

## New record of two species belonging to superfamily Sphaerularioidea (Nematoda: Rhabditida) from Iran

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**Abstract:** Morphological and morphometric characters of two species of superfamily Sphaerularioidea, recovered in southwestern Iran namely *Deladenus durus* and *Prothallonema obtusum* are given. The recovered specimens of *D. durus* are characterized by having a valvular chamber in metacarpus and excretory pore posterior to the hemizonid. The Iranian population of *P. obtusum* is characterized by having four lines in the lateral field, excretory pore anterior to the end of pharynx, not bulgy vulva region and short subcylindrical tail.

**Keywords:** *Deladenus durus*, *Prothallonema obtusum*, *Pistacia atlantica*, Iran.

### Introduction

More than 30 nematode families have been known to be insect-associated or parasites of insects (Nickle, 1972, Poinar, 1975, 1983, 1990, Maggenti, 1981 and Kaya and Stock, 1997). Some groups of nematodes are obligate insect parasites or entomopathogens which can infect and kill a broad range of insect hosts. However, due to biocontrol potential of these nematodes, several studies have focused on seven families namely Mermithidae Braun, 1883, Allantonematidae Pereira, 1931, Neotylenchidae Thorne, 1941, Sphaerularidae Lubbock, 1861, Rhabditidae Öerley, 1880, Steinernematidae Filipjev, 1934 and Heterorhabditidae Poinar, 1975 (Grewal *et al.*, 2005). Members of Steinernematidae and Heterorhabditidae have attracted more attention as biocontrol agents of insect pests, compared to the members of the other families (Lacey *et al.*, 2001).

The superfamily Sphaerularioidea with its 232 species comprising three families Neotylenchidae, Sphaerularidae and Allantonematidae belongs to the suborder Hexatylinea Siddiqi, 1980 (with 298 species). Members of this superfamily usually possess two types of generation *i.e.* entomoparasitic (insect or mite haemocoel parasitic) and free-living (fungus-feeder or plant-parasitic) forms (Andrássy, 2007). However, some may have only one type of generation (Siddiqi, 2000).

The main characters of the family Neotylenchidae are, having long pharyngeal overlapping and a well developed bursa. The seven genera of this family contain 46 species (Andrássy, 2007). Currently, three of these species namely *Fergusobia indica* (Jairajpuri, 1962) Siddiqi, 1986, *Gymnotylenchus zae* Siddiqi, 1961 and *Hexatylus mulveyi* Das, 1964 have been recorded from Iran (Barooti *et al.*, 2002, Barooti and Golzar, 1993 and Kheiri, 1972a, respectively).

The main characters of the family Sphaerulariidae are presence of a very long uterus in fertilized females and bulb-like

Handling Editor: Dr. Majid Pedram

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Received: 21 April 2013, Accepted: 30 September 2013

pharyngeal glands, forming a short lobe over intestine; often with a stem-like extension. This family has ten genera and 47 species (Andrássy, 2007).

There are reports on occurrence of three species of this family namely *Prothallonema asymmetricum* (Thorne, 1941) Siddiqi, 1986, *P. intermedium* (Christie, 1938) Siddiqi, 1986 and *P. mucronatum* (Thorne and Malek, 1968) Siddiqi, 1986 in Iran (Mirzaee-qomi *et al.*, 2010, Motamedi *et al.*, 2004 and Gharakhani *et al.*, 2010, respectively).

This study aims to discuss on morphological characters and morphometric features of two species belonging to the superfamily Sphaerularioidea, recovered from Jiroft region, Kerman province of Iran.

## Materials and Methods

A total number of 100 soil and root samples were collected from several orchards during 2007-2008. The nematodes were extracted using sieving and centrifugal-flotation techniques (Jenkins, 1964). The permanent slides of nematodes were prepared after fixation and transferring to anhydrous glycerin (De Grisse, 1969). Identification of the recovered nematodes was carried out using morphological and morphometric characters. The measurements and drawings were performed using a drawing tube attached to an Olympus BH-2 light microscope.

## Results and discussion

Among several genera belonging to different orders, two species belonging to the suborder Hexatylinea (superfamily Sphaerularioidea) were recovered and identified. One of the recovered species, *Deladenus durus* (Cobb, 1922) Thorne, 1941 belongs to the family Neotylenchidae and the other species, *Prothallonema obtusum* (Thorne, 1941) Siddiqi, 1986 belongs to the family Sphaerulariidae. The morphological and morphometric characters of the two above mentioned species are presented herein for the first time.

