

# RESEARCH/INVESTIGACIÓN

## DESCRIPTION OF *AGLENCHUS MICROSTYLUS* N. SP. (NEMATODA, TYLENCHIDAE) FROM IRAN WITH A MODIFIED KEY TO THE SPECIES OF THE GENUS

Manouchehr Husseinvand<sup>1</sup>, Mohammad Abdollahi<sup>1\*</sup>, and Akbar Karegar<sup>2</sup>

<sup>1</sup>Faculty of Agriculture, Yasouj University, Yasouj, Iran, <sup>2</sup>Faculty of Agriculture, Shiraz University, Shiraz, Iran.  
\*Corresponding author: Mohammad Abdollahi (mdabdollahi@gmail.com)

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### ABSTRACT

Husseinvand, M., M. Abdollahi, and A. Karegar. 2016. Description of *Aglenchus microstylus* n. sp. (Nematoda, Tylenchidae) from Iran with a modified key to the species of the genus. *Nematropica* 46:38-44.

A new species of the genus *Aglenchus* is described from the rhizosphere of a Montpellier maple tree in the Sepidar region, Kohgiluyeh and Boyer-Ahmad province, Iran. *Aglenchus microstylus* n. sp. is a small nematode characterized by the presence of three incisures in each lateral field, stylet of 7–8  $\mu\text{m}$  length, short post-vulval uterine sac, lateral vulval flaps, straight vagina and elongated-conoid tail, with pointed to finely rounded terminus. The new species differs from all the known species of the genus, including more closely related *A. dakotensis* and *A. geraerti*, by having a short stylet (7-8  $\mu\text{m}$ ), a short post-vulval uterine sac and straight vagina. A modified key to identification of the species of genus *Aglenchus* is presented.

*Key words:* *Acer monspessulanum* subsp. *cinerascens*, *Aglenchus microstylus*, morphology, morphometrics, new species, taxonomy.

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### RESUMEN

Husseinvand, M., M. Abdollahi, y A. Karegar. 2016. Descripción de *Aglenchus microstylus* n. sp. (Nematoda, Tylenchidae) de Irán con una clave de especies modificada. *Nematropica* 46:38-44.

Se describe una nueva especie del género *Aglenchus* de la rizosfera del árbol de arce de Montpellier, en la región de Sepidar, Kohgiluyeh y provincia Boyer-Ahmad, Irán. *Aglenchus microstylus* n. sp. es un pequeño nematodo que se caracteriza por la presencia de tres incisuras en cada campo lateral, estilete de 7-8  $\mu\text{m}$  de longitud, saco uterino post-vulvar corto, aletas laterales vulvares, vagina recta y la cola alargada conoide, con punta terminal finamente redondeada. La nueva especie se diferencia de todas las especies conocidas del género, y de los más estrechamente relacionados *A. dakotensis* y *A. geraerti*, por tener un estilete corto (7-8  $\mu\text{m}$ ), un corto saco uterino posterior a la vulva y la vagina recta. Se presenta una clave modificada para la identificación de las especies del género *Aglenchus*.

*Palabras claves:* *Acer monspessulanum* subsp. *cinerascens*, *Aglenchus microstylus*, morfología, morfometría, nueva especie, taxonomía.

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### INTRODUCTION

First described as *Tylenchus* (*Aglenchus*), Andrassy (1954) proposed *Aglenchus* as one of three sub-genera of *Tylenchus* Bastian, 1865, which was then raised to genus by Meyl (1961). Goodey (1962) regarded *Aglenchus* as a sub-genus of *Tylenchus*, but with some amendments and addition of one more species. Later on, Andrassy (1963) revalidated *Aglenchus* as a genus and designated *A. agricola*

as its type species (Andrassy, 1980). Bello (1971), Golden *et al.* (1971), and Siddiqi (1971) regarded *Aglenchus* as a valid genus and differentiated it from the genus *Tylenchus* by the presence of offset head, well-marked labial annules, prominent stylet knobs, median pharyngeal bulb, and the presence of lateral vulval flaps.

