

A New and a Known Species of *Deladenus* Thorne, 1941 (Nematoda: Neotylenchidae) from Iran, with an Updated Species Checklist of the Genus

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Abstract: *Deladenus persicus* sp. n. and *D. apopkaetus* from Iran are described and illustrated. The new species is characterized by having a body length of 0.47-0.60 mm and 0.38-0.51 mm in females and males, respectively, seven incisures in the lateral field, excretory pore 30-38 μ m anterior to hemizonid, median chamber present in pharynx, and short conoid tail with narrowly to broadly rounded terminus males with short spicule and peloderan bursa. Regarding body size, lateral lines, excretory pore and hemizonid position, the new species closely resembles *D. minimus* but is differentiated from it by having a median chamber, shorter tail length, absence of deirids and peloderan bursa. A population of *D. apopkaetus* was found for the first time from Iran. An updated checklist for species of *Deladenus* is presented.

Key words: *Deladenus*; taxonomy; Iran; morphology; new species

Introduction

THORNE (1941) first proposed the genus *Deladenus* in the subfamily Neotylenchinae and designated *D. durus* (COBB, 1922) THORNE, 1941 as the type species. He characterized the genus as having a posterior vulva (V ratio always greater than 90%), with the pharyngo-intestinal junction immediately behind the nerve ring, the presence of a median pharyngeal chamber in some species and absence of a post-uterine sac. All species of *Deladenus* were thought to be mycetophagous until BEDDING (1968) found the infective form for some species that have two life cycles: a free-living form with typical neotylenchoid life cycle involving a mycetophagous stage and an insect-parasitic form with a typical sphaerulariid cycle. BLINOVA & KORENCHENKO (1986) transferred those species with both free-living and insect-parasitic forms to a new genus, *Beddingia*, belonging new family Phaenopsitylenchidae. However, this was not accepted by CHITAMBAR (1991), SIDDIQI (2000), FORTUNER & RASKI (1987) and ANDRASSY (2007). Currently, the genus *Deladenus* has 27 valid

species. Of these, 13 are known to have both mycetophagous and infective forms. *Deladenus* has a world-wide distribution, being recorded from Europe, Asia, Africa, North America and Australia. Up to now, only one species, *D. durus*, has been recorded from Iran (JAHANSHAHI AFSHAR *et al.* 2014).

Two populations of the genus *Deladenus* were found in northern and northwest Iran during a nematode survey in 2012-2014. One population closely resembled *Deladenus apopkaetus* CHITAMBAR, 1991 but the other one did not match any described *Deladenus* species. The occurrence of *D. apopkaetus* is reported here for the first time from Iran, and the new species is illustrated and described as *D. persicus* sp. n. A checklist for valid species of *Deladenus* is also provided.

Materials and Methods

More than 100 soil, root, bark and wood samples were collected from several orchards and pastures during

2012-2014. Nematodes were extracted from samples using the tray method (WHITEHEAD & HEMMING, 1965). The specimens were heat killed and fixed by adding boiling 4% formalin solution and transferred to anhydrous glycerin according to DE GRISSE (1969). Permanent slides were examined using an Olympus BH2 light microscope. Measurements and drawings were made with the same microscope equipped with a drawing tube and a digital camera.

Results

Deladenus persicus sp. n.

(Figs 1, 2)

Measurements: see Table 1.

Free-living female. Small sized nematodes, 0.47-0.60 mm long, fusiform, straight to irregularly curved upon heat relaxation, obese in some specimens (a = 19 and 20, respectively, in two specimens). Cuticle with indistinct transverse annulations, about 0.67–1.00 μm wide at mid-body. Lateral field 4-6 μm in diameter at mid-body, hardly visible and with seven incisures at vulval region. Cephalic region low, truncate, continuous with body contour, appearing smooth under light microscope. Stylet short, 6-8 μm long, delicate with symmetrical minute rounded knobs, cone occupying 31-43% of total stylet length. Dorsal gland orifice (DGO) just posterior to basal knobs. Pharyngeal corpus fusiform, expanding slightly to form median chamber, clearly distinct in older specimens, oval or elliptical, isthmus narrow, cylindrical, encircled by nerve ring. Pharyngeal glands overlapping anterior end of intestine, sub-ventral glands reduced, obscure in some specimens. Dorsal pharyngeal gland elongate, occupying 46-56% (70-87 μm) of total pharynx length. Excretory pore with cuticularized duct, opening always located between median chamber and nerve ring. Hemizonid poorly developed, 30 to 38 μm posterior to excretory pore. Deirids not seen. Intestinal lumen narrow in young females, broader in older females especially in posterior region. Rectum and anus distinct.

Genital system mono-prodelphic with outstretched ovary, reaching to pharyngeal glands in some specimens, oocytes arranged in single to double rows. Spermatheca not seen, crustaformeria a long tube formed by a large number of cells in four rows. Vulva posterior, at 92.0–93.7% of body length, at less than a vulval body width from anus. No post-vulval uterine sac. Uterus thick-walled, vagina anteriorly directed, vulva a wide transverse slit, vulval lips protuberant. Body constricted posterior to vulva. Phasmids absent, tail short and conoid, length always greater than vulva to anus distance, with narrowly

rounded terminus in younger and broadly rounded terminus in older specimens, without mucron.

