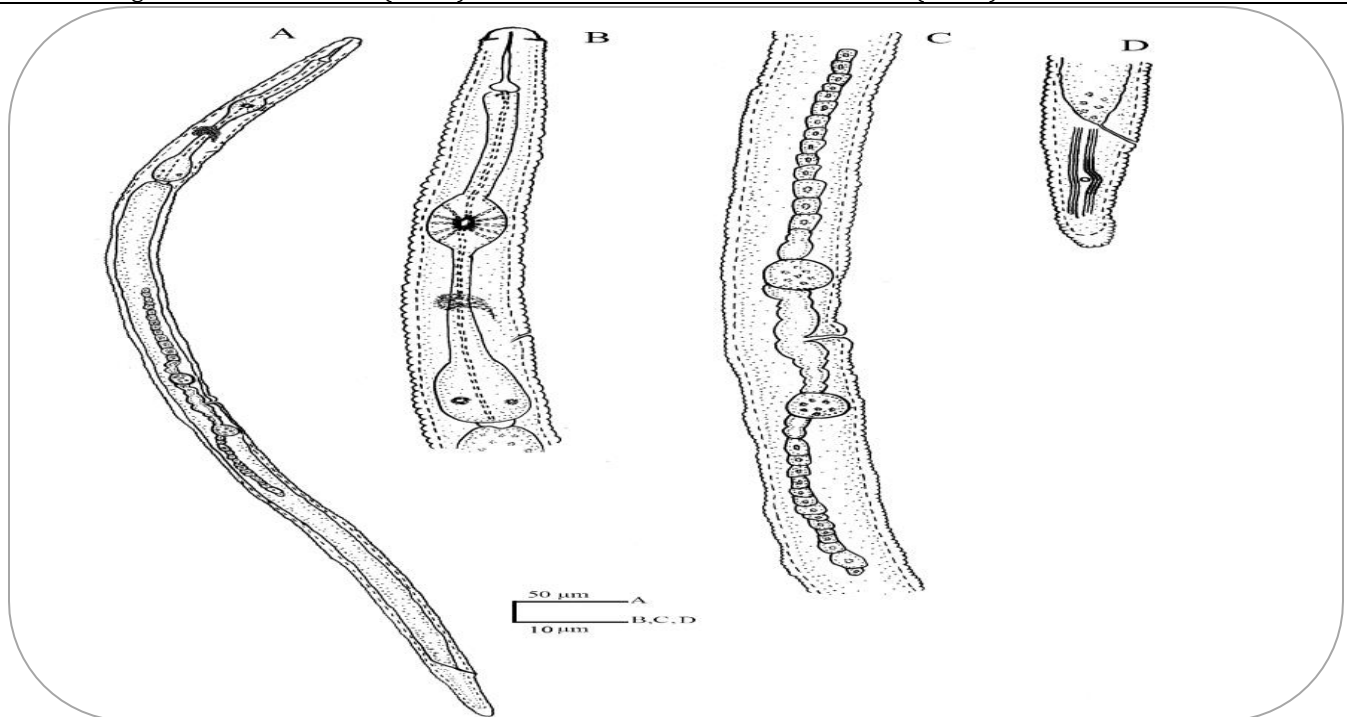


Figure 2. *Geocenamus koreanus*, A: whole of body; B: Head region with oesophagus; C: Ovary; D: Tail of female

Body straight or slightly curved ventrally. Longitudinal striations on body prominent, 7-8 dorsal, 8-9 central; longitudinal striate are formed by involutions of the coarse transverse striations 1.6-2 μm wide. The lateral field is prominent, occupying 1/4th of the body width and with 8 incisures visible in cross-sections (the outer 2 lines, however, not visible from a lateral view). Head slightly offset, with 5-6 annuli; cephalic framework slightly sclerotized, situated 2-3 head annuli anteriorly than the level of the head-constriction; head divided longitudinally into 5-6 sectors corresponding. Conical part of style cery fone and delicate, measuring about 60% of the spear length, and surrounded by a guiding apparatus consisting of a tubular and an oval part; stylet knobs well developed and flattened anteriorly. Median bulb oval. Terminal bulb cylindrical. Excretory pore opposite anterior end of terminal bulb. Vulva not than 4-4.2 μm wide and provided with anterior and posterior epiptygma. Spermathecae rounded, filled with sperm. Tail sub-cylindrical, slightly tapering towards the rounded, coarsely annulated tip. Plasmids at about 1 and diamerer posterior to the anus (17.5-32% of tail length). This species was compared with close species *Geocenamus hexincisus*. Studied species was similar to the close species number of longitudinal grooves on the surface of the body, but differ in style and tail length, so that the length of stylet in studied species was more than *Geocenamus hexincisus* (The length of the stylet in the discussed species is 22-25 and in the similar species is 19-19 μm). In addition, the length of tail and factor C differ in these species. C Factor is 11-16 in studied species and 18-23 in the close species. Therefore, population under study was identified as *Geocenamus koreanus*.

CONCLUSION

In this study, the fauna of plant parasite nematodes in pistachio root rhizosphere of Khorasan Razavi province, Iran, were investigated and 19 species belonging to 8 genera were identified. During this study, two nematode species *Geocenamus koreanus* and *Merlinius gatevi* were described for the first time from Iran nematode fauna.

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Contribution of Authors:

Reyhane Hadadfar	:	Write original manuscript and conduct research
Esmat M. Moghadam	:	Help in writing manuscript
Sareh Baghaee	:	Data analysis
Majid S. Bajestani	:	Review manuscript