

Plant parasitic nematodes from rhizosphere of saffron (*Crocus sativus* L.) with two new records of *Geocenamus squamatus* and *Filenchus pratensis* from Iran

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Abstract

For the investigation and identification of plant parasitic nematodes associated with saffron, 88 soil samples were collected in the autumn of 2012 from the rhizosphere of saffron in different areas of southern Khorasan province, Iran. Nematodes were extracted by centrifugal flotation technique. The extracted nematodes were transferred to glycerine for preparing permanent slides. Then plant parasitic nematodes were identified by using morphological and morphometric characters. In this study, a total of 30 species of nematodes were identified. Among them, two species viz., *Geocenamus squamatus* and *Filenchus pratensis* are recorded for the first time in Iran.

Saffron is a spice derived from the flower of *Crocus sativus* L., belongs to the family Iridaceae. Iran is the largest producer of saffron in the world. The southern Khorasan in Iran is the main area of saffron production. This important crop has long been cultivated in Iran and Kashmir. Today, the area under saffron cultivation is high from the western Mediterranean (Spain) to India (Kashmir). All continents outside this zone except Antarctica produce smaller amounts. Iran is the largest producer of it accounting for more than 85 % of the global saffron production. Spain is the second largest producer (35-40 tons/year) while India, which produces 7.0 tons, ranks third. So, Spain and Iran are the largest producers, accounting together for more than 80 % of the world's production (Dharmananda, 2005). Annual worldwide production amounts to some 300 tons (Anon., 2012).

In recent years, cultivation of this crop has enormously increased and Iran now produces more saffron than Spain and accounts for approximately 90 % of the world production of saffron (Ghorbani, 2008). The name saffron comes from the Arabic name "zafaran", which means yellow. It is a spice commonly found in Mediterranean, Arabic and Asian dishes. Saffron is also the world's oldest medicinally used plants. Because flower's stigmas need to be collected by

hand and there are only a few per flower, this plant is the most expensive spice in the world (Kafi *et al.*, 2006). A new plant parasitic nematode species was reported from saffron (Fotadar & Handoo, 1977). Two new records of the genera *Geocenamus* Thorne & Malek, 1968 and *Filenchus* Andrassy, 1954 were detected during the survey.

The objective of this study was to investigate the plant parasitic nematodes associated with saffron fields in southern Khorasan province of Iran, as very little is known about the plant parasitic nematode associated with saffron in Iran.

Materials and Methods

Soil samples were collected from the rhizosphere of saffron (*Crocus sativus* L.) fields in southern Khorasan province. About 88 soil samples were taken from 30-40 cm from different areas with a soil auger. From each field several small samples were taken from all areas of field.

Then the soil samples were put in a polyethylene bags with pertinent information about each sample and then brought to the laboratory and kept in the refrigerator at about 4 °C, until they were processed for nematode extraction and other activities.

Nematodes were extracted from all soil samples by using the Jenkins (1964) method. They were then handpicked, killed and fixed according to De Grisse (1969) method and permanent slides were prepared. Genera and species found were identified based on morphological and morphometrical characters. Species were identified by using keys given by Geraert (2008).

Results and Discussion

fo latot A 30 species of nematodes were identified and are given in Table 1, of which two species are recorded for the first time in Iran.

Geocenamus squamatus
Eroshenko & Volkova, 1988
(Fig. 1, Table 2)

Female: Body about 774.5-955 μm long, slightly ventrally curved when relaxed. Body cuticle coarsely annulated. Lateral field with 6 lines. Head hemispherical with 6 small annuli. Stylet thin, about 24.5-26 μm long. Orifice of dorsal gland about 2-3 μm behind stylet base. Median bulb oval. Isthmus elongate, slender. Basal bulb pyriform, offset from the intestine. Excretory pore at the anterior end of the posterior bulb. Vulva at the 50.22-58.88 % body length. Spermatheca obvious, axial, filled with sperms. Tail cylindrical, narrowing to the terminus, ventrally curved with 25-27 annuli, tail terminus annulated and rounded. Phasmids large, located at 1/3 of the tail length behind the anus.

Male: Not found.

Remarks: General description and morphometric measurements of this species closely fit to the original description of Eroshenko & Volkova (1988). The species represents a new country record for Iranian nematode fauna.

