

# Discovery of a New Fungivorous Nematode, *Deladenus golestanicus*, from Northern Iran: Morphological and Phylogenetic Evidence

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## INTRODUCTION AND AIM

Neotylenchidae (Thorne, 1941) is a family of nematodes that includes candidates for biological control in agriculture, with members exhibiting dual life cycles comprising parasitic stages on insects or plants and free-living, fungus-feeding stages in soil and organic matter. Within this family, *Deladenus*—particularly *D. siricidicola*—has been successfully employed against the pine pest *Sirex noctilio* in Australia, South America, and South Africa. In Iran, eleven *Deladenus* species are known: nine described (*D. bonabensis*, *D. brevis*, *D. gilanicus*, *D. hebetocaudatus*, *D. hyrcanus*, *D. maraghensis*, *D. persicus*, *D. ramianensis*, and *D. tonekabonensis*) and two reported (*D. apopkaetus* and *D. durus*). The present study, conducted in 2023 on samples of bark of pine trees collected from the natural Golestan Forest Park in Golestan Province, northern Iran, aims to (1) describe the morphology of a newly recovered *Deladenus* species and (2) elucidate its molecular phylogenetic relationships using the 18S rDNA region.

## MATERIALS AND METHODS

1

### Sample Collection and Preparation

Wood and bark-inhabiting beetles were collected from a dead *Acer velutinum* trunk. The trunk was chopped (<1 cm), and nematodes were extracted using the tray method. Extracted nematodes were fixed in hot 4% formaldehyde, processed to anhydrous glycerin, and examined under an Olympus BX51 microscope for measurements, drawings, and photomicrographs.

2

### DNA Extraction and Amplification

DNA was extracted from a single female nematode. The 18S rDNA region was amplified using two pairs of primers: 1096F/1912R and 1813F/2646R. Sequencing was performed bidirectionally, and sequences were compared using BLAST.

4

### Phylogenetic Analysis

Phylogenetic analysis was conducted under the GTR+G+I model using Bayesian inference in MrBayes (6×10<sup>6</sup> generations). *Acrobeles ciliatus* and *Acrobeloides maximus* were used as outgroups. The resulting trees were visualized in Dendroscope and finalized in CorelDRAW.

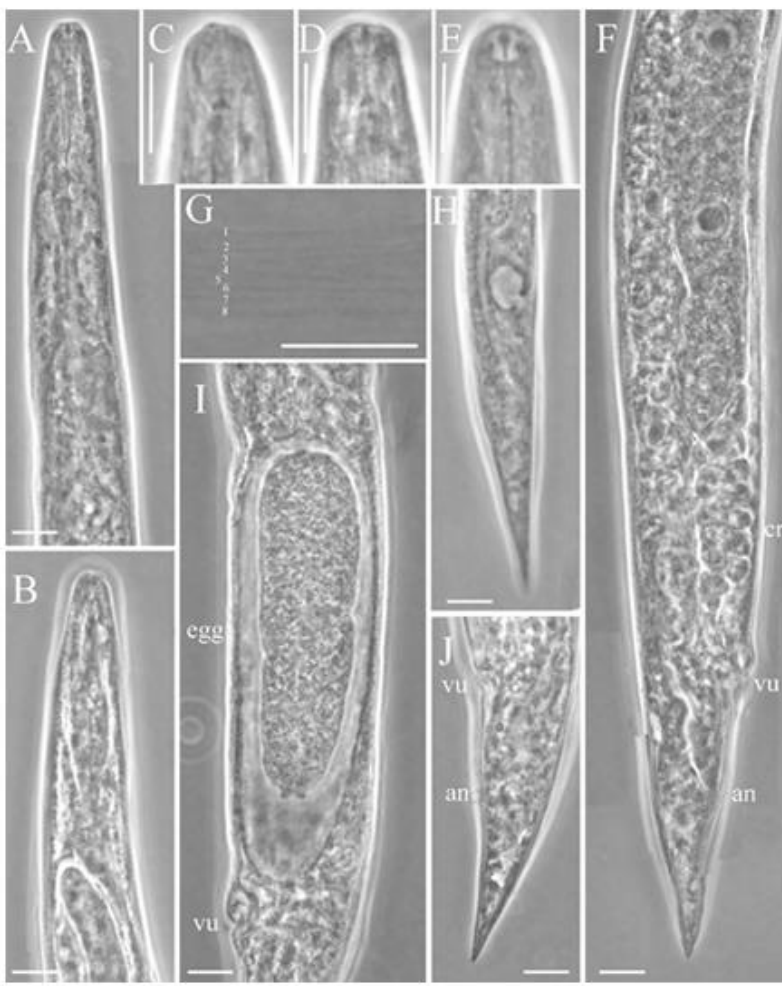
3

### Sequence Alignment and Refinement

Sequences were aligned with MAFFT (Q-INS-i), refined using Gblocks, and prepared for phylogenetic analysis.