## *Deladenus durus* (Cobb, 1922) Thorne, 1941 (Table 1; Fig. 1)

### Description

*Mycetophagous female*: Body slender, tapering gradually toward both ends, straight or slightly curved ventrally after fixation. Cuticle with fine annuli, about 1  $\mu\text{m}$  wide at mid-body. Lateral field with seven incisures. Lip region smooth, low and continuous with body contour. Head framework weakly sclerotized. Stylet delicate, 8-10  $\mu\text{m}$  long with distinct basal knobs. Dorsal pharyngeal gland orifice near to the base of stylet. Procorpus cylindrical, metacarpus slightly swollen, with oval median chamber, 5  $\mu\text{m}$  long and 2.5  $\mu\text{m}$  wide, isthmus narrow, cylindrical, dorsal pharyngeal gland elongate, overlapping intestine dorsally with distinct nucleus, the subventral glands obscure. Excretory pore 128  $\mu\text{m}$  from the anterior end, its duct heavily sclerotized. Deirids located near excretory pore. Hemizonid 4  $\mu\text{m}$  long, 5  $\mu\text{m}$  anterior to the excretory pore. Nerve ring at the posterior portion of isthmus, about 88  $\mu\text{m}$  from the anterior end. Intestine joins pharynx at the level of the nerve ring. Ovary outstretched, vulva situated posterior at about 90% of the body length with protuberant lips, post-vulval uterine sac absent, vagina anteriorly directed, spermatheca axial, elongate and filled with rounded sperm. Tail conoid, slightly longer than vulva-anus distance, with a small mucro.

*Male*: Similar to mycetophagus female with exception in reproductive system. Body more slender. Spicules and gubernaculum tylenchoid, bursa terminal, not enveloping the mucronate tip of the tail.

### Habitat and locality

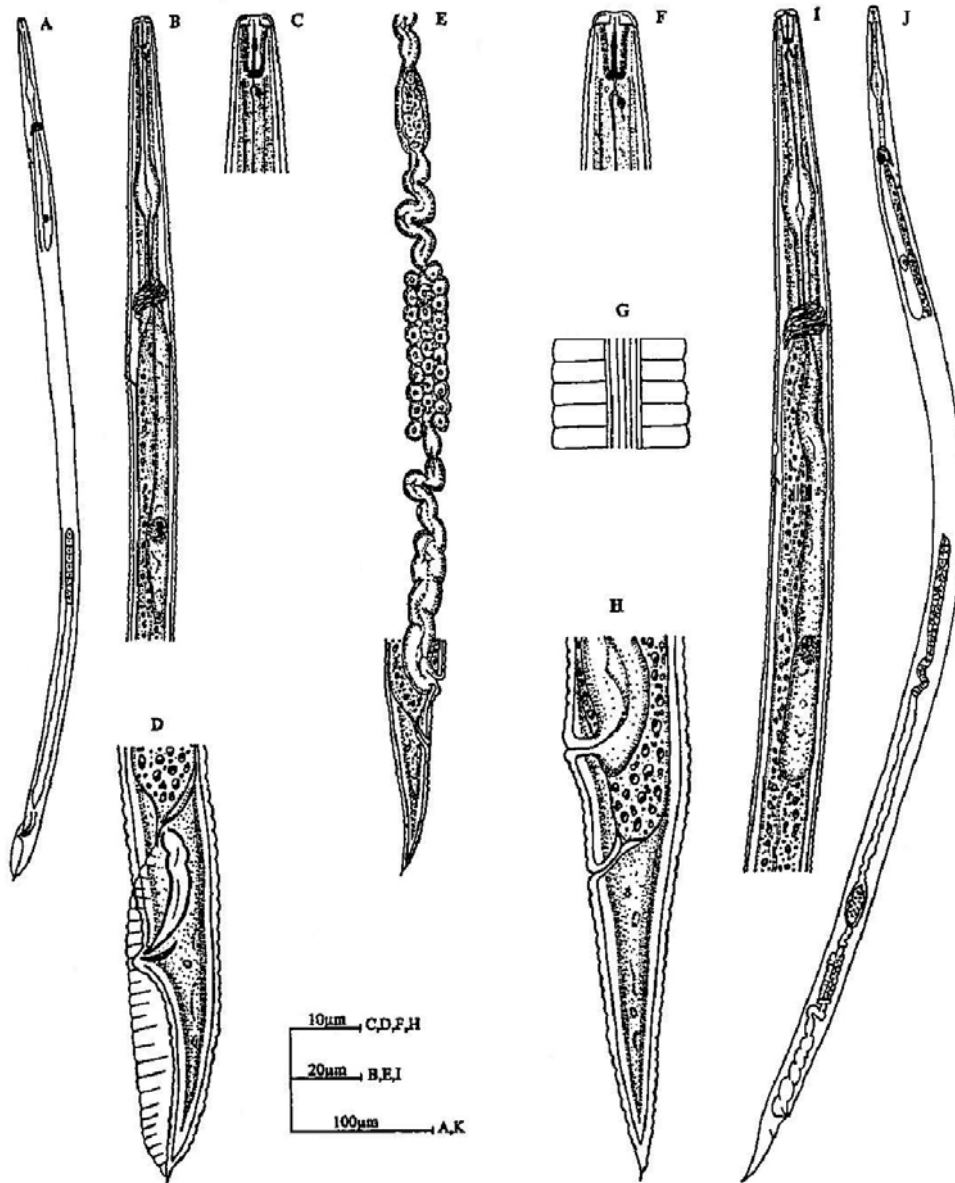
*Deladenus durus* was originally described as *Tylenchus durus* by Cobb (1922), recovered from galls of swamp chestnut-oak (*Quercus prinus* L.) and decomposing fungi (*Pleurotus* sp.) beneath dead bark of Fremont poplar (*Populus fremonti* S. Wats) from Virginia in USA. It was later recovered by Thorne (1941) from the rhizosphere of Lucerne in Utah and Oregon (USA). It has also been found in sandy