***Filenchus pratensis* (Sumenkova, 1987)**
Ebsary, 1991
(Fig. 2, Table 2)

Female: Body slender, about 580-720 μm , almost straight to ventrally curved, cuticle annulated,

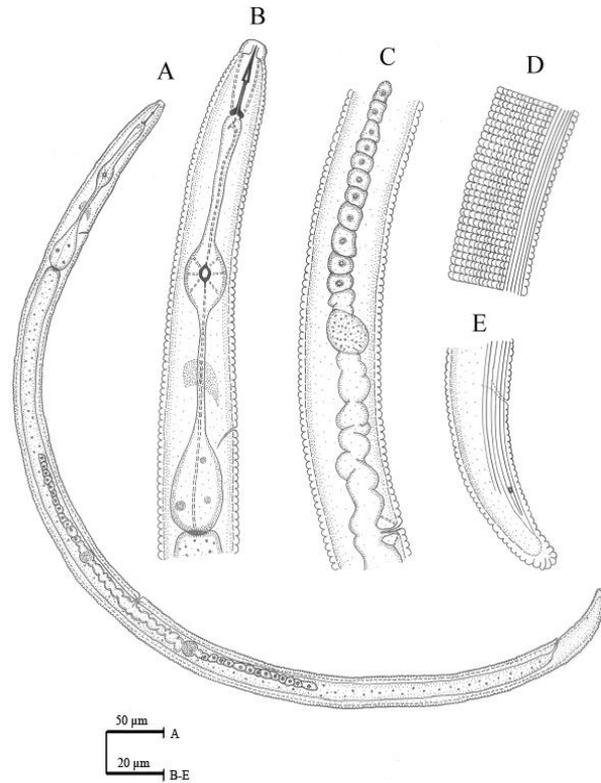


Fig. 1 (A-E). *Geocenamus squamatus*. A. Whole body; B. Head region with oesophagus; C. Ovary; D. Lateral field with longitudinal striae; E. Tail with phasmid.

annuli 1.3-1.4 μm wide at mid-body. Lateral field with two lines. Head short, trapezoid, non-annulated. Stylet delicate with slightly posteriorly knobs. Orifice of dorsal gland 1.5-2 μm . Median bulb rounded-oval. Basal bulb oval-pear-shaped. Excretory pore at the anterior end of the basal bulb. Vulva simple, without epiptygma.

Vulva at 57.74-62.23 % body length, ovary outstretched, oocytes in a single row, spermatheca rounded, filled with small sperms. Post-vulval uterine sac coniform. Tail thread-like, narrowing to the end.

Male: Not found.

Remarks: General description and measurement of this species closely fit the original description

Table 1. Nematode species and collection areas in southern Khorasan province, Iran.

Nematode species	Collection area
<i>Amplimerlinius icarus</i>	Nimblook, Harivand, Hasanabad, Khosf
<i>Amplimerlinius macrurus</i>	Sede, Khosf, Darmian
<i>Amplimerlinius socialis</i>	Sede
<i>Aphelenchoides asterocaudatus</i>	Sarayan, Birjand, Sarbishe
<i>Aphelenchoides besseyi</i>	Birjand, Nimblook
<i>Aphelenchoides curiolis</i>	Khosf
<i>Criconemoides deccipiens</i>	Birjand, Boghabad, Kale-esgholi
<i>Ditylenchus dipsaci</i>	Qaen, Sede, Boghong
<i>Ditylenchus myceliophagus</i>	Zirkooh, Birjand, Sarayan
<i>Filenchus hamatus</i>	Khosf, Dehek
<i>Filenchus pratensis</i> *	Boghong, Razgh
<i>Geocenamus squamatus</i> *	Bojd, Saghi
<i>Geocenamus tenuidens</i>	Tangal, Nehbandan, Aliabad
<i>Helicotylenchus crassatus</i>	Zirkooh, Qaen
<i>Helicotylenchus exallus</i>	Qaen, Sede
<i>Merlinius bavaricus</i>	Boghong, Nimblook, Khonik
<i>Merlinius brevidens</i>	Sarayan, Harivand, Birjand
<i>Merlinius graminicola</i>	Fanod, Nehbandan
<i>Merlinius microdorus</i>	Darmian, Mood
<i>Merlinius nanus</i>	Qaen
<i>Merlinius pseudobavaricus</i>	Qaen, Nimblook, Gaz
<i>Pratylenchus coronatus</i>	Gaz, Birjand, Shosf
<i>Pratylenchus coffeae</i>	Shosf, Qaen
<i>Pratylenchus loosi</i>	Mood, Sarayan, Qaen
<i>Pratylenchus penetrans</i>	Birjand, Zirkooh, Sede, Mood
<i>Pratylenchus thornei</i>	Zirkooh, Gaz, Boghabad
<i>Psilenchus elegans</i>	Zirkooh, Qaen, Avizh
<i>Psilenchus minor</i>	Nehbandan
<i>Tylenchorhynchus brassicae</i>	Kale-esgholi, Ariyanshahr
<i>Tylenchus parvus</i>	Darmian, Nimblook

*New records from Iran

Table 2. Morphometric characters of the Iranian population of *Geocenamus* and *Filenchus* species and their comparison with type population (measurements are in μm).