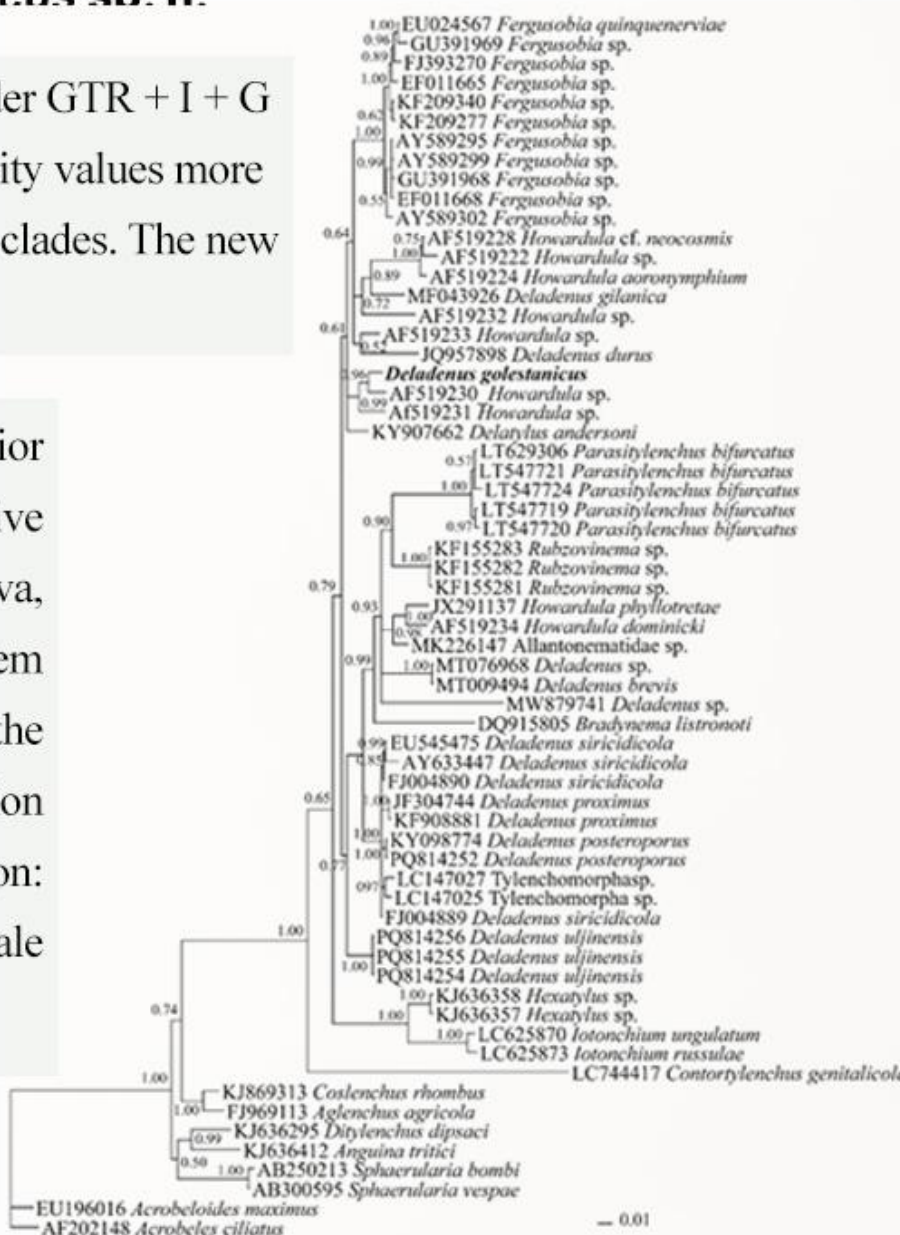
## RESULTS AND DISCUSSION

1 Figure 1: Mycetophagous female of *Deladenus golestanicus* sp. n.



2 Figure 2: Bayesian 50% majority rule consensus tree of *Deladenus golestanicus* sp. n.

based on 18S rDNA sequences under GTR + I + G model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. The new sequence is indicated in bold.



