

Further Studies on Soil Nematode Fauna in North Western Iran with the Description of One New Species

MAJID PEDRAM, EBRAHIM POURJAM, MOHAMMAD REZA ATIGHI, AND YOUSEF PANAHADEH

Abstract: *Heterodorus youbertghostai* n. sp. is described and illustrated based on morphological, morphometric, and molecular data. The new species was found in two geographically distant points in northwestern Iran and is characterized by having angular lip region, separated from the rest body by a constriction, body length of 1,432.5 to 1,751.3 μm , odontostyle length of 24 to 28 μm , rod-like odontophore, 37.0 to 42.5 μm long, lacking flanges at base, double guiding ring at 14 to 16 μm distance from anterior end, pharyngeal bulb comprising 40% to 48% of pharynx, intestine usually containing green material, female reproductive system amphidelphic with less divided short uterus, specific structure of pars distalis vaginae, bluntly conical tail, dorsally convex and ventrally flat, with rounded tip and saccate bodies in ventral side. The new species comes more close to *H. conicaudatus* and *H. irregularis* by its morphology and morphometric characters. Compared to former, it has remarkable difference in vulva position and tail characters, and compared to the latter, it could be separated by shorter body, posteriorly located vulva, wider lip region, and longer tail. In phylogenetic analyses using partial sequences of 28S rDNA D2-D3, the new species formed a fully supported clade with several isolates of *H. brevidentatus*, prevalent in Iran. The other nordiid taxon, *Enchodorus dolichurus*, already reported from Iran, was also sequenced for the same genomic region and included in phylogenetic analyses.

Key words: 28S rDNA, city of Kaleibar, description, Eastern Azarbaijan, Nordiidae, Sabalan, taxonomy.

In his excellent review on the genus *Heterodorus* Alther, 1952, Andr assy (2011) discussed its taxonomic status, presented diagnostic features, and listed the name of the valid species. Some new species were also described, comments were given on some species, and an identification key and compendium of morphometric data of valid species were provided (*op. cit.*). Till now, this is the most comprehensive taxonomic study on the genus. The studies on nordiid taxa occurring in Iran is given by Pedram et al. (2011). During our study on dorylaim nematode fauna in northwestern Iran, two populations of a new nordiid species belonging to the genus *Heterodorus* were recovered in Eastern Azarbaijan and Ardabil provinces and described herein as *H. youbertghostai* n. sp.

MATERIALS AND METHODS

Several soil samples were collected from northwestern Iran, mostly from high-altitude mountainous regions of Eastern Azarbaijan and Ardabil provinces. The tray method (Whitehead and Hemming, 1965) was employed to extract nematodes. Nematodes of interest were hand-picked using a Nikon SMZ1000 stereomicroscope (Nikon Corp., Tokyo, Japan). The specimens were killed by adding hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. Observations were made using a Nikon E600 light microscope (Nikon Corp., Tokyo, Japan), equipped with a drawing tube for preparing of drawings and performing morphometrics. The drawings were re-

drawn with CorelDRAW Graphics Suite 12. An Olympus BX51 light microscope (Japan) equipped with differential interference contrast, and a DP72 digital camera (Japan) was used to get digital images. The information of the valid species of the genus *Heterodorus*, given by Andr assy (2011), were used for morphological and morphometric comparisons. The location of pharyngeal glands nuclei were calculated according to Andr assy (1998).

For molecular studies, one isolate from each population of the new species and two isolates of the species *E. dolichurus* Vinciguerra, 1976 previously reported from Iran (Pourjam et al., 2010) were heat killed on temporary slides, studied for their correct selection, transferred to 1.0 μl AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean slide, and squashed using a clean slide cover. Each suspension was collected by adding 24 μl AE buffer. DNA samples were stored at -20°C until used as a PCR template. Each isolate was sequenced for its 28S rDNA D2-D3 segments using the forward D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse D3B (5'-TGCGAAGGAACCAGCTACTA-3') primers (Nunn, 1992). Details of PCR and sequencings are according to Yaghoubi et al. (2014). The newly obtained sequences were deposited into the GenBank database (accession numbers KR184124 and KR184125 for isolates 2 and 1 of *E. dolichurus*, and KR184126 and KR184127 for isolates Arasbaran and Sabalan of the new species). The sequences used for reconstructing of the phylogenetic relationships were selected from GenBank. They were aligned using ClustalX2 (<http://www.clustal.org/>) software. Manually editing of the alignments performed using MEGA5.05 software (Tamura et al., 2011). A general time-reversible model including among-site rate heterogeneity (GTR + G) was selected using the MrModeltest 2 (Nylander, 2004) according to Akaike criterion and used in the phylogenetic analysis. Bayesian analysis was used to infer a phylogenetic tree using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003), running the chains for 10^6 generations. After discarding

Received for publication April 23, 2015.

Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

We thank the Iranian National Science Foundation (INSF) and Tarbiat Modares University (Iran) for financial support. The first author deeply appreciates the kind help of two librarians, Mrs. Martina-Christine Koschwitz and Mrs. Beate Peters-Mergner, for their warm help with finding some rare publications.

E-mail: majid.pedram@modares.ac.ir; mj_pedram@yahoo.com.

This paper was edited by Zafar Handoo.

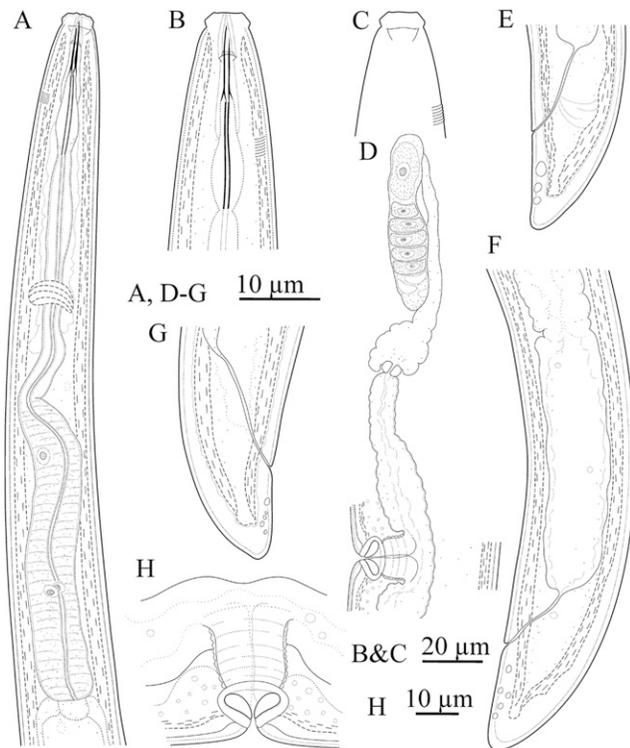


FIG. 1. *Heterodorus youbertghostai*, n. sp. A. Pharynx. B. Details of anterior end. C. Amphidial fovea, opening, and cuticle transverse striation. D. Anterior genital tract. E and G. Tail. F. Posterior end, H. Details of vagina.

burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov chain Monte Carlo method within a Bayesian framework was used to determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic tree (Larget & Simon, 1999) using the 50% majority rule. The Bayesian posterior probabilities (BPP) higher than 50% were given on appropriate clades. The output file of the phylogenetic program was visualized using Dendroscope V.3.2.8 (Huson & Scornavacca, 2012) and redrawn in CorelDRAW software version 12. For Bayesian inference (BI), the species *Aporcelainmellus obtusicaudatus* (Bastian, 1865) Altherr, 1968 (AY601632) was used as outgroup taxon.

*Heterodorus youbertghostai** n. sp.
(Figs. 1,2)

Measurements
See Table 1.

DESCRIPTION

Females (type population)

Slender nematodes: Body, slightly ventrally bent after fixation, tapering gradually toward both ends. Cuticle, with two layers, 2 to 4 µm thick in anterior region