soil infested with wild grass rosettes from Canada (Das, 1964), Italy (Vinciguerra, 1972), and India (Bajaj et al., 1981). In present study,

this species was recovered from the rhizosphere of wild pistachio or Baneh (*Pistacia atlantica*) in Jiroft region of Iran.

**Table 1** Morphometric data of the Iranian population of *Deladenus durus* and data of other populations reported by different authors (All measurements are given in micrometer and in the form mean ± SD (range)).

Species (the original names are used)	<i>D. durus</i> (Cobb, 1992) Thorne, 1941		<i>Tylenchus durus</i> Cobb (1922)		<i>D. durus</i> Thorne (1941)		<i>D. durus</i> Das (1964)	<i>D. andrassyi</i> Vinciguerra (1972)	<i>D. durus</i> (Massey, 1974) Chitamber, 1991	<i>D. paradurus</i> Bajaj (1981)	<i>D. durus</i> Chitamber (1991)
Habitat	rhizosphere of <i>Pistacia atlantica</i>		Galls of ( <i>Quercus prinus</i> L.)		Rhizosphere of Lucerne		wild grass rosettes	Moss on rock	<i>Dendroctonus adjunctus</i> in ponderosa pine	Soil around roots of wheat	Population cultured on <i>Coleophoma</i> sp.
locality	Kermen, Iran		Virginia (USA)		Utah and Oregon		Canada	Italy	Ruidoso New Mexico	India	Las Cruces, New Mexico
Characters	♀	♂	♀	♂	♀	♂	♀	♀	♀	♀	♀
n	5	2	1	1	-	-	8	3	4	7	45
L	880±25 (840-910)	525-605	820	1000	1000	1000	980-1360	1080-1120	874±100.6 (796-944)	620-910	915±35 (724-1232)
L'	842±25 (800-867)	500-575	-	-	-	-	-	-	-	-	-
a	45.4±2.0 (42.0-46.8)	50.4-52.3	37	37	30-50	50	29-39	27-32	38±6.6 (34-44)	35-47	35±2.2 (21-50)
b	9.6±0.4 (9.1-10.2)	7.7-7.9	7.7	7.7	8-10	8-10	7.8-9.3	?	9.8±1.3 (8.6-10.4)	3.7-5	10.7±0.4 (8.7-16)
b'	4±0.5 (3.4-4.7)	3.7-3.7	-	-	-	-	-	-	4.4±0.8 (3.7-4.8)	-	5.9±0.5 (1.6-12.1)
c	23±1.3 (21.5-25)	20.2-20.9	20.8	62.5	25	24	19.5-23.6	22-24	24.6±2.4 (22.4-25.9)	21-28	27.8±1.3 (20.9-39.3)
c'	3.4±0.2 (3.2-3.6)	2.3-2.7	2.5	0.8	-	-	-	-	2.9±0.5 (2.6-3.3)	-	2.2±0.1 (1.6-2.8)
V or T	93.4±0.2 (93.2-93.7)	36.2-42.8	93	-	93	65	92-93	90	93±1.2 (91-93)	90-93	93±0.3 (90-95)
V'	97.7±0.3 (97.4-98.1)	-	-	-	-	-	-	-	-	-	-
Stylet length	8-9	8	6	10	8	-	8.4-10.8	10-11	8.5±0.2 (8.4-8.6)	7-8	9.6±0.2 (8-11)
Pharyngeal length	91.6±3.2 (86-94)	68-77	-	-	-	-	-	-	-	-	-
Overlapping	131±20.9 (100-155)	75-85	-	-	-	-	-	-	-	-	-
Max. body diam.	19.4±0.5 (19-20)	10-12	22	-	-	-	-	-	23±3.6 (22-26)	-	26±2.3 (16-52)
Head-vulva	822±2 (785-845)	-	-	-	-	-	-	-	-	-	-
Vulva-anus distance	19.8±2.9 (15-22)	-	-	-	-	-	-	40	31±3.5 (28-33)	-	28±1.3 (18-41)
Tail	38.4±2.1 (35-40)	25-30	33	16	-	41.7	-	33	36±2.9 (34-38)	-	34±1.5 (21-43)
Tail/ vulva-anus (%)	2±0.4 (1.7-2.6)	-	-	-	-	-	-	-	-	-	-
body diam. at anus	11.4±0.5 (11-12)	11	14	20	-	-	-	-	12±1.4 (12-13)	-	16±0.7 (11-22)