Morphological characters	<i>Geocenamus squamatus</i>		<i>Filenchus pratensis</i>	
	Southern Khorasan, Iran (n = 10)	Eroshenko & Volkova, 1988	Southern Khorasan, Iran (n = 8)	Ebsary, 1991
L	878.55±65.8 (774.5-955)	890-920	642.83±54.2 (580-720)	550-690
a	30.34±2.5 (25.03-33.67)	30-34	32.22±2.21 (29.19-35.2)	27-38
b	5.4±0.79 (4.42-7.33)	-	5.65±0.75 (4.71-6.63)	-
c	16.1±1.69 (14.34-20.29)	17-18	4.18±0.24 (3.9-4.59)	3.7-5.2
c'	3.02±0.17 (2.75-3.36)	2.8-2.9	13.22±1.49 (11.91-15.72)	-
V	54.15±3.18 (50.22-58.88)	51-55	59.96±1.6 (57.74-62.23)	57-64
V'	57.78±3.56 (53.48-63.27)	-	78.83±2 (74.54-81.15)	-
Stylet	25.25±0.56 (24.5-26)	26	11.28±0.72 (10-12)	11-12
Oesophageal length	167.1±6.5 (158-179)	154	116.75±8.54 (99-127)	104-118
Excretory pore from ant. end	122.8±9.96 (104.5-133)	-	82.68±4.97 (72-88)	71-87
DGO	2.35±0.41 (2-3)	3	1.81±0.25 (1.5-2)	-
Body width	29±1.5 (27-32.5)	30	19.93±1.14 (18-22)	-
MB	46.71±1.64 (44.69-49.37)	49	41.28±1.27 (39.37-42.98)	41
Head-vulva	474.35±22.08 (443-502)	-	385.5±33.92 (347-435)	-
Vulva-anus	349.35±52.77 (277.5-405)	-	103.65±15.42 (90-140)	-
G ₁	15.53±1.5 (13.11-17.81)	-	-	-
G ₂	16.19±1.39 (14.21-18.34)	-	-	-
PVS	-	-	11.66±0.89 (10.5-13)	10.5-13
Width at vulva	28.7±0.97 (27-30)	-	17.75±1.01 (16.8-20)	-
Tail length	54.85±5 (44-63)	51	153.68±14.77 (140-184)	-
Width at anus	18.13±1.45 (16-21.5)	-	11.96±0.6 (11-13)	-
T/VA	0.154±0.02 (0.12-0.2)	-	1.49±0.19 (1.14-1.82)	1.1-1.7

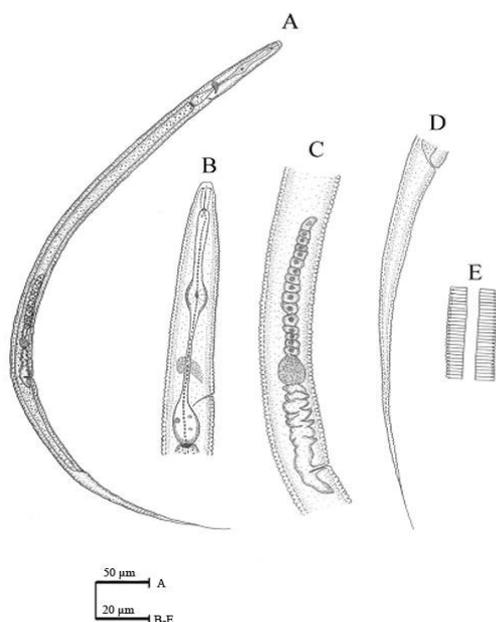


Fig. 2 (A-E). *Filenchus pratensis*. A. Whole body; B. Head region with oesophagus; C. Ovary; D. Tail end; E. Lateral field.

of Ebsary (1991). This species represents a new country record for Iranian nematode fauna and a new host from saffron.

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