Andrassy (1968) transferred *A. machadoi* (Andrassy, 1963) Andrassy, 1968 to a new genus *Malenchus* and designated *A. machadoi* as its type

species, *M. machadoi*. The genus *Coslenchus* was proposed by Siddiqi (1978) for *A. costatus* de Man, 1921, which was characterized by longitudinal cuticular ridges on the body and the position of vulva at right angles to its body axis. Geraert (2008) discussed *Aglenchus* in detail and summarized a list of eight well-established species, *A. agricola* (de Man, 1884) Meyl, 1961; *A. ainakamurae* Mizukubo, 1989; *A. andrassyi* Sultan, 1986; *A. dakotensis* Geraert and Raski, 1988; *A. geraerti* Mizukubo, 1989; *A. mardanensis* Maqbool, Shahina and Zarina, 1984, under *Aglenchus*. The two other species, *A. agricola* var. *aquaticus* (Micoletzky 1922) Ebsary 1991 and *A. parvulus* Husain 1967 are regarded as *species inquirendae*.

There is only one report of the genus *Aglenchus* from Iran (Panahandeh *et al.*, 2014). They characterized the Iranian population of *A. agricola* by its body length (542–659  $\mu\text{m}$ ), stylet length (10–12  $\mu\text{m}$ ), distance of anterior end to excretory pore (70–87  $\mu\text{m}$ ), tail length (170–198  $\mu\text{m}$ ), prominent lateral fields (with four lines), and rare males with 15–20  $\mu\text{m}$  long spicules. In the course of a study on plant-parasitic nematodes associated with forest trees in Kohgiluyeh and Boyer-Ahmad province, Iran, an undescribed species belonging to the genus *Aglenchus* was recovered. The new species is described and illustrated herein as *A. microstylus* n. sp.

## MATERIALS AND METHODS

Soil samples were collected from several places in the province of Kohgiluyeh and Boyer-Ahmad. After labelling, samples were transported to the laboratory and stored at 5–10°C until the nematodes were extracted by using the tray method (Whitehead and Hemming, 1965). The collected nematodes were killed by adding 4% formaldehyde solution and mounted on the glass slides using the Seinhorst method (Seinhorst 1959, 1962, 1966). Photomicrographs were taken with a USB AM7023 Dino-Eye digital camera coupled to an Olympus BX31 compound light microscope, and the nematode drawings were made with the aid of a drawing tube attached to the microscope. Measurements were taken using Dino-Lite Pro software. Identification was done using available key (Geraert, 2008).

## RESULTS

### SYSTEMATICS

#### *Aglenchus microstylus* n. sp.

(Table 1; Fig. 1 and 2)

#### Description

*Female.* Small nematodes, straight or slightly ventrally curved when heat killed. Body cuticle