Spicule length	-	15-19	-	24	-	-	-	-	-	-	-
Gubemaculum	-	6	-	-	-	-	-	-	-	-	-



**Figure 1** *Deladenus durus*: Male (A-D), Female (E-J). A & J: Entire body; B & I: Oesophageal region; C & F: Anterior; D: Tail; E: Reproductive system; G: Lateral field; H: Posterior end.

### Discussion

The morphological and morphometric characters of this species is similar to those given in the original description (Cobb, 1922) and those given in the revision of the species (Thorne 1941) except for shorter body length of

male (525-605 vs. 1000 µm). Chitamber (1991) synonymised *D. paradurus* Massey, 1974 and *D. andrassy* Vinciguerra, 1972 with *D. durus*. Iranian population of *D. durus* resembles *D. ipini* Massey, 1974, *D. ulani* Sultanalieva, 1983, *D. obesus* Thorne, 1941, *D. aridus* Andrassy, 1957 and *D. indicus* Singh, 1976 but

it differs from *D. ipini* and *D. ulani* by absence of post-vulval uterine sac. It also differs from *D. obesus*, *D. aridus* and *D. indicus* by having an oval chamber in the median bulb. In addition, this species is closely related to *D. nevexii* Bedding, 1974 and *D. apopkaetus* Chitamber, 1991 but differs from those species by anterior location of excretory pore to the hemizonid vs posterior (Chitamber, 1991, Sumenkova, 1989).

***Prothallonema obtusum* (Thorne, 1941) Siddiqi, 1986**  
(Table 2; Fig. 2)

**Description**

*Mycetophagous female:* Body relatively long, slightly ventrally arcuate after fixation, obese (the widest part of body is anterior to the vulva), gradually tapering toward both ends. Cuticle with fine transverse striae (1.5 µm wide). Lip region rounded, smooth, continuous, 9-11 µm wide and 2-3 µm high. Lateral field with four incisures. Stylet slightly longer than lip region width with asymmetrical knobs (ventro-submedian knobs larger than the dorsal knob). The dorsal pharyngeal gland orifice located at the base of the stylet. Procorpus cylindrical, metacarpus slightly swollen without a valve, basal bulb of pharynx oval with conical extension. Excretory pore anterior to the junction of pharynx with the intestine almost at the level of base of pharynx, excretory duct sclerotized. Hemizonid 5 µm long and 9 µm anterior to excretory pore. Deirids between hemizonid and excretory pore. Vulva as a depressed transverse slit, vagina extending about one-third the body width into the body, uterus broad, thin-walled, spermatheca elongate, axial and filled with large (2.5-3.5 µm) sperm, ovary anteriorly directed with reflexed tip, post-vulval uterine sac absent. Tail subcylindrical with rounded terminus, about twice as long as the anal body diameter.