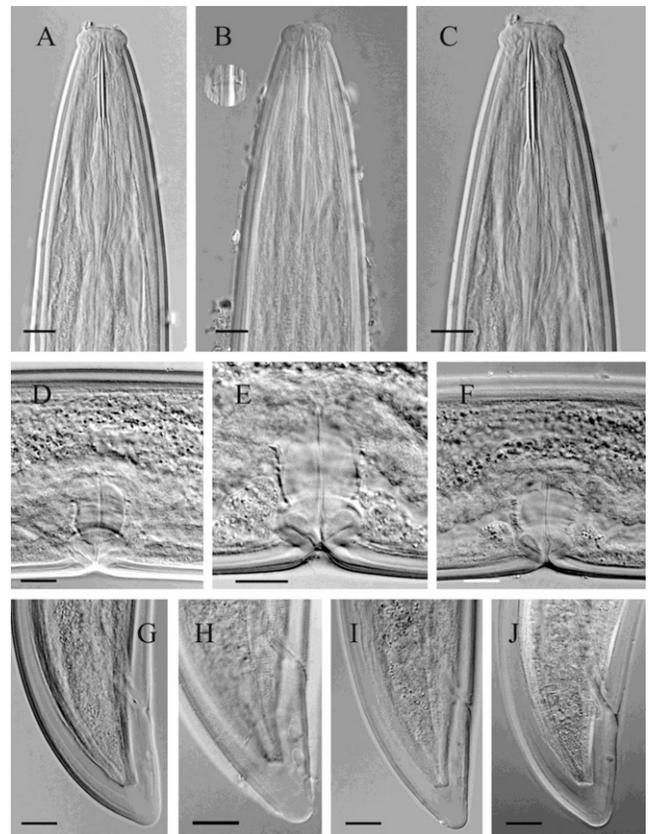


FIG. 2. *Heterodorus youbertghostai*, n. sp. A-C. Details of anterior end (B with detail of double guiding ring). D-E. Vagina (E. in detail), G-J. Tail (H. in detail, showing saccate bodies) (all scale bars = 10 µm).

(distance between anterior end and guiding ring), 3 to 4 µm at mid-body and 4 to 6 µm on tail (anterior lip of anus), outer layer with very fine transverse striations, more visible at anterior end and dorsal side of the tail. Lateral chord, about 22% to 33% corresponding body width, wide. Lip region, angular in lateral view, separated from the rest body by a constriction, 2.1 to 3.2 times wider than high. Amphidial fovea, cup-shaped, their opening at level of labial constriction, ca. 70% of lip region diam. Odontostyle, slender, needle like, 1.5 to 1.7 times longer than lip region diam. Odontophore, road-like, 1.5 times longer than odontostyle, lacking basal flanges. Guiding ring, double. Pharynx, consisting of slender but muscular anterior region and a basal bulb, 5 to 7 times as long as wide, occupying about 40% to 48% of total pharynx; gland nuclei located as follows: dorsal nucleus (D) = 61–66, anterior subventral pair of pharyngeal nuclei (AS), obscure, posterior subventral pair (PS) = 52–58. Nerve ring at 120 to 147 µm distance from anterior end. Cardia, rounded, 10–20 × 12–15 µm sized, almost as long as wide. Intestine, usually with green material in most examined individuals. Pre-rectum, variable in length, about 3.0 to 6.5 times anal body diam. long. Rectum, about as long as (0.9–1.1 times) anal body diam. Reproductive system, didelphic-amphidelphic, with both branches more or less

* The new species is named in honor of Dr. Youbert Ghosta, the first plant pathology professor of the first author.

TABLE 1. Morphometric data for *Heterodorus youbertghostai* n. sp. All measurements are in μm and in the form: mean \pm standard deviation (range).

	Females		
	Type population from Arasbaran		
	Holotype	Paratypes	Ardabil population
<i>n</i>		10	9
L	1,500	1,530 \pm 69 (1,432.5–1,612.5)	1,680 \pm 61 (1,573.8–1,751.3)
a	26.8	56.3 \pm 1.2 (24.3–28.5)	28.2 \pm 2.5 (24.0–31.8)
b	4.6	4.3 \pm 0.5 (3.7–5.0)	4.4 \pm 0.1 (4.1–4.5)
c	39.5	40.5 \pm 2.8 (36.5–46.0)	40.2 \pm 2.2 (36.7–43.6)
c'	1.2	1.2 \pm 0.1 (1.1–1.3)	1.2 \pm 0.1 (0.9–1.3)
V	49.3	48.5 \pm 1.3 (45.8–50.5)	49.5 \pm 2.2 (45.9–53.7)
Anterior end-vulva	738.8	741.0 \pm 30.5 (695–800)	831 \pm 35 (761.5–892.5)
Lip region diam.	15	15.5 \pm 0.5 (15–16)	15.5 \pm 0.5 (15–16)
Lip region height	7	5.6 \pm 0.7 (5–7)	5.8 \pm 0.4 (5–6)
Odontostyle length	25	26.2 \pm 1.2 (25–28)	24.7 \pm 0.7 (24–26)
Odontophore length	38	39.5 \pm 2.0 (37.0–42.5)	39.7 \pm 1.7 (37–42)
Stylet total length	63	65 \pm 4 (62–69)	64.3 \pm 2.0 (61–67)
Ods/lip width	1.7	1.7 \pm 0.1 (1.6–1.8)	1.6 \pm 0.1 (1.5–1.7)
Guiding ring from anterior end	14	15 \pm 1 (14–16)	14.0 \pm 0.5 (14–15)
Neck length	325	360 \pm 43 (287–420)	386 \pm 8 (372.5–397.5)
Pharyngeal expansion length	142.5	157 \pm 22 (120–180)	173.5 \pm 6.0 (165–185)
Pharyngeal expansion width	25	26 \pm 1 (25–27)	25.5 \pm 2.0 (23–29)
Diam. at guiding ring level	21	21.5 \pm 0.5 (20–22)	21.5 \pm 1.0 (20–23)
At neck base	47	54.5 \pm 6.0 (47–59)	59 \pm 7 (51–70)
At mid-body	56	58.0 \pm 3.5 (54–65)	60 \pm 5 (55–70)
At anus	32	32 \pm 2 (29–35)	36.5 \pm 3.5 (32–44)
Prerectum length	195	163 \pm 35 (100–195)	178 \pm 18 (157–202)
Rectum length	34	32 \pm 3 (28–38)	33 \pm 3 (29–40)
Tail length	38	38.0 \pm 1.5 (35–40)	42 \pm 3 (37–45)

equal in length, each *ca.* 150 μm long, composed of a 35 to 60 μm long reflexed ovary, 60 to 90 μm long oviduct, 63 to 72 μm long uterus, less divided to wider proximal and narrower slender distal section. Vagina, extending inwards for 45% to 60% of corresponding body width, composed of pars proximalis vaginae almost as long as wide (19–22 \times 14–21 μm), with sigmoid walls, surrounded by weak musculature; pars refringens vaginae composed of two drop-shaped sclerotized pieces (in lateral view), their tip slopping towards vulval opening; pars distalis vaginae well developed, in the shape of a spherical structure completely surrounding the pars refringens. Vulva, a transverse slit (sperm observed within the genital tracts of one female of the second population). Tail, conical, dorsally convex, ventrally flat, with rounded terminus and medium to large-sized saccate bodies in ventral side, its hyaline part *ca.* 13.5 μm thick.

Males

Not found.

TYPE HABITAT AND LOCALITY

The type population was recovered from soil samples collected in vicinity of Babak Fort, Arasbaran forests, city of Kaleybar, Eastern Azarbayjan province, northwestern Iran about the rhizosphere of grasses in depth of 15 to 20 cm. GPS coordinates: 38°50'12.814" N, 46°58'52.007" E.

OTHER LOCATIONS

The second population was recovered from soil samples collected in grasslands of Sabalan mountains.

TYPE MATERIAL

Holotype female and three paratype females deposited in the Nematode Collection at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Two female paratypes deposited in each of the following collections: CABI Europe-UK, Egham, UK; USDA Nematode Collection, Beltsville, MD, and WANECO collection, Wageningen, The Netherlands (<http://www.waneco.eu/>).

DIAGNOSIS AND RELATIONSHIPS

H. youbertghostai n. sp. is characterized by having angular lip region separated from the rest body by a constriction, body length of 1,432.5 to 1,751.3 μm , odontostyle length of 24 to 28 μm , rod-like 37.0 to 42.5 μm long odontophore, lacking flanges at base, double guiding ring at 14 to 16 μm distance from anterior end, pharyngeal bulb, comprising 40% to 48% of pharynx with D at 61% to 66%, AS inconspicuous, PS distinct and lying far from the base of the bulb at 52% to 58%, didelphic–amphidelphic nature of female reproductive system with less divided short uterus, vagina, with

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28:2731–2739.

Vinciguerra, M. T. 1976. *Enchodorus*, a new genus of Dorylaimoidea from Italy. *Nematologica* 22:10–14.

Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods for extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55:25–38.

Yaghoubi, A., Pourjam, E., Pedram, M., Siddiqi, M. R., and Atighi, M. R. 2014. Molecular and morphological characterization of *Abursanema iranicum* n. gen., n. sp. (Nematoda: Hexatylinea, Sphaerularioidea) from Iran. *Zootaxa* 3826:301–314.