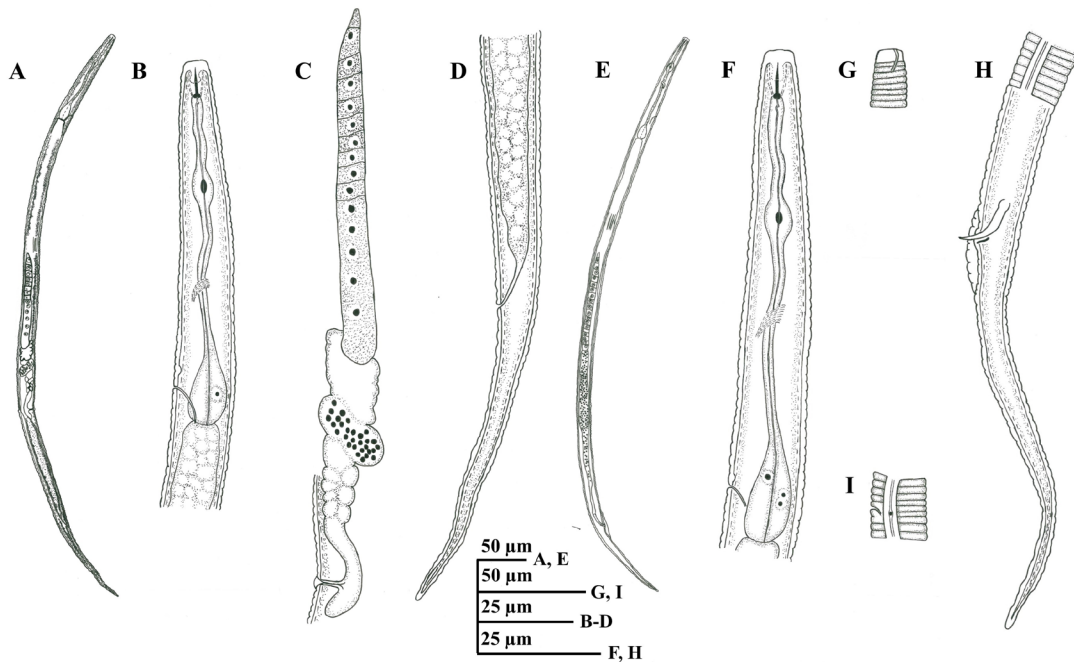


Fig. 1. *Aglenchus microstylus* n. sp. A-D: Female. A: Whole body; B: Pharyngeal region; C: Reproductive system; D: Posterior end. E-I: Male. E: Whole body; F: Pharyngeal region; G: Anterior end; H: Posterior end; I: Deirid and secretory-excretory pore.