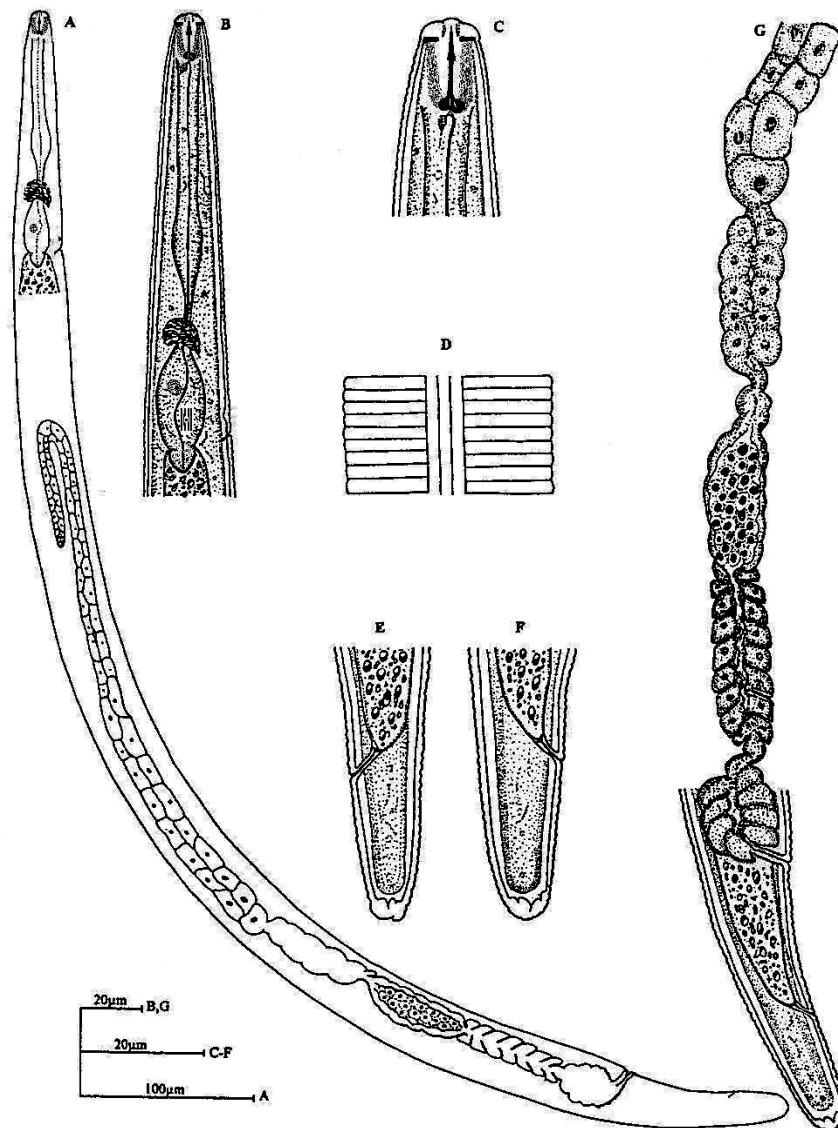
*Male:* Not found.

**Table 2** Measurements of the Iranian population of *Prothallonema obtusum* and its comparison with type population (All measurements are given in micrometer).

Species	<i>P. obtusum</i> (Thorne, 1941) Siddiqi, 1986	<i>Stictylus obtusum</i> , Thorne (1941)
Habitat	rhizosphere of peach and apple	rhizosphere of sugar beets
locality	Kermen, Iran	Utah (USA)
Characters	Female	Female
n	5	-
L	807 ± 85.2 (680-900)	1000
L'	767.4 ± 86.9 (636-860)	-
a	26.2 ± 1.8 (24.3-29)	25
b	6.3 ± 0.5 (5.7-7.1)	6.4
b'	5.6 ± 0.5 (4.8-6.1)	-
c	19.6 ± 1.7 (17-20.9)	18
c'	2.3 ± 0.3 (2.0-2.8)	-
V	88.8 ± 0.9 (87.5-89.7)	90
V'	93.5 ± 0.2 (93.3-93.8)	-
Stylet length	11.9 ± 0.7 (11-13)	-
Pharyngeal length	129 ± 13.5 (110-143)	-
Overlapping	14.6 ± 6.5 (5-21)	-
Excretory pore	123 ± 10.4 (107-133)	-
Max. body diam.	30.8 ± 2.4 (28-34)	-
Head-vulva	718 ± 81.7 (595-807)	-
Vulva-anus distance	50 ± 5.5 (41-55)	-
Tail	41.2 ± 1.6 (39-43)	-
Tail/ Vulva-anus (%)	0.8 ± 0.1 (0.8-1)	-
Body diam. at anus	17.4 ± 0.9 (16-18)	-

**Habitat and locality**

This species was originally described by Thorne (1941), recovered from the rhizosphere of sugar beets in Utah (USA). In the present study, this species was collected from the rhizosphere of peach and apple trees in Jiroft region of Iran.



**Figure 2** *Prothallonema obtusum*: Female (A-G). A: Entire body; B: Pharyngeal region; C: Anterior end; D: Lateral field; E and F: Tail; G: Part of reproductive system and the posterior end.

### Discussion

Thorne (1941), originally described the species *Stictylus obtusus*. Siddiqi (1986) synonymized the genus *Stictylus* Thorne, 1941 with *Prothallonema* Christie, 1938, because infective

females and adult female of some species of *Stictylus* (*S. hastatus* Khan, 1957) have everted uterus similar to that in *Prothallonema*. Fortuner and Raski (1987) did not accept this synonymization and proposed the genus *Stictylus* as *genus incertae sedis* in Tylenchida. Again,

Siddiqi (2000) considered *Stictylus* as a synonym of *Prothallonema*, that was accepted by Andrassy (2007).

Iranian population of *P. obtusum* (Thorne, 1941) Siddiqi, 1986 closely resembles the originally described population (Thorne, 1941). This species comes close to *P. intermedium* (Christie, 1938) Siddiqi, 1986 and *P. asymmetricum* (Thorne, 1941) Siddiqi, 1986. It can be separated from *P. intermedium* by having four lines in the lateral field (vs. six in *P. intermedium*), location of excretory pore (at the base of terminal bulb vs. anterior part of terminal bulb) and the shorter tail. Moreover, in *P. intermedium* (female) intestine continues posterior to the rectum forming a blind sac that occupies one third to one half of the tail which was not seen in *P. obtusum* (Geraert et al., 1984). Iranian population of *P. obtusum* differs from *P. asymmetricum* by having lower c value (20.7 vs. 27).

### Acknowledgements

Authors are grateful to Late Prof. István Andrassy, Dr. Akbar Karegar and Dr. Zahra Tanha Maafi for their valuable comments and Dr. Majid Pedram who read critically and improved the manuscript. The financial support of Tarbiat Modares University is also appreciated.

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## گزارش جدید از دو گونه نماتد متعلق به بالا خانواده (Nematoda: Rhabditida) Sphaerularioidea

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دریافت: ۱ اردیبهشت ۱۳۹۲؛ پذیرش: ۸ مهر ۱۳۹۲

**چکیده:** خصوصیات مرفولوژیکی و مرفومتري دو گونه *Prothallonema obtusum* و *Deladenus durus*

متعلق به بالا خانواده Sphaerularioidea از جنوب شرق ایران ارائه می‌شود. جمعیت به‌دست آمده از *D. durus* به‌واسطه وجود محفظه دریچه مانند در حباب میانی و قرار گرفتن منفذ ترشحي بعد از همی‌زونی قابل تشخیص است. جمعیت *P. obtusum* به‌واسطه داشتن چهار شیار طولی در سطح جانبی، قرار گرفتن منفذ ترشحي در جلوی بخش انتهایی مری، متورم نبودن ناحیه ولوا و کوتاه بودن دم نیمه استوانه‌ای شکل مشخص می‌شود.

**واژگان کلیدی:** *Pistacia atlantica*, *Prothallonema obtusum*, *Deladenus durus*، ایران