

## Research Article

# Description of *Ditylenchus azarbaijanensis* n. sp. (Tylenchomorpha: Anguinidae) from West Azarbaijan province, northwest Iran

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**Abstract:** *Ditylenchus azarbaijanensis* n. sp. is described and illustrated based upon morphological and morphometric characters. The new species is mainly characterized by having six lines in the lateral field, rudimentary postuterine sac (PUS) ca. 0.2 vulval body width long, anteriorly inclined vagina and conical female tail with a pointed terminus, 48-70  $\mu\text{m}$  long ( $c = 15-21$ ,  $c\% = 2.4-3.7$ ). It is further characterized by having fine stylet 7.5-10.0  $\mu\text{m}$  long with small knobs, small pyriform pharyngeal bulb offset from the intestine, and males with 26.5-31.0  $\mu\text{m}$  long spicules. By having a rudimentary PUS, the new species is morphologically compared with three closely related species, namely *Ditylenchus apus*, *D. deiridus* and *D. khani*. Moreover, morphological differences of the new species with eight similar species of the genus, namely *D. arachis*, *D. convallarie*, *D. dauniae*, *D. medicaginis*, *D. silvaticus*, *D. tenuidens*, *D. valveus*, and *D. virtudesae* are presented. The status of the rudimentary or absent PUS of *D. deiridus* and the new species is also discussed.

**Keywords:** *Diptenchus*, *Ditylenchus deiridus*, *D. khani*, morphology, morphometric data, new species, taxonomy

## Introduction

The genus *Ditylenchus* Filipjev, 1936 belongs to the family Anguinidae Nicoll, 1935, and the superfamily Tylenchoidea Örley, 1880. Several authors have already revised the genus and have given the list of the valid species (Siddiqi, 2000; Andrassy, 2007; Brzeski, 1991). Hashemi and Karegar (2019) presented the list of 63 species under the genus *Ditylenchus sensu stricto*. The species lacking a post-vulval-uterine sac (PUS) is already placed under the genus *Diptenchus* Khan, Chawla & Seshadri, 1969. Accordingly, in the present study, it was regarded as the synonym of *Ditylenchus*. From plant pathological points of

view, only a few species of *Ditylenchus* are parasites of higher plants, and the majority have a mycophagous habit (Sturhan and Brzeski, 1991). The majority of plant-parasitic species are endoparasites of aerial parts, roots, rhizomes, tubers, stolons, and tubers. They dissolve the middle lamellae of the cell walls so that the cells separate and the tissues swell (Sturhan and Brzeski, 1991). The type species of *Ditylenchus*, *D. dipsaci* (Kühn, 1857) Filipjev, 1936, infects more than 500 plant species worldwide (Caubel and Pedron, 1976).

Representatives of the genus are widely distributed over the country, and so far, 38 species of the genus *Ditylenchus* have been described or reported from Iran (Aliverdi *et al.*, 2021; Esmaeili *et al.*, 2017a, 2017b; Mirbabaei Karani *et al.*, 2017; Hashemi *et al.*, 2017; Hashemi *et al.*, 2018; Shokoohi *et al.*, 2018 a,b; Yaghoubi *et al.*, 2018; Hashemi and Karegar, 2019).

Handling Editor: Zahra Tanhamaafi

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Received: 19 April 2021, Accepted: 11 October 2021  
Published online: 22 November 2021

During our survey in West Azarbaijan province, a new species of the genus *Ditylenchus* was obtained from the sun spurge's rhizosphere. In the present study, it is described and illustrated by using morphological criteria.