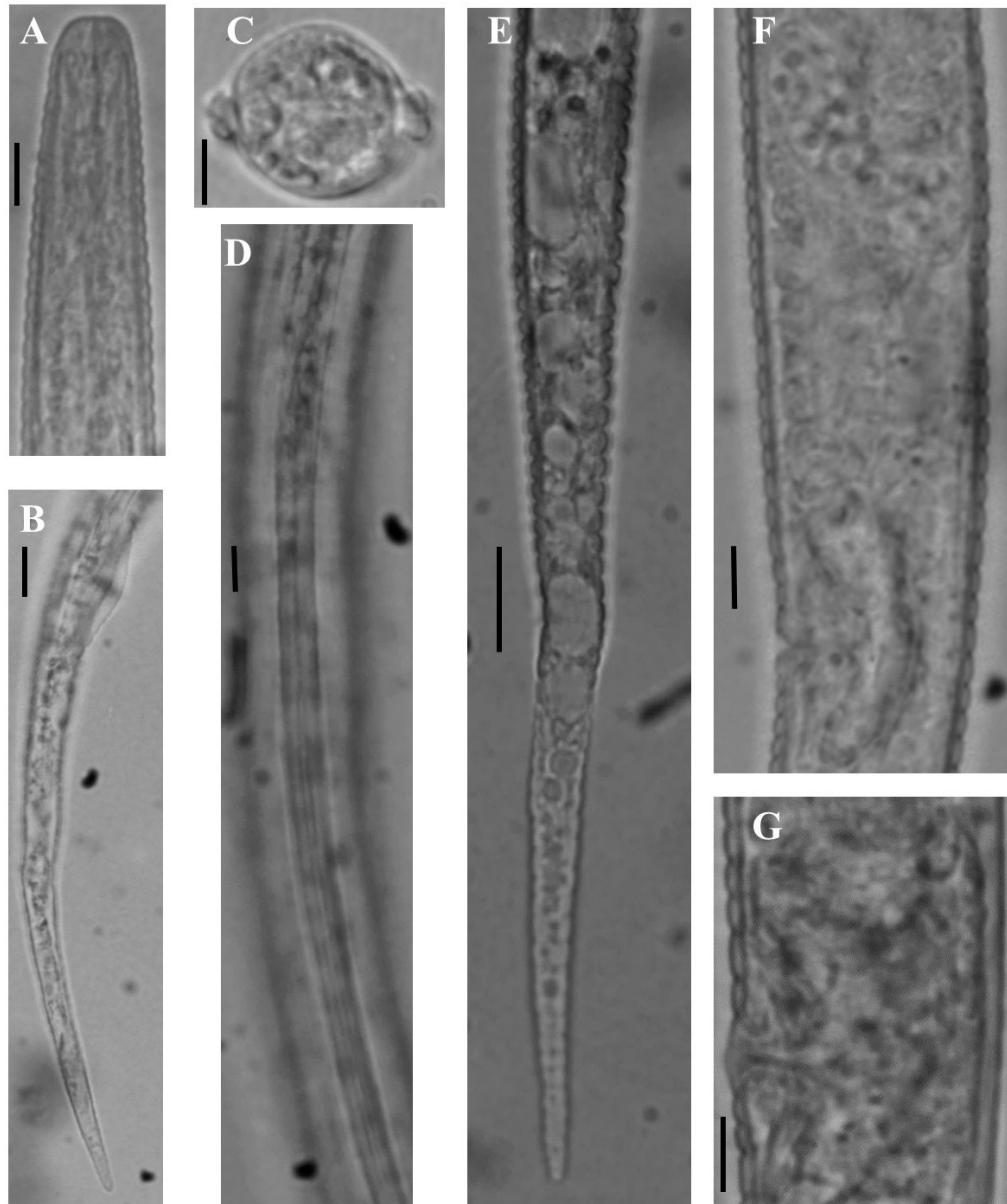


Fig. 2. Light micrographs of *Aglenchus microstylus* n. sp. A, B: Male. A: head region; B: tail region showing bursa and tail tip; C-G: Female. C: mid-body cross section; D: lateral field; E: tail; F: vulval region showing post-vulval uterine sac, spermatheca and crustaformeria; G: vulval region showing vulval flaps, vagina and post-vulval uterine sac (scale bars: A, C, D, F, G = 5 µm; B, E = 10 µm).

coarsely annulated, annuli 1.7–2.3 µm wide at mid-body and 1.8–2.5 µm wide at the tail region. Lateral field 2.6–4.5 µm wide, three lines and two bands in lateral field, in some specimens these two bands separate, resulting in appearance of four lines. Head elevated, not offset, without annulation; about as high as wide at its base; cephalic framework weakly sclerotized. Lip region with prominent and amalgamated lips, 6–7 µm wide and 2.2–3.3 µm high. Amphidial aperture crescent-shape, starts from near the oral opening. Stylet short, 7–8 µm,

with rounded small basal knobs, conus as long as or slightly shorter than half of total stylet length (ca. 38–47%). Distance between stylet base and dorsal pharyngeal gland orifice (DGO) is short (1.1–1.3 µm). Procorpus cylindrical, shorter than the slender isthmus. Median bulb oval at 40–54 µm from anterior end, 5–7 µm in width, with distinct valve. Terminal bulb pyriform, 17–26 µm long and 8–12 µm wide, abutting intestine, cardia inconspicuous. Nerve ring 56–94 µm from the anterior end, located at mid-isthmus. Hemizonid 1–2.5 µm anterior to excretory

pore. Deirid at the level of or posterior to excretory pore, in the middle of lateral field. Excretory pore at the level of basal bulb or just anterior. Reproductive system monodelphic, ovary outstretched, oocytes in a single row, quadricolumellate crustaformera with three or four cells in each row. Spermatheca with two slightly offset lobes, usually filled with rounded sperm cells, followed by oviduct, which is as wide as or slightly wider than ovary. Vulva with distinct lateral flaps. Vagina 4–8  $\mu\text{m}$  long, perpendicular to body axis. Post-vulval uterine sac short, 8–10  $\mu\text{m}$  long. Rectum long and thin, anus distinct; tail elongated, conical; tail tip variable in shape, from conical pointed to finely rounded.