3 Table 1: Morphometrics of *Deladenus golestanicus* sp. n. All measurements in µm and in the form: mean ± S.D. (range).

Character	Present study		Jalalinasab et al., 2020
	Holotype	Female	Females
n	-	10	10
L	554	554 ± 97.4 (450-733.5)	364.8 ± 33.7 (314-422)
a	19.8	18.8 ± 2.7 (15.4-22.2)	21.1 ± 2.6 (18.1-26.5)
b	8.7	8.4 ± 1.1 (7.0-10.8)	7.1 ± 0.9 (5.9-8.5)
b'	5.3	5.1 ± 0.5 (4.4-6.0)	5.0 ± 0.9 (4.1-6.9)
c	12.6	12.7 ± 1.3 (9.9-14.0)	12.4 ± 1.1 (10.8-14.6)
c'	3.1	2.9 ± 0.5 (2.3-3.8)	3.3 ± 0.4 (2.4-3.8)
v	86.8	86.6 ± 1.0 (85.0-88.7)	86.6 ± 0.7 (85.4-87.4)
Lip region height	1.5	1.7 ± 0.6 (1.0-2.3)	1.4 ± 0.2 (1.0-1.5)
Lip region width	7.5	7.7 ± 0.6 (6.5-8.5)	6.5 ± 0.5 (6.0-7.0)
Stylet length	7.5	7.2 ± 0.5 (6.0-7.5)	8.0 ± 0.2 (7.5-8.0)
Stylet conus	2.5	2.5 ± 0.2 (2.0-2.5)	-
Dorsal pharyngeal gland orifice from anterior	1.0	1.0 ± 0.2 (0.5-1.2)	0.5-1.0
Excretory pore from anterior end	108	107.2 ± 14.5 (91.5-131)	68.4 ± 5.2 (61.0-76.0)
Hemizonid from anterior end	109.5	106 ± 13.3 (90.0-129)	68.4 ± 5.2 (61.0-76.0)
Excretory pore to hemizonid	1/5	0.9 ± 1.6 (-2.0-2.5)	-
Pharynx base from anterior end	64.0	65.5 ± 5.5 (58.0-78.0)	51.4 ± 4.9 (46.0-63.0)
Nerve ring from anterior end	65.0	63.0 ± 5.8 (54.0-73.0)	-
Pharynx overlapping	40.0	43.8 ± 15.5 (22.5-83.0)	23.1 ± 7.5 (11.0-33.0)
Maximum body diam.	28.0	29.7 ± 3.9 (23.5-34.5)	17.5 ± 2.8 (14.0-22.0)
Head-vulva	481	480 ± 88.8 (384.5-641)	316 ± 30.8 (268-367)
Vulval body diam. (VBD)	24.0	24.6 ± 3.7 (19.0-31.0)	-
Vulva-body end	73.0	73.6 ± 9.7 (63.0-95.0)	-
Vulva to anus	29.0	29.6 ± 4.3 (20.0-37.0)	19.4 ± 3.4 (15.0-26.0)
Anal (cloacal) body diam.	14.0	15.3 ± 1.7 (12.0-18.0)	-
Tail length	44.0	44.0 ± 7.6 (34.0-58.0)	29.4 ± 1.3 (27.0-32.0)

This species is characterized in females by a body length of 450–733 µm; a short stylet measuring 6.0–7.5 µm; a dorsal gland orifice 0.5–1.2 µm behind the knobs; seven to eight lateral field incisures, sometimes with oblique, discontinuous middle lines; a fusiform median bulb lacking a median chamber; an excretory pore at the level of the hemizonid and near the terminus of the basal esophageal bulb; and a monodelphic-prodelphic reproductive system with an outstretched ovary (oocytes in 1–3 rows), a long tube-like oviduct, an inconspicuous spermatheca, a crustaformeria of 8 cells × 4 rows, a thick-walled uterus, an anteriorly oblique vagina, a transverse vulva, and no posterior uterine sac. The rectum and anus are distinct. The tail is elongate-conoid, 1.1–2.3 times the vulva–anus distance, ending in a rounded to pointed tip.

The new species closely resembles *D. gilanicus* and *D. hyrcanus* but can be distinguished from *D. gilanicus* by its longer body length (450–773 vs. 314–422 µm), shorter stylet (6.0–7.5 vs. 7.5–8.0 µm), longer tail (34–58 vs. 27–32 µm), greater vulva–anus distance (20–37 vs. 15–26 µm), and more posteriorly positioned excretory pore (opposite the end of the dorsal esophageal gland). It differs from *D. hyrcanus* in body length (450–773 vs. 718–806 µm), stylet length (6.0–7.5 vs. 7.5–10.0 µm), excretory pore position (near the end of the basal bulb vs. anterior to the basal bulb), and tail length (34–58 vs. 58–72 µm).

Additionally, sequences of the small and large subunit ribosomal DNA regions (SSU and LSU D2–D3) were obtained, and phylogenetic analyses were conducted. The proposed new species, *Deladnus golestanicus*, is supported by the morphological and morphometric distinctiveness of the resulting phylogenetic trees.

## CONCLUSION

This study describes a new *Deladenus* species from Iran, indicating greater genus diversity in the country than previously recognized. The species is characterized from its free-living phase, as with several prior Iranian records; notably, *D. gilanicus* was described from both free-living and infective phases. Consistent with earlier work, phylogenetic analysis of the 18S rDNA region places sequenced *Deladenus* species into several distinct clades.

## REFERENCES

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