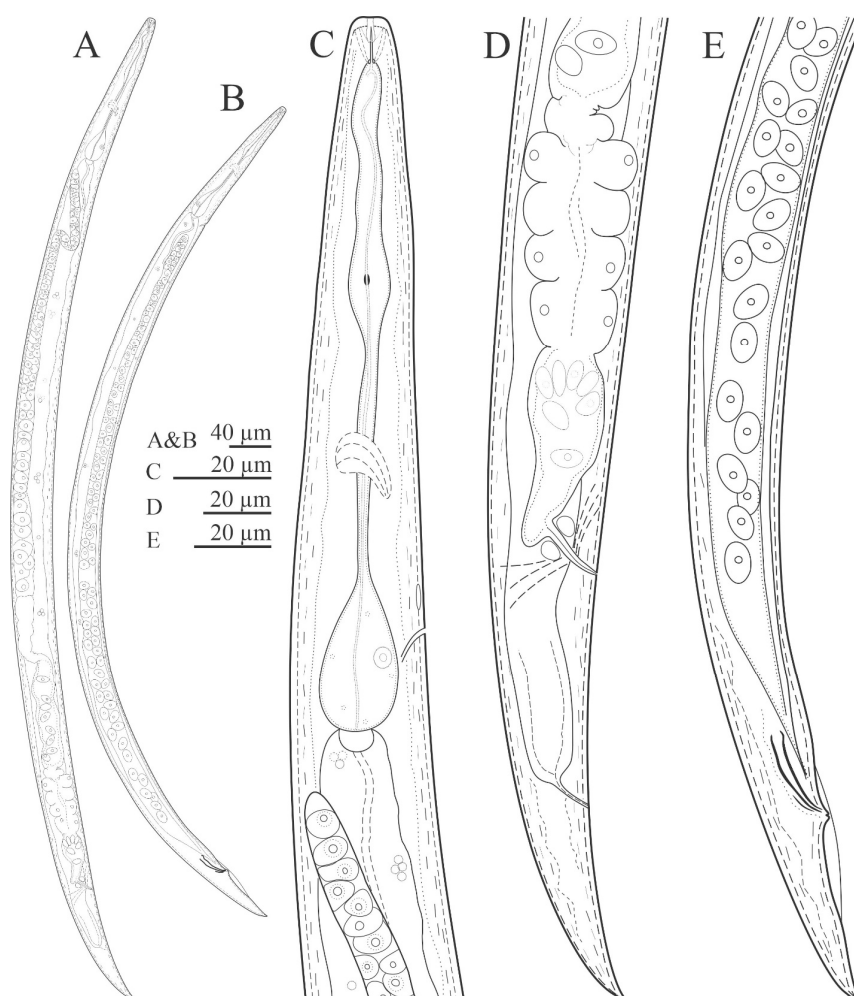
# Materials and Methods

Several soil samples were collected from grasslands in West Azarbaijan province, northwest Iran, during 2015. The tray method (Whitehead and Hemming, 1965) was used to extract the nematodes from soil samples. Nematodes of interest were handpicked under a Nikon SMZ1000 stereomicroscope, heat-killed by adding hot 4% formalin solution, transferred

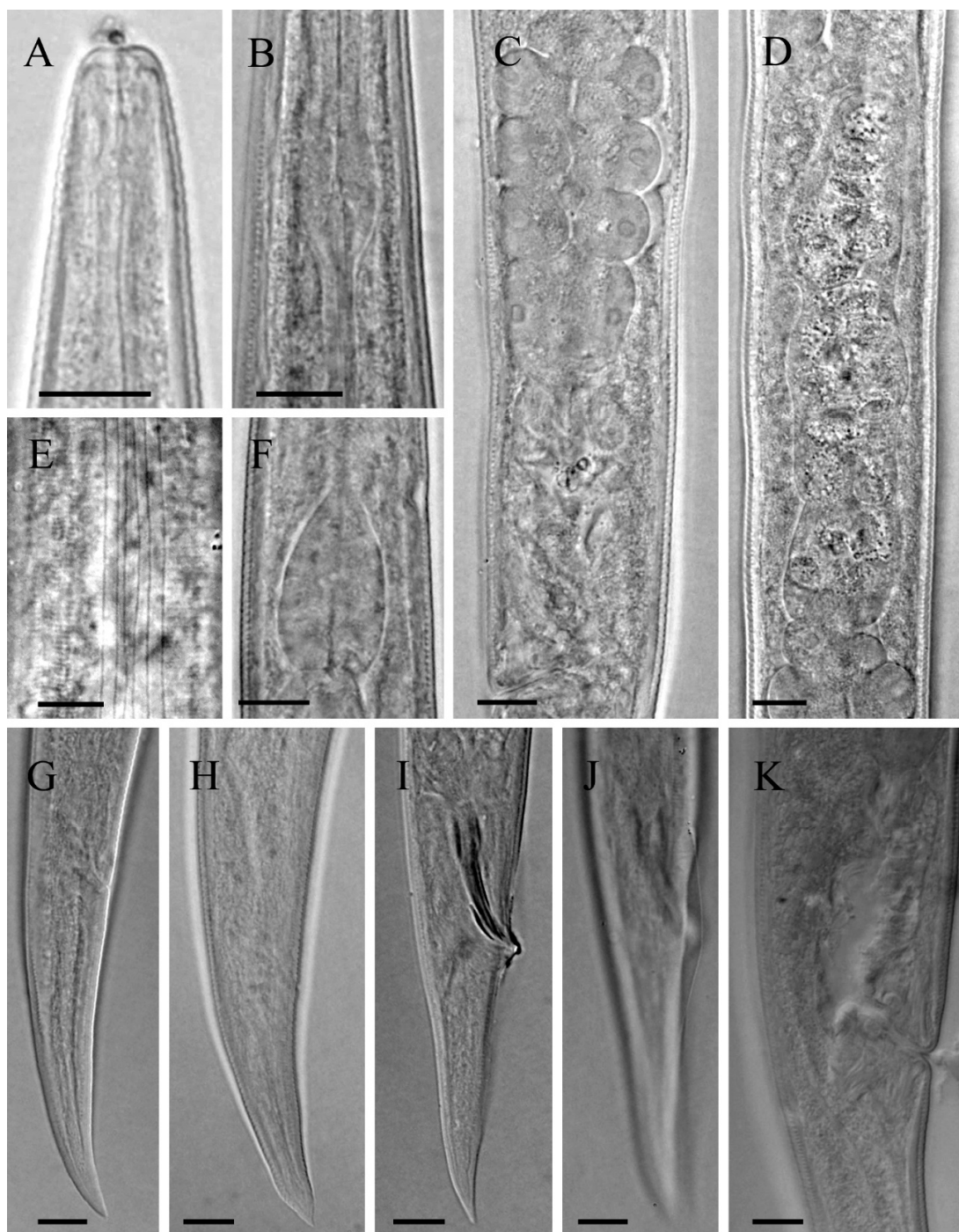
to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. The permanent slides were examined using a Nikon Eclipse E600 light microscope. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope powered with differential interference contrast (DIC). Drawings were made using a drawing tube attached to the microscope and were redrawn using the CorelDRAW® software version 18.

# Results

*Ditylenchus azarbaijanensis* n. sp.  
(Figs 1 and 2).



**Figure 1** Line drawings of *Ditylenchus azarbaijanensis* n. sp. A & B: Entire body of female and male; C: Anterior body region of female; D: Posterior body region of female; E: Posterior body region of male.



**Figure 2** Light microphotographs of *Ditylenchus azarbaijanensis* n. sp. A-H, K: Female, I & J: Male. A: Anterior body region; B: Part of the pharynx, showing procorpus; C: Part of the reproductive system, the quadricolumella crustaformeria; D: Spermatheca; E: Lateral field; F: Basal pharyngeal bulb; G-J: Tail; K: Vulval region. (All scale bars = 10  $\mu$ m).

### Measurements

See Table 1.

**Table 1** Morphometrics of *Ditylenchus azarbaijanensis* n. sp.

| Entries                   | Holotype | Paratypes                   |                          |
|---------------------------|----------|-----------------------------|--------------------------|
|                           | Female   | Females                     | Males                    |
| n                         | 1        | 14                          | 3                        |
| L                         | 999      | 1041 ± 98 (781 - 1184)      | 926 ± 20 (906 - 947)     |
| a                         | 24.4     | 26.8 ± 4.4 (19.5 - 35.5)    | 26.4 ± 2.4 (23.7 - 28.0) |
| b                         | 6.8      | 6.7 ± 0.3 (6.0 - 7.2)       | 6.5 ± 0.5 (6.0 - 7.0)    |
| c                         | 20       | 18.0 ± 1.7 (15 - 21)        | 15.1 ± 0.3 (14.8 - 15.4) |
| c□                        | 2.5      | 3.0 ± 0.4 (2.4 - 3.7)       | 3.2 ± 0.3 (3.0 - 3.5)    |
| V                         | 88.8     | 87.3 ± 1.5 (82.6 - 88.9)    | —                        |
| V□                        | 93.5     | 92.4 ± 1.3 (88.5 - 93.5)    | —                        |
| Cephalic region width     | 8        | 8.2 ± 1.0 (7 - 10)          | 6.3 ± 0.6 (6 - 7)        |
| Cephalic region height    | 2        | 2.2 ± 0.4 (2 - 3)           | 2.0 ± 0.0 (2 - 2)        |
| Stylet length             | 10.1     | 8.5 ± 0.7 (7.5 - 10.0)      | 9.0 ± 0.2 (9.0 - 9.2)    |
| Conus                     | 3.8      | 3.3 ± 0.4 (2.5 - 3.8)       | 3.5 ± 0.2 (3.3 - 3.6)    |
| m                         | 38.2     | 38.8 ± 3.6 (33.5 - 44.5)    | 38.3 ± 1.8 (36.5 - 40.0) |
| MB%                       | 38.1     | 37.5 ± 1.4 (35.0 - 39.5)    | 40.8 ± 1.2 (39.5 - 42.0) |
| Nerve ring                | 98.6     | 102.3 ± 12.6 (75.2 - 126.3) | 111.3 ± 0.3 (108 - 114)  |
| Excretory pore            | 125      | 139 ± 13 (103 - 159)        | 137.5 ± 7.2 (130 - 144)  |
| Hemizonid                 | 120.8    | 132.0 ± 12.3 (99.7 - 152.0) | 130.8 ± 7.4 (123 - 138)  |
| Pharynx                   | 147      | 155 ± 12 (131 - 174)        | 144 ± 10 (132 - 152)     |
| Head - vulva              | 887      | 909 ± 96 (645 - 1041)       | —                        |
| Vulval/maximal body width | 41       | 39.4 ± 5.3 (28 - 46)        | 34 ± 4 (33 - 40)         |
| Anal body width           | 20       | 19.5 ± 1.7 (17 - 22)        | 19.3 ± 2.0 (17 - 21)     |
| Spicules                  | -        | —                           | 28.2 ± 2.5 (26.5 - 31.0) |
| Gubernaculum              | -        | —                           | 9.8 ± 1.8 (8.0 - 11.5)   |
| Tail                      | 50       | 58.0 ± 6.5 (48 - 70)        | 61.3 ± 2.3 (60 - 64)     |

All measurements are in  $\mu\text{m}$  and the form: mean  $\pm$  s. d. (range).

Abbreviations: L = total body length, a = body length/greatest body diameter, b = body length/pharynx, c = body length/tail length, c□ = tail length/tail diameter at anus or cloaca, V = The position of vulva from anterior end as a percent of body length, V□ = distance from anterior end to vulva as a percent of anterior end to anus, m = conus of stylet as a percent to the total stylet length (Siddiqi, 2000).

## Description

### Female

Body cylindrical, tapering at both ends, slightly ventrally arcuate when killed by heat. Lip region continuous with body contour, narrower than the adjacent body, about 3.5 times wider than high. Cephalic framework weakly sclerotized. The cuticle is finely annulated. Lateral field with six lines. Stylet delicate, short, the conus 33.5-44.5% of the total stylet length. Procorpus cylindroid, metacorpus

ellipsoid with a small central valve located at 38-39% of the pharyngeal length, isthmus narrow, pharyngeal bulb small, pyriform, offset from the intestine. Nerve ring enveloping isthmus at about its middle. Secretory-excretory pore (S-E pore) opening posterior to nerve ring. Hemizonid anterior to the S-E pore. Reproductive system monodelphic-prodelphic, ovary outstretched, reflexed in few specimens, oocytes arranged in a single row at the anterior, two rows at the posterior portion of ovary, oviduct tubular, spermatheca elongate, axial,



with or without sperm, crustaformeria quadricolumellate, the cells well visible, large with a distinct nucleus, vagina anteriorly inclined, extending into the body for 50-60% corresponding body width, vulva a wide transverse slit, an embryonic egg sometimes present in the uterus, PUS rudimentary, *ca.* 0.2 vulval body width long. Vulva-anus distance 62-48  $\mu\text{m}$  long, or 1.0-1.6 times the tail length, tail conoid, dorsally convex, ventrally concave, with a pointed tip.

#### Male

General morphology was similar to that of females, except for the sexual organs. Spicules paired, ventrally arcuate, weakly cephalate, with elongate manubrium and pointed distal tip. Gubernaculum simple, arcuate, less than one-third of total spicules length. Bursa extending to about mid-tail. Tail conoid, dorsally convex, with a pointed tip.

#### Type host and locality

*Ditylenchus azarbaijanensis* n. sp. was recovered from a soil sample collected around the rhizosphere of *Euphorbia helioscopia* L., in West Azarbaijan province, northwest Iran, during October 2015. GPS coordinates: 38°58.159 N, 44°54.920 E.

#### Etymology

The specific epithet refers to the province name where the new species was recovered.

#### Type material

Holotype female, 14 paratype females, and three paratype males were deposited at Nematode Collection of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. The ZooBank Life Science Identifier (LSID) for this publication is as follows: urn:lsid:zoobank.org:pub:BF336EA1-1C19-4331-9717-98F774F40952.

#### Diagnosis and relationships

*Ditylenchus azarbaijanensis* n. sp. is mainly characterized by having a rudimentary PUS and anteriorly directed vagina. It is further characterized by 1041 (781-1184)  $\mu\text{m}$  females

length with six lines in lateral field, fine stylet with small knobs, small pyriform offset pharyngeal bulb, tail conical with the pointed terminus, and males with 28.2 (26.5-31.0)  $\mu\text{m}$  spicules length. By having a rudimentary PUS, the new species is comparable with three closely related species, *D. apus* Brzeski, 1991, *D. deiridus* Thorne & Malek, 1968, and *D. khani* Fortuner, 1982. It was further compared with six-lined species of the genus having similar morphology, including *D. arachis* Zhang, Liu, Janssen, Zhang, Xiao, Li, Couvreur & Bert, 2014, *D. convallarie* Sturhan & Friedman, 1965, *D. daunia* Brzeski & Palmisano, 1990, *D. medicaginis* Wasilewska, 1965, *D. silvaticus* Brzeski, 1991, *D. tenuidens* Gritzenko, 1971, *D. valveus* Thorne & Malek, 1968 and *D. virtudesae* Tobar-Jimenez, 1964. *Ditylenchus azarbaijanensis* n. sp. differs from *D. apus* by having an offset pharyngeal bulb (*vs.* long overlap), conical tail with pointed tip (*vs.* conical with an almost cylindrical distal part, ending to a rounded tip), greater *c* value (18 (15-21) *vs.* 10.8 (9.6-10.9)), lower *c* $\square$  value (3.0 (2.4-3.7) *vs.* (4.1-5.3)) and greater *V* value (87.3 (82.6-88.9) *vs.* (75-76)). It could be separated from *D. deiridus* by having six *vs.* four lateral lines and a lower *c* $\square$  value (3.0 (2.4-3.7) *vs.* 3.9). Compared to *D. khani*, the new species has a different number of lateral lines (six *vs.* four), lower *c* value (18 (15-21) *vs.* (12-15)), and longer spicules (28.2 (26.5-31.0) *vs.* 22  $\mu\text{m}$ ). The new species differs from *D. arachis* by having slightly longer females (1041 (781-1184) *vs.* 893 (680-1007)  $\mu\text{m}$ ), offset pharyngeal bulb *vs.* shortly overlapping intestine, vulval slit inclined anteriorly *vs.* perpendicular to the body axis, rudimentary *vs.* well developed PUS 57 (41-65)  $\mu\text{m}$  long, tail tip not ventrally bent and longer spicules (28.2 (26.5-31.0) *vs.* 21 (16-24)  $\mu\text{m}$ ). Compared to *D. convallarie*, it has a lower *a* value (26.8 (19.5-35.5) *vs.* 47(36-54)), greater *c* value (18 (15-21) *vs.* 13.5 (13-14)), greater *V* value (87.3 (82.6-88.9) *vs.* 77 (74-79)), shorter stylet (8.5 (7.5-10.10) *vs.* 12.7 (11.5-13.5)  $\mu\text{m}$ ), rudimentary *vs.* well-developed PUS, tail with pointed *vs.* sharp terminus and longer spicules (28.2 (26.5-31.0)

vs. 20-26  $\mu\text{m}$ ). *Ditylenchus azarbaijanensis* n. sp. differs from *D. daunia* by slightly longer females (1041 (781-1184) vs. 748 (640-942)  $\mu\text{m}$ ), rudimentary vs. well-developed PUS 39 (32-57)  $\mu\text{m}$  long, vagina inclined anteriorly (vs. perpendicular) and longer spicules (28.2 (26.5-31.0) vs. 17 (15-19)  $\mu\text{m}$ ). It differs from *D. medicaginis* by longer females (1041 (781-1184) vs. 720 (650-680)  $\mu\text{m}$ ), greater c value (18.0 (15-21) vs. 11.2 (10.1-12.5)), greater V value (87.3 (82.6-88.9) vs. 77 (76-81)), longer spicules (28.2 (26.5-31.0) vs. 16.5  $\mu\text{m}$ ) and rudimentary vs. well-developed PUS, 2.2-2.5 times vulval body width long.

It differs from *D. silvaticus* by longer females (1041 (781-1184) vs. 602 (560-660)  $\mu\text{m}$ ), with longer pharynx (155 (131-174) vs. 104 (96-108)  $\mu\text{m}$ ), posteriorly located S-E pore (139 (103-159) vs. 90 (82-97)  $\mu\text{m}$  from anterior end), greater c value (18 (15.0-21.0) vs. 10.6 (9.2-12.6)), lower c $\square$  value (3.0 (2.4-3.7) vs. 5.0 (4.2-6.3)), greater V value (87.3 (82.6-88.9) vs. 79 (78-81)) and rudimentary vs. developed PUS. Compared to *D. tenuidens*, it could be separated by greater c value (18 (15-21) vs. (9.0-12.7)), lower c $\square$  value (3.0 (2.4-3.7) vs. (4.1-6.8)), longer spicules (28.2 (26.5-31.0) vs. (15-19)  $\mu\text{m}$ ) and rudimentary vs. small PUS. It differs from *D. valveus* (data after Anderson, 1983) by greater V value (87.3 (82.6-88.9) vs. 79-82), greater c value (18 (15-21) vs. 8.4-12.0) and rudimentary vs. 23-36  $\mu\text{m}$  long PUS. And finally, the new species has longer females (1041 (781-1184) vs. 400 (370-450)  $\mu\text{m}$ ), rudimentary vs. well-developed PUS, greater V value (87.3 (82.6-88.9) vs. (79.9-81.7)  $\mu\text{m}$ ), pointed vs. rounded to dull tail tip, and longer spicules (28.2 (26.5-31.0) vs. 11.4  $\mu\text{m}$ ) compared to *D. virtudesae*.

### Remarks

In the original description of *Ditylenchus deridus*, Thorne and Malek (1968) described the species without PUS, unlike the drawing showing a rudimentary PUS. However, it was regarded as absent by Hashemi and Karegar (2019). Similarly, the new species also has a rudimentary PUS. The term "postuterine sac"

was used in the present study instead of "post-vulval uterine sac", as the vagina of the new species is long and anteriorly directed, yielding to an anterior placement of PUS to the vulva.

### Acknowledgments

This research was financially supported by Tarbiat Modares University.

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**Ditylenchus azarbaijanensis n. sp. (Tylenchomorpha: Anguinidae) توصیف گونه از استان****آذربایجان غربی، شمال غرب ایران**

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دریافت: ۳۰ فروردین ۱۴۰۰؛ پذیرش: ۱۹ مهر ۱۴۰۰

**چکیده:** گونه *Ditylenchus azarbaijanensis* n. sp. براساس خصوصیات ریخت‌شناختی و ریخت‌سنجی توصیف شده است. گونه جدید عمده‌تاً به دلیل داشتن شش شیار در باند جانبی، کیسه عقبی رحم (PUS) کوچک و رشد نیافته به طول حدود ۲/۰ عرض بدن در ناحیه فرج، واژن مایل به سمت جلو و دم مخروطی نماتد ماده به طول ۴۸ تا ۷۰ میکرومتر (۷/۳ تا ۴/۲ c و ۱۵ تا ۲۱ c) با انتهای تیز شناخته می‌شود. سایر ویژگی‌های این گونه به قرار زیر است: داشتن استایلت ضعیف به طول ۵/۷ تا ۱۰/۰ میکرومتر با گره‌های کوچک، مری گلابی شکل کوچک مماس با روده و طول اسپیکول ۲۶/۵ تا ۳۱ میکرومتر. براساس وجود کیسه عقبی رحم رشد نیافته، گونه جدید با سه گونه مرتبط به نام‌های *D. khani* و *D. deiridus* *Ditylenchus apus* مورد مقایسه ریخت‌شناسی قرار گرفت. تفاوت‌های ریخت‌شناسی و ریخت‌سنجی گونه جدید و هشت گونه مشابه شامل *D. convallarie* *D. arachis* *D. virtudesae* *D. valveus*، *D. tenuidens* *D. silvaticus* *D. medicaginis* *D. daunia* قرار گرفته است.

**واژگان کلیدی:** داده‌های ریخت‌سنجی، تاکسونومی، گونه جدید، ریخت‌شناسی، *Ditylenchus*

*D. khani* *Ditylenchus deiridus*