*Male.* Morphology similar to that of female, but amphids are longer and laterally elongated. Testis single and outstretched, occupying 52–58% of body length; spermatocytes in a single row. Sperm rounded with amoeboid movement. Spicules tylenchoid, ventrally arcuate, with offset head; gubernaculum simple; bursa adanal, 18–35  $\mu\text{m}$  long. Cloaca simple, papilla-like structure near the cloaca is absent. Cloacal lips 2–2.3  $\mu\text{m}$  long forming a pointed tube. Tail elongate-conoid, with annulated terminus; tail tip variable in shape, from finely to broadly rounded.

#### *Type specimens*

Holotype female has been deposited in the Yasouj University Nematode Collection (YUNC) (slide no. 121). Paratypes on slide WT 3647 at WaNeCo, Plant Protection Service, Department of Nematology, Wageningen, The Netherlands (three females and two males) and on slides numbers 122–124 (fifteen females and ten males) in Yasouj University Nematode Collection (YUNC).

#### *Type host and locality*

*IRAN:* Sepidar region, 34 km from Yasouj, the capital city of Kohgiluyeh and Boyer-Ahmad Province in Iran (30°31'40.7" N; 51°28'55.7" E). Nematodes were taken from a soil sample collected from the rhizosphere of Montpellier maple tree, *Acer monspessulanum* L. subsp. *cinerascens* (Boiss.) Yalt., by Manouchehr Husseinvand, 7.viii.2013.

#### *Diagnosis and relationships*

*Aglenchus microstylus* n. sp. is a small-sized nematode characterized by its small stylet, 7–8  $\mu\text{m}$  long; monodelphic with a short post-vulval uterine sac, lateral vulval flaps, and straight vagina. On the basis of stylet length, the genus *Aglenchus* contains two groups of species. In one group, stylet length is

9–13  $\mu\text{m}$  and in the other 13.5–16.5  $\mu\text{m}$  (Geraert, 2008). The length of stylet in *Aglenchus microstylus* n. sp. is 7–8  $\mu\text{m}$ , so it can be placed in the first group. The other species with relatively small stylet are: *A. dakotensis*, *A. mardanensis*, *A. agricola*, *A. mukhtii*, *A. ainakamurae*, and *A. geraerti*. In the other two species of this genus, *A. siddiqii* and *A. andrassyi*, the stylet is longer. Tail length is a good character for separating the species of the genus *Aglenchus*. In *A. microstylus* n. sp., tail length is 83–99  $\mu\text{m}$ , similar to *A. dakotensis* with a tail length of 79–107  $\mu\text{m}$ , but the stylet in *A. microstylus* n. sp. is shorter (7–8 vs. 9–11  $\mu\text{m}$  in *A. dakotensis*). Other differences between these two species include length of pharynx (98–150 vs. 72–83  $\mu\text{m}$ ) and spicule length (15–18 vs. 11–14  $\mu\text{m}$ ).

*Aglenchus microstylus* n. sp. differs from *A. ainakamurae* in MB% (36–46.4 vs. 46–50), including *A. geraerti* in this comparison, they differ in V (60.5–64.7 vs. 46–50.4 and 52–56, respectively) and c (6.1–7.2 vs. 2.5–2.8 and 2.9–3.4, respectively). The post-vulval uterine sac is an important character for diagnosing *A. microstylus* n. sp. from other species of the genus. Based on Geraert and Raski (1987), in the genera *Aglenchus* and *Coslenchus* Siddiqi, 1978, the post-vulval uterine sac is either rudimentary or absent, but Siddiqi (2000) believed that *Aglenchus* lacks a post-vulval uterine sac. Although a short post-vulval uterine sac is mentioned for the genus *Aglenchus* in the diagnostic key for the genera of Tylenchidae (Geraert 2008), none of the described species have any post-vulval uterine sac. Members of the genus *Aglenchus* can be confused with the species of the genus *Allotylenchus* Andrassy 1984, but *Allotylenchus* has two lateral lines as opposed to three in *Aglenchus*. This is a critical character that separates these two genera from each other.

#### *Etymology*

The species epithet refers to the short stylet of the species.