Free-living male. General morphology similar to that of female. The body is thinner and smaller than that of females and is always straight when relaxed. Genital system monorchic, testis outstretched, occupying 52-67% of the body length, with developing spermatocytes in single to double rows, never reaching dorsal pharyngeal gland lobe. Spicules and gubernaculum typically tylenchoid and weakly developed. Bursa arising at level of anterior end of spicules, poorly developed, reaching to tail terminus. Tail short and conoid with narrowly rounded to slightly pointed terminus without mucron, slightly ventrally curved.

Infective female. Not found.

Diagnosis and relationships. *Deladenus persicus* sp. n. is characterized by its short body, 0.47-0.60 mm and 0.38-0.51 mm in female and male, respectively, delicate stylet 6-8 μm long, seven incisures in lateral field at vulval region, excretory pore opening 46-59 μm from anterior end, hemizonid 30-38 μm posterior to excretory pore. Presence of median chamber in pharynx, pharyngeal gland lobe dorsally overlapping intestine, weakly developed spicule and gubernaculum, poorly developed peloderan bursa and short conoid tail with narrowly to broadly rounded terminus.

The new species is closely related to *D. apopkaetus* CHITAMBAR, 1991, *D. cocophilus* NASIRA, SHAHINA & FIROZA, 2013, *D. minimus* CHIZHOV & STURHAN, 1998, *D. nevexii* BEDDING, 1974 and *D. valveus* YU, POPOVIC & GU, 2014. It is distinguished from *D. apopkaetus* by having a shorter body length (475-610 vs 612-840 μm and 385-515 vs 504-796 μm in female and male, respectively), shorter stylet (6-8 vs 8.7-13.5 μm long), shorter spicule (10-12 vs 14-22 μm long) and the shape of the tail terminus (narrowly to broadly rounded without mucron vs narrowly rounded or mucronate). *Deladenus persicus* sp. n. differs from *D. cocophilus* by having a slightly shorter stylet (6-8 vs 8-10 μm), presence of a median chamber in the pharynx, differing numbers of lateral lines (7 vs 6), and a shorter spicule (10-12 vs 14-16 μm). *D. minimus* is another closely related species that originally described based on mycetophagous male and infective female. It differs from the new species by lacking a pharyngeal median chamber, longer tail length ($c' = 2.9-4.4$ vs $2-2.5$), different type of bursa in male (leptoderan vs peloderan) and presence of dot-like deirids. It differs from *D. nevexii* by having a shorter body length (0.47-0.6 vs 1.42-1.98 mm and 0.38-0.51 vs 1.03-1.83 mm in female and male, respectively), slightly

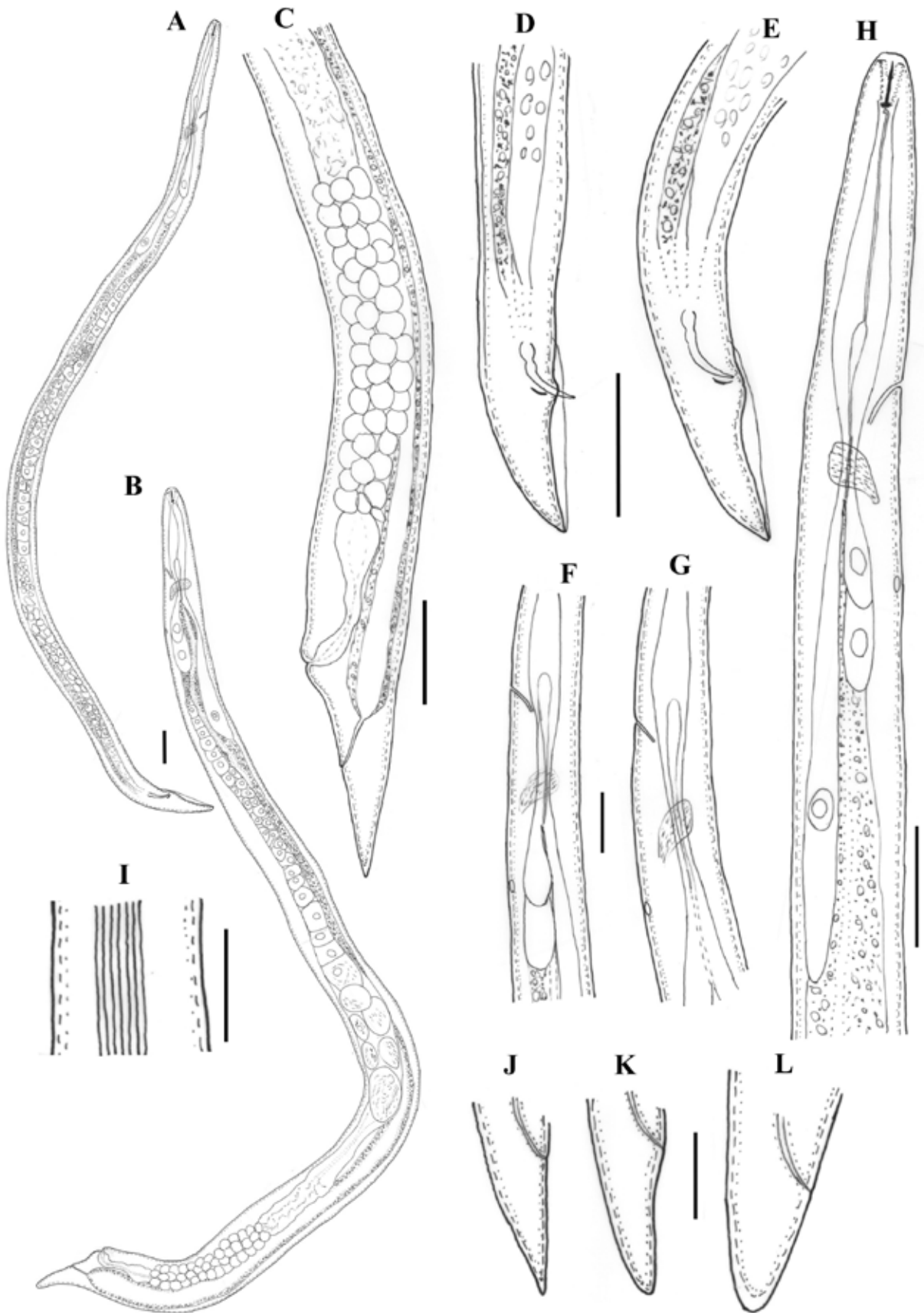


Fig. 1. *Deladenus persicus* sp. n. A, B: Entire body of male and female. C: Posterior region of female. D, E: Posterior region of males. F, G: Position of excretory pore and hemizonid. H: Female pharynx. I: Lateral field at vulval region. J-L: Tail variation. Scale bars, A-E, H: 20 μ m, F, G, I-L: 10 μ m

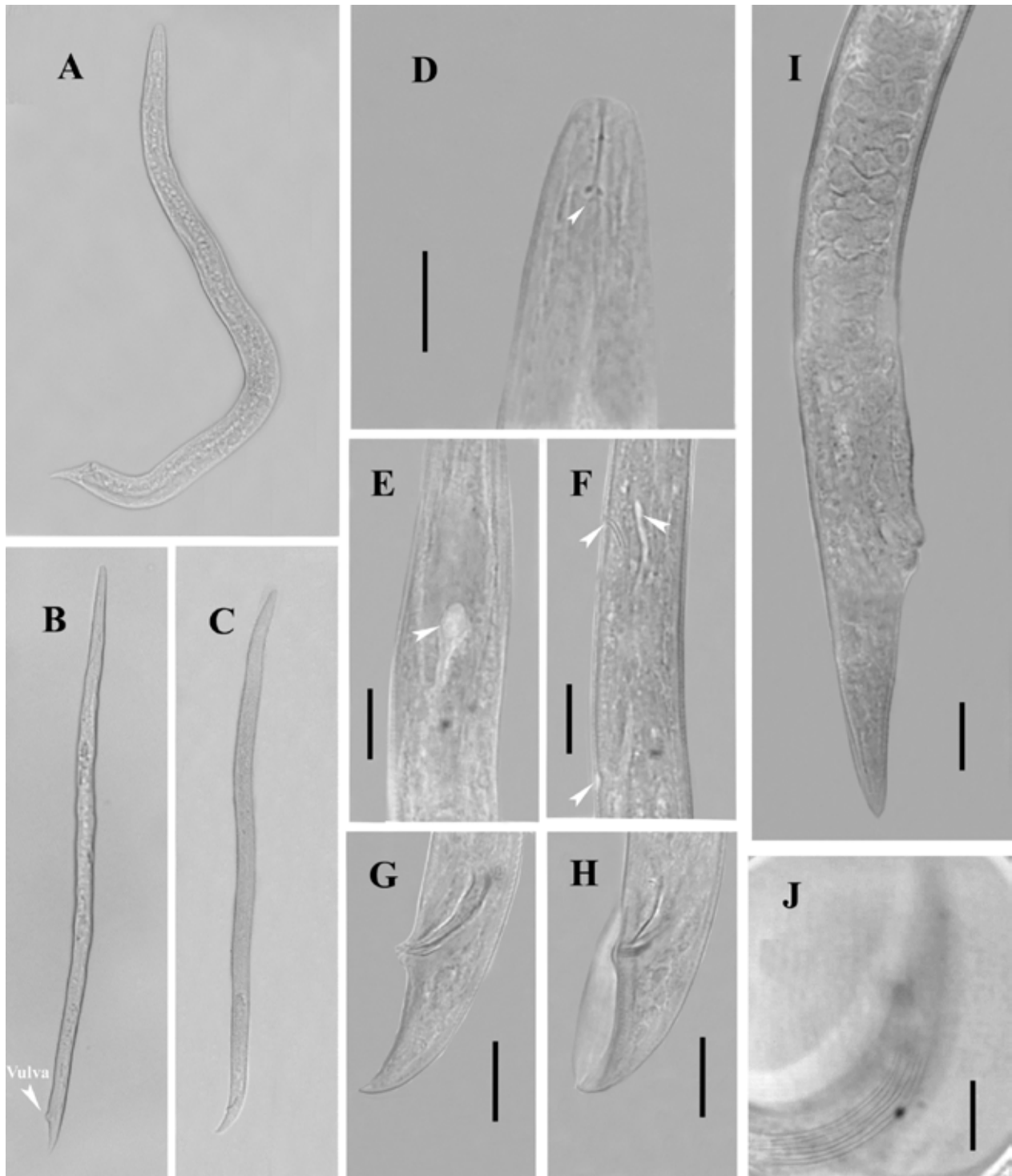


Fig. 2. Light microscopy observation of *D. persicus* sp. n. A-C: Females and male entire body. D: Anterior end (Arrow showing DGO). E, F: Position of median chamber, excretory pore and hemizonid (Arrows). G, H: Male posterior end showing bursa and spicule. I: Female genital tract. J: Lateral lines. Scale bars, D-J: 10 μ m

shorter stylet (6-8 vs 9-10 μ m) and excretory pore closer to hemizonid (30-38 vs 64-87 μ m). The new species is differentiated from *D. valveus* by having a shorter stylet (6-8 vs 9-13 μ m), differing number of lateral lines (7 vs 8), shape of the tail terminus (narrowly to broadly rounded vs pointed), poorly

developed vs well developed bursa in male, shorter spicule (10-12 vs 20-27 μ m), and presence of a sclerotized valve extending from the pharynx into the intestinal lumen in *D. valveus*.

Remarks. PCR/DNA sequencing on 28S and ITS ribosomal DNA was not successful.

Table 1. Morphometric data for *Deladenus persicus* sp. n. All measurements are in μm and in the form: mean \pm S.D. (range)

Population Characters	Holotype female	Paratype females Mycetophagous stage	Paratype males Mycetophagous stage
N		8	6
L	568	548 \pm 45 (474-609)	446 \pm 64 (384-516)
A	28.5	27 \pm 6.1 (19-36)	32 \pm 2.9 (28-35)
B	7.6	7.3 \pm 0.5 (6.5-8)	6.8 \pm 0.5 (6-9)
b'	3.7	3.6 \pm 0.3 (3-4.2)	3.4 \pm 0.3 (3-3.7)
c	28.4	26 \pm 2.6 (22-31)	21 \pm 0.4 (19-24)
c'	2	2.3 \pm 0.5 (1.8-3.3)	2.2 \pm 0.2 (2-2.5)
V or T	92.6	93 \pm 0.6 (92-93.7)	61 \pm 4.6 (60-67)
Head height	1.5	1.8 \pm 0.4 (1-2)	2.3 \pm 1 (2-4)
Head width	5	6 \pm 0.6 (5-7)	4.5 \pm 1.6 (2-5.5)
Stylet	7	7 \pm 0.5 (6-8)	7.2 \pm 0.5 (7-8)
m	35.7	36 \pm 3.3 (31-43)	36 \pm 4.8 (29-43)
Anterior end to beginning of median chamber	39	42 \pm 6.3 (35-54)	38 \pm 8 (25-44)
Nerve ring from anterior body	63	64 \pm 6.7 (52-76)	58 \pm 9.5 (43-65)
Excretory pore from anterior body	55	54 \pm 4.7 (46-59)	44 \pm 6.9 (37-52)
Distance between ex. pore to hemizonid	35	32 \pm 3.5 (28-38)	27 \pm 1.2 (25-29)
Maximum body width	20	21 \pm 5 (16-30)	14 \pm 1 (12.5-15)
Vulva – anus	22	17.5 \pm 1.4 (16-22)	—
Oesophagus	75	76 \pm 5.3 (68-85)	66 \pm 10.6 (51-75)
Overlap	79	78 \pm 5.8 (70-87)	67 \pm 8 (56-87)
Testis or ovary length	336	354 \pm 61 (252-449)	278 \pm 19 (224-313)
Anal (cloacal) body width	10	9.5 \pm 1.5 (7-12)	10 \pm 1 (9-11)
Tail length	20	21 \pm 1.5 (18-23)	21.7 \pm 3.3 (18-26)
Spicule length (arc line)	—	—	11.1 \pm 1 (10-12)
Gubernaculum	—	—	3.5 \pm 0.5 (3-4)

Type locality and habitat. Recovered from sawdust collected from holes caused by wood-boring insects in a living beech tree (*Fagus orientalis* LIPSKY) in Shamushak Forest, south of Kordkuy (GPS coordinates: N 36°72', E 54°28'), Golestan Province, northern Iran; August 2013.

Type material. Holotype female, slide NDP001 together with eight paratype specimens: four females, four males (Slides NDP001, NDP002) deposited in the Nematode Collection of the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. Two female and one male paratypes deposited at Department of Nematology, WANECO collection, Wageningen (<http://www.waneco.eu/>). One female and one male paratypes deposited at the National Nematode Collection of the Department of Nematology, Iranian Research Institute of Plant Protection, Tehran, Iran.

Deladenus apopkaetus CHITAMBAR, 1991

(Figs 3, 4)

Measurements: see Table 2.

Free-living female. Body slender or semi-

obese, straight or slightly ventrally curved after fixation. Lip region flattened, smooth and continuous with body contour. Lateral field with narrow band 5 to 6 μm wide, with six incisures. Deirids and phasmids not seen. Procorpus cylindrical, metacarpus slightly swollen, with oval median chamber. Isthmus narrow and surrounded by nerve ring *c.* 83-103 μm from anterior end. Pharyngo-intestinal junction located immediately posterior to nerve ring. Excretory pore situated 80-91 μm from head. Hemizonid 10-20 μm posterior to excretory pore. Pharyngeal glands overlapping intestine on dorsal side. Vulva a transverse slit, opening near anus, protruded only in older females. Reproductive tract prodelphic, gonad outstretched, oocytes in single row, spermatheca elongate. Crustiformeria a quadricolumella with 8 cells in each row. Post-vulval uterine sac absent. Tail conoid with narrowly rounded terminus, length equal to vulva-anus distance.

Male. Similar to females in general characteristics, with exception of reproductive system. Tail conoid, 40-42 μm long. Testis single, outstretched. Spicule tylenchoid, ventrally arcuate, 20-21 μm long. Bursa smooth, reaching tail terminus.

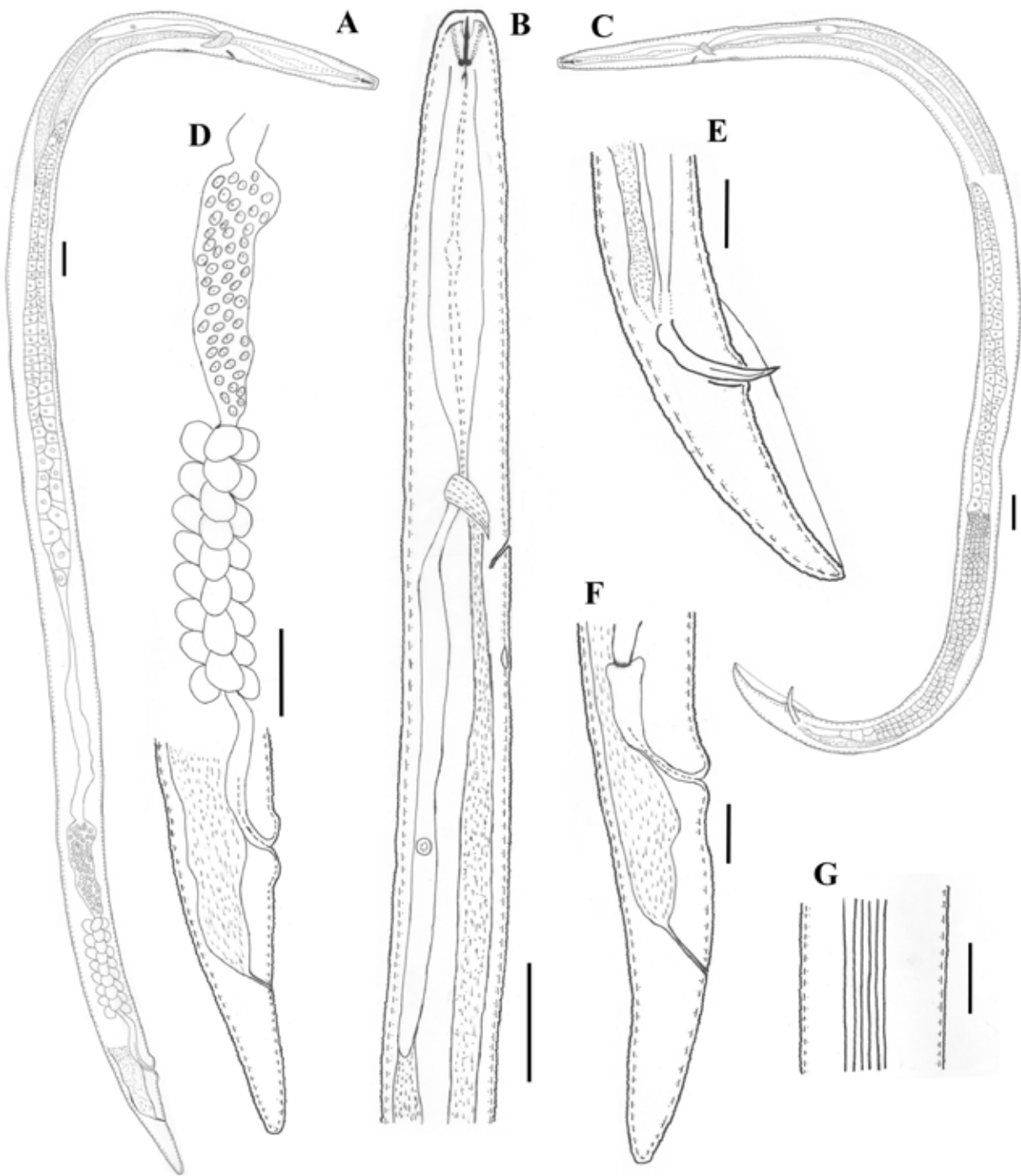


Fig. 3. *Deladenus apopkaetus*. A, C: Entire body of male and female. B: Female pharynx. D: Female genital tract. E, F: Posterior region of male and female. G: Lateral field at vulval region. Scale bars, A-D: 20 μm, E-G: 10 μm

Infective female. Not found.

Remarks. The morphological details and dimensions of the present specimens matched with those previously reported in the original description of *D. apopkaetus* by CHITAMBAR (1991) from Florida, USA except for some variations in stylet length (stylet = 8.7– 13.5 μm in CHITAMBAR 1991).

The Iranian population of *D. apopkaetus* is closest to *D. durus* (COBB, 1922) THORN, 1941. It differs from *D. durus* by the anterior vs posterior position of the excretory pore in relation to the hemizonid, and the posterior position of the nerve ring related to the excretory pore in *D. apopkaetus*. *Deladenus apopkaetus* was originally recovered from the rhizo-

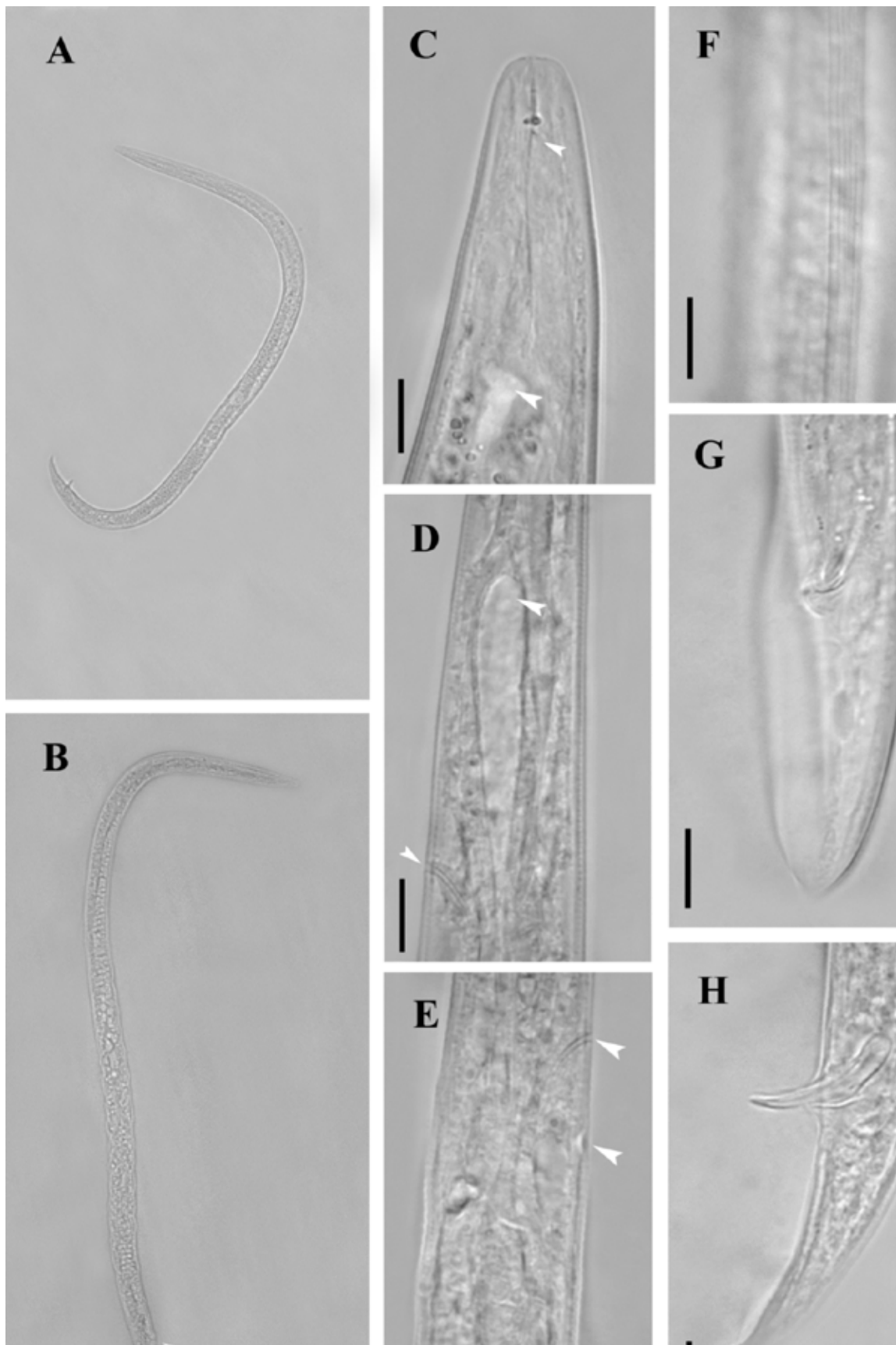


Fig. 4. Light microscopy observation of *D. apopkaetus*. A, B: Males and female entire body. C: Anterior end (Arrow showing DGO and median chamber). D, E: Position of median chamber, excretory pore and hemizonid (Arrows). F: Lateral lines. G, H: Male posterior end showing bursa and spicule. Scale bars, C-H: 10 μ m. scale

Table 2. Comparison of morphometrics of *D. apopkaetus* as described by CHITAMBAR (1991) and the present study. All measurements are in μm and in the form: mean \pm S. D. (range)

Population Characters	Iranian population		Chitambar (1991)	
	Female	Male	Female	Male
n	7	2	43	36
L	770 \pm 102 (654-900)	(605-708)	743 \pm 15.6 (612-840)	649 \pm 23 (504-796)
a	31 \pm 2.6 (26-33)	(33.6-37)	32 \pm 1.4 (21-42)	30 \pm 1.4 (21-42)
b	7.8 \pm 0.6 (6.7-7.6)	(6.3-6.6)	7.5 \pm 0.3(6.4-10)	6.9 \pm 0.2 (5.6-7.9)
c	24 \pm 5.1 (18.7-31)	(15-17)	20.3 \pm 0.6 (16.5-24.8)	17.5 \pm 0.6 (14.6-21.2)
c'	2.5 \pm 0.4 (2-3)	(3-3.3)	2.8 \pm 0.1 (2-3.5)	2.8 \pm 0.1 (2.1-3.1)
V or T	91 \pm 1.4 (90-93)	(53-55)	91 \pm 0.2 (90-93)	-
Stylet	8.1 \pm 0.2 (8-8.5)	(8-8.5)	10.9 \pm 0.5 (8.7-13.5)	9.9 \pm 0.2 (8.3-11.6)
Excretory Pore	87 \pm 5 (80-91)	(71-77)	81 \pm 2.7 (50-95)	76 \pm 2.4 (60-88)
Oesophagus	99 \pm 7.5 (90-108)	(91-112)	-	-
Hemizonid	104 \pm 9.4 (90-111)	-	(71-131)	-
Overlapping	77.6 \pm 9.5 (62-90)	(55-72)	-	-
Head-Vulva	700 \pm 97 (587-817)	-	-	-
Vulva-Anus	32 \pm 4 (30-41)	-	30 \pm 0.8 (24-35)	-
Tail	32.6 \pm 3 (29-36)	(40-42)	37 \pm 0.8 (31-43)	37 \pm 1.2 (30-46)
Spicule	-	(20-21)	-	19 \pm 0.5 (14-22)
Gubernaculum	-	(6-7)	-	-

sphere of *Dieffenbachia* sp. at Apopka, Florida, USA. In the present study, this species was collected from the rhizosphere of unidentified grasses, in Naqadeh (GPS coordinates: N 36°56', E 45°24'), West Azerbaijan Province, Northwestern Iran.

Discussion

MULVEY (1969) proposed the genus *Hadrodenus* by introducing one new species (*H. megacondylus*) and transferring *H. saccatus* (ANDRÁSSY, 1954) from *Deladenus* THORNE, 1941 and placed it in the subfamily Nothotylenchinae of the family Anguinidae *sensu* SIDDIQI (1986). He characterized *Hadrodenus* as having a well developed post-vulval uterine sac, vulva at about 77-80% of body length, and absence of insect-parasitic stages. The differences noted between the species in *Deladenus* and *Hadrodenus* were not accepted as sufficient diagnostic characters at generic level by SUMENKOVA (1975) and CHITAMBAR (1991). They proposed *Hadrodenus* as a junior synonym of *Deladenus* THORNE, 1941. SIDDIQI (2000) regarded *Hadrodenus* as genus *inquirendum* and placed its name after the family Neotylenchidae and subfamily Neotylenchinae. However, ANDRASSY (2007) accepted it as a valid genus of the family Neotylenchidae and the subfamily Neotylenchinae. Molecular studies on these genera should be performed as part of an integrated generic revision of subfamily Neotylenchinae.

ANDRASSY (2007) listed 22 valid species of

Deladenus and considered one species as *species inquirendum*. He ignored *D. ulani* SULTANALIEVA, 1983 as a valid species for either *Deladenus* or *Hadrodenus*. Subsequently, eleven new species of *Deladenus* have been described (NASIRA et al. 2013, YU et al. 2013, 2014, TOMAR et al. 2015; BAJAJ 2015; KANZAKI et al. 2016). An updated checklist of *Deladenus* spp. is presented (after ANDRASSY 2007, with additions):

Updated checklist of species of the genus *Deladenus* THORNE, 1941

Genus *Deladenus* THORNE, 1941

Synonyms: *Dotylaphus* ANDRÁSSY, 1958; *Physitylenchus* RAO & REDDY, 1982; *Beddingia* BLINOVA & KORENTCHENKO, 1986; *Phaenopsitylenchus* BLINOVA & KORENTCHENKO, 1986

Type species

1. *Deladenus durus* (COBB, 1922) THORNE, 1941

Synonyms: *Tylenchus durus* COBB, 1922; *Anguillulina dura* (COBB) GOODEY, 1932; *Ditylenchus durus* (COBB) FILIPJEV, 1936; *Deladenus andrassyi* VINCIGUERRA, 1972; *Deladenus paradurus* MASSEY, 1974

Other species

2. *Deladenus aenea* (RAO & REDDY, 1982) EBSARY, 1991¹

Synonym: *Physitylenchus aenea* RAO & REDDY, 1982

3. *D. albizicus* TOMAR, SOMVANSHI & BAJAJ, 2015*

4. *D. apopkaetus* CHITAMBAR, 1991

5. *D. arboricola* (COBB, 1922) GOODEY & FRANKLIN

¹ For those species, the infective stage is known.

in GOODEY, 1956

Synonyms: *Tylenchus arboricola* Cobb, 1922; *Anguillulina arboricola* (COBB) GOODEY, 1932; *Ditylenchus arboricola* (COBB) FILIPJEV & SCHUURMANS STEKHOVEN, 1941

6. *D. aridus* ANDRÁSSY, 1957

Synonym: *Deladenus crassus* ZELL, 1985

7. *D. canii* BEDDING, 1974*

Synonym: *Beddingia canii* (BEDDING, 1974) BLINOVA & KORENTCHENKO, 1986

8. *D. cocophilus* NASIRA, SHAHINA & FIROZA 2013*

9. *D. imperialis* BEDDING, 1974*

Synonym: *Beddingia imperialis* (BEDDING, 1974) BLINOVA & KORENTCHENKO, 1986

10. *D. indicus* SINGH, 1976

11. *D. ipini* MASSEY, 1974

12. *D. laricis* (BLINOVA & KORENTCHENKO, 1986) EBSARY, 1991

Synonym: *Phaenopsitylenchus laricis* BLINOVA & KORENTCHENKO, 1986

13. *D. leptosoma* GAGARIN, 2001

14. *D. minimus* CHIZHOV & STURHAN, 1998*

15. *D. nevexii* BEDDING, 1974*

Synonym: *Beddingia nevexii* (BEDDING, 1974) BLINOVA & KORENTCHENKO, 1986

16. *D. nitobei* KANZAKI, TANAKA, FITZA, KOSAKA, SLIPPERS, KIMURA, TSUCHIYA & TABATA, 2016*

17. *D. norimbergensis* RÜHM, 1956

18. *D. obesus* THORNE, 1941

19. *D. obtusicaudatus* BAJAJ, 2015*

20. *D. oryzae* BAJAJ, 2015*

21. *D. pakistanensis* SHAHINA & MAQBOOL, 1992

22. *D. parvus* Zell, 1985

23. *D. persicus* sp. n.

24. *D. pinusus* BAJAJ, 2015*

25. *D. processus* TOMAR, SOMVANSHI & BAJAJ, 2015

26. *D. prorsus* YU, GU & YE, 2013*

27. *D. proximus* BEDDING, 1974*

Synonym: *Beddingia proximus* (BEDDING, 1974) BLINOVA & KORENTCHENKO, 1986

28. *D. rudyi* BEDDING, 1974*

Synonym: *Beddingia rudyi* (BEDDING, 1974) BLINOVA & KORENTCHENKO, 1986

29. *D. ruehmi* (ANDRÁSSY, 1958) FORTUNER & RASKI, 1987

Synonym: *Dotylaphus ruehmi* ANDRÁSSY, 1958

30. *D. siricidicola* BEDDING, 1968*

Synonym: *Beddingia siricidicola* (BEDDING, 1968) BLINOVA & KORENTCHENKO, 1986

31. *D. uteropinusus* BAJAJ, 2015*

32. *D. valveus* YU, POPOVIC & GU 2014*

33. *D. wilsoni* BEDDING, 1968*

Synonym: *Beddingia wilsoni* (BEDDING, 1968) BLINOVA & KORENTCHENKO, 1986

34. *D. zyzyphus* BAJAJ, 2015*

Species inquirendum

35. *Deladenus lonchites* (MASSEY, 1974) FORTUNER & RASKI, 1987

Synonym: *Dotylaphus lonchites* MASSEY, 1974

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