## DISCUSSION

In reviewing the genus *Tylenchus*, Bastian (1865) and Andrassy (1954) established four subgenera: *Tylenchus*, *Aglenchus*, *Filenchus* Andrassy, 1954 and *Lelenchus* Andrassy, 1954. Meyl (1961) raised these subgenera to generic rank. Considering the presence of lateral vulval membranes, Wood (1973) agreed with Meyl (1961) on the generic status of *Aglenchus*. Based on the characteristics of the head (broad cephalic region, head is continuous or slightly offset and weakly sclerotized) and the presence of lateral



Table 1. Morphometric characters of *Aglenchus microstylus* n. sp. from Iran. Measurements are in micrometers ( $\mu\text{m}$ ) and in the form: mean  $\pm$  sd (range).

Character	Holotype female	Holotype male	Paratype females	Paratype males
n			10	7
L	603	595	603 $\pm$ 22.1 (573-645)	578 $\pm$ 36 (532-618)
L'	504	498	513 $\pm$ 23.8 (483-553)	482 $\pm$ 34 (431-516)
a	38.9	47.6	34.9 $\pm$ 2.8 (30.9-38.9)	42 $\pm$ 4.2 (36.2-47.6)
b	5.7	5.8	5.6 $\pm$ 0.6 (4.1-6.1)	5.5 $\pm$ 1.7 (4.6-6.3)
c	6.1	6.1	6.7 $\pm$ 0.4 (6.1-7.2)	6.1 $\pm$ 0.4 (5.3-6.8)
c'	9.9	8.4	9.1 $\pm$ 0.8 (7.9-10.3)	8.7 $\pm$ 0.5 (8.1-9.2)
V	63.5	-	63.3 $\pm$ 1.2 (60.5-64.7)	-
V'	76	-	74.9 $\pm$ 1.6 (70.5-76)	-
Stylet	7	7.7	7.7 $\pm$ 0.4 (7-8.3)	7.8 $\pm$ 0.3 (7.3-8)
m <sup>2</sup>	44.3	45.5	42.5 $\pm$ 3 (37.8-47.4)	39.2 $\pm$ 3.6 (35.4-45.5)
DGO	1.7	0.9	1.4 $\pm$ 0.3 (1-1.8)	1.2 $\pm$ 0.3 (0.9-1.7)
Pharynx	106	102	110 $\pm$ 15.7 (98-150)	105 $\pm$ 10 (89-118)
MB%	40.6	47.1	40.8 $\pm$ 2.8 (36-46.4)	43.7 $\pm$ 3 (40.2-47.2)
S.E. pore	85	90	91.4 $\pm$ 15.2 (81-130)	85.6 $\pm$ 8.9 (77-103)
Deirid	89	92	81 $\pm$ 13.4 (60-94)	79 $\pm$ 10.8 (69-92)
Head-vulva	383	-	382 $\pm$ 12.5 (364-398)	-
Tail/vulva-anus	0.8	-	0.7 $\pm$ 0.1 (0.6-0.8)	-
Tail length	99	97	91 $\pm$ 4.3 (83-99)	96 $\pm$ 6.5 (87-103)
Spicule	-	16	-	16.3 $\pm$ 0.9 (15-17.7)
Gubernaculum	-	4.6	-	4.1 $\pm$ 0.7 (2.7-4.7)
Body width	15.5	12.5	17.4 $\pm$ 1.5 (15.4-19.5)	13.9 $\pm$ 1.6 (12-16.5)
Vulval body width	13.8	-	15.1 $\pm$ 1.1 (13.8-17)	-
Anal body width	10	11.6	10.1 $\pm$ 0.6 (9-10.9)	11 $\pm$ 0.5 (9-10.9)
Lateral field/BW%	19.4	21.6	20.5 $\pm$ 3.3 (15.6-25)	26.2 $\pm$ 2.9 (21.6-30)

<sup>2</sup>m: (conus/stylet%)

vulval flaps in females, as well as large papilla-like structures at the cloacal opening of males, Geraert and Raski (1987) concluded that *Atylenchus* Cobb, 1913; *Aglenchus* Andr ssy, 1954; *Antarctenchus* Spaul, 1972; *Coslenchus* and *Pleurotylenchus* Szczygiel 1969, can be put in one subfamily, Atylenchinae Skarbilovich 1959. *Antarctenchus* and *Aglenchus* can be separated from the other three genera in that it contains two ovaries and lacks longitudinal cuticular ridges on the body (Geraert 2008). Brzeski (1998) followed the classification of Geraert and Raski (1987), but Siddiqi (2000) kept the genus *Aglenchus* in the subfamily Tylenchinae, and Andr ssy (2007) followed the latter classification. The genus *Aglenchus* can be separated from the genera *Filenchus* and *Tylenchus* by the presence of

lateral vulval flaps. It may be separated from *Fraglenchus* Siddiqi 2000 by having three lines vs. four lines in the lateral fields and longer amphid apertures in males, which do not extend on lateral sides of the cephalic region in *Fraglenchus*.

Our population differs from all known species of the genus *Aglenchus* by having a shorter stylet, a short post-vulval uterine sac, and a straight vagina. However, some species in the genus *Coslenchus* have a short post-vulval uterine sac and the vagina is perpendicular to the body axis (e.g., *C. turkeyensis* Siddiqi 1980, *C. pastor* Andr ssy 1982), while many of the species of *Coslenchus* completely lack the sac, and the vagina is bent anteriorly. The vulval flaps in our population are not prominent, when compared with other known species of *Coslenchus*, but such

reduction of the vulval flaps also can be observed in some species of *Coslenchus* (e.g., *C. rugosus* Andrassy 1982, *C. rhombus* Andrassy, 1982, *C. alacinatus* Siddiqi, 1981); a similar trend may occur in the genus *Aglenchus*. Considering the elongated amphidial aperture in males, lateral field structure and presence of vulval flaps, it may be concluded that the morphometric characters of the present

population fit well with the diagnostic characters of the genus *Aglenchus*.

#### ACKNOWLEDGMENTS

This research was supported by Post Graduate Office of Yasouj University, Iran. The authors thank staff of this office for their valuable assistance.

#### Key to species of *Aglenchus*

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|--|-----------------------|
| 1 A short post-vulval uterine sac present; Stylet < 9 $\mu\text{m}$    | <i>A. microstylus</i> |
| A post-vulval uterine sac completely lacking; Stylet > 9 $\mu\text{m}$ | 2                     |
| 2 Stylet = 13.5–16.5 $\mu\text{m}$                                     | 3                     |
| Stylet = 9–13 $\mu\text{m}$  | 4                     |
| 3 Pharynx = 119 $\mu\text{m}$  | <i>A. siddiqii</i>    |
| Pharynx = 71–77 $\mu\text{m}$  | <i>A. andrassyi</i>   |
| 4 Tail = 79–107 $\mu\text{m}$ ; stylet = 9–11 $\mu\text{m}$            | <i>A. dakotensis</i>  |
| Tail = 96–136 $\mu\text{m}$ ; stylet = 10.5–12 $\mu\text{m}$           | <i>A. mardanensis</i> |
| Tail = 134–179 $\mu\text{m}$ ; stylet = 11–13 $\mu\text{m}$            | <i>A. agricola</i>    |
| Tail = 178–276 $\mu\text{m}$ ; stylet = 10.5–13 $\mu\text{m}$          | 5                     |
| 5 Tail terminus finely rounded   | <i>A. muktii</i>      |
| Tail terminus hair-like to needle-like                                 | 6                     |
| 6 MB = 46–50%; c = 2.5–2.8; V = 46–50%, V' = 74–79%                    | <i>A. ainakamurae</i> |
| MB = 52–56%; c = 2.9–3.4; V = 52–56%, V' = 78–82%                      | <i>A. geraerti</i>    |

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Received:

22/II/2016

Accepted for publication:

05/IV/2016

Recibido:

Aceptado para publicación: