

# New data on predatory dorylaimids (Nematoda: Dorylaimida) from Iran

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**Summary.** Two species of predatory dorylaimid nematodes were recovered from different parts of northern and northwestern Iran. The first species, *Carcharolaimus banaticus*, was recovered from Noshahr city, Mazandaran province, and is characterised by 1.8-2.0 mm long females having 20.3-21.8 µm long odontostyle. It was recorded from Iran for the first time. Compared to the type population from Banat (former Yugoslavia), the body of the Iranian population is longer (1.8-2.0 vs 1.23-1.76 mm). The second species, *Aetholaimus rotundicauda*, was recovered from grasslands of Kaleibar city, East Azarbaijan province, and is characterised by 1.3-1.6 mm long females having 9.5-11.5 µm long tooth. Its occurrence in Iran is a new record. Compared to the type population from The Netherlands, its body is shorter (L mean = 1.4 vs 1.7 mm). Both recovered species represent Iran as a new geographical distribution area for the two genera *Carcharolaimus* and *Aetholaimus*. Phylogenetic relationships of these two species with other selected dorylaimid taxa were studied using small subunit ribosomal DNA sequences. In the inferred tree, representatives of the two suborders Dorylaimina and Nygolaimina formed two separate clades. The Iranian population of *Carcharolaimus banaticus* formed a clade with formerly sequenced isolate of the species inside the former major clade, and Iranian population of *Aetholaimus rotundicauda* formed a clade with formerly sequenced isolate of the species inside the latter major clade.

**Key words:** *Aetholaimus rotundicauda*, *Carcharolaimus banaticus*, Dorylaimina, Nygolaimina, phylogeny, taxonomy.

The taxonomic studies on Dorylaimida Pearse, 1942 in Iran in last ten years, have increased (Hadi Alijanvand *et al.*, 2013; Peña-Santiago *et al.*, 2014, 2022; Konani *et al.*, 2016; Vinciguerra *et al.*, 2016; Zahedi Asl *et al.*, 2016; Kazemi *et al.*, 2018; Naghavi *et al.*, 2019, 2020; Vazifeh *et al.*, 2019, 2020, 2022; Asgari *et al.*, 2020, 2023; Álvarez-Ortega *et al.*, 2020, Jahanshahi Afshar, 2020; Jabbari *et al.*, 2021). Most studies however have focused on the suborder Dorylaimina Pearse, 1936 and a few representatives of the suborder Nygolaimina Ahmad & Jairajpuri, 1979 have been reported from Iran (Olia *et al.*, 2004).

The two genera *Carcharolaimus* Thorne, 1939 and *Aetholaimus* Williams, 1962 have 20 and five species, respectively, prevalent in several parts of the world (Andrássy, 2009). These two predatory dorylaimid genera have not been reported from Iran.

During 2020-2023, several soil samplings were performed in northern Iran. Two species belonging to two aforementioned genera were recovered, representing Iran as a new geographical distribution region for them. The morphological and molecular characterisations of these two species are given in this paper.

## MATERIALS AND METHODS

**Soil sample collection and nematode extraction.** Several soil samples were collected from grasslands of Kaleibar and Noshahr cities in East Azarbaijan and Mazandaran provinces, during 2020-2023. The nematodes were extracted from soil samples using the tray method (Whitehead & Hemming, 1965). The large sized specimens were extracted using two 841 and 250 µm opening mesh

sieves (the first one to collect root particles and debris, the second one to collect the nematodes).

**Morphological study.** The extracted specimens were heat-killed by adding boiling 4% formalin; and permanent slides were prepared after the nematodes were transferred to dry glycerol (De Grisse, 1969). The morphology and morphometrics of the prepared specimens were studied using light microscope Nikon E600 (Nikon, Tokyo, Japan). The light microphotographs were prepared using a DP72 Olympus digital camera attached to an Olympus BX51 microscope (Olympus, Tokyo, Japan).

**Molecular and phylogenetic analyses.** The crude DNA samples of two species were prepared using the fast method. Each specimen (one female specimen from each species) was washed, placed on a drop of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0, 100 QIAGEN Inc., Valencia CA), heat killed, and a cover slip was placed on the specimen and photographed. The crude DNA sample was obtained using pressure of a plastic pipette tip on the cover slip to rupture the specimen. For each species, one DNA sample was prepared and stored at -20°C. The SSU rDNA was amplified and sequenced using two primer pairs: i) forward 988F (5' - CTC AAA GAT TAA GCC ATG C - 3'), reverse 1912R (5' - TTT ACG GTC AGA ACT AGG G - 3'), and ii) forward 1813F (5' - CTG CGT GAG AGG TGA AAT - 3') and reverse 2646R (5' - GCT ACC TTG TTA CGA CTT TT - 3') (Holterman *et al.*, 2006). The newly generated sequences were deposited in the GenBank database under the accession numbers: OQ682609 for Iranian population of *Aetholaimus rotundicauda* (de Man, 1880) Coomans & Loof, 1978 and OQ682611 for Iranian population of *Carcharolaimus banaticus* Krnjaic & Loof, 1975. Each sequence was compared with available sequences in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) using the basic local alignment search tool (BLAST). The relevant sequences were retrieved, and the outgroup sequences were selected from order Mononchida Jairajpuri, 1969. The SSU dataset was aligned using ClustalX2 (<https://clustalx.software.informer.com/2.1/>) and was manually trimmed in both sides using MEGA7 (Kumar *et al.*, 2016). The best-fit model of base substitution was selected using PAUP\* (Swofford, 2002) and MrModeltest v2 (Nylander, 2004). The Akaike-supported model, a general time-reversible model including among-site rate heterogeneity and estimates of invariant sites (GTR+G+I), was used in the phylogenetic analysis using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) with a starting random tree and running the chains for five million generations. Having

discarded 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 estimate the posterior probabilities of the phylogenetic tree (Larget & Simon, 1999) based on the 50% majority rule. The Bayesian posterior probability values exceeding 0.5 are indicated on each clade. To visualize and digitally draw final trees, Dendroscope V.3.5.9 (Huson & Scornavacca, 2012) and CorelDraw® software version 20 were used.

## RESULTS AND DISCUSSION

### *Carcharolaimus banaticus* Krnjaic & Loof, 1975 (Fig. 1)

**Measurements.** See Table 1.

**Female.** Body relatively long and cylindrical, tapering very indiscernibly at anterior region. Habit of fixed specimens straight. Cuticle two-layered, 3-4 µm thick at guiding ring level, 3.0-4.5 µm at mid-body and 4.8-5.1 µm at anus. Lip region set off from the rest of the body by a deep constriction, the stomatal cavity spacious, composed of two parts, the anterior part basket-like, 10-12 µm high and 20-24 µm wide, its walls with sclerotised ribs, fine and larger denticles (Fig. 1 B, C) at base, the posterior part 5-7 µm high and 10-12 µm wide. Lips separated and somewhat angular. Amphidial fovea cup-shaped. Guiding ring simple. Odontostyle dorylaimoid, 0.6-0.7 times the lip region diameter. Odontophore rod-like, 1.3-1.4 times longer than the odontostyle. Pharynx with two typical parts separated by a constriction, anterior part narrow but muscular with two swellings: one at junction with odontophore and one at junction with the pharyngeal bulb. The pharyngeal bulb muscular, with the dorsal gland nucleus (DGN) at 49-52% and the nuclei of other glands not well observed due to muscular texture. Cardia dome-shaped about 19×22 µm in size. Reproductive system didelphic-amphidelphic, each branch composed of reflexed vary (63-93 µm long), oviduct joining the ovary underneath and consisting of a slender part with invisible lumen and an underdeveloped *pars dilalata*, a weak sphincter, uterus in the shape of a short tube without special differentiation, vagina cylindrical that extends inwards one third of the corresponding body width, its walls adjacent to the vulva weakly sclerotised, and vulva in the shape of a longitudinal slit. Prerectum 1.7-1.9 times longer than body width at anus level, and rectum slightly smaller than the diameter of the body at anus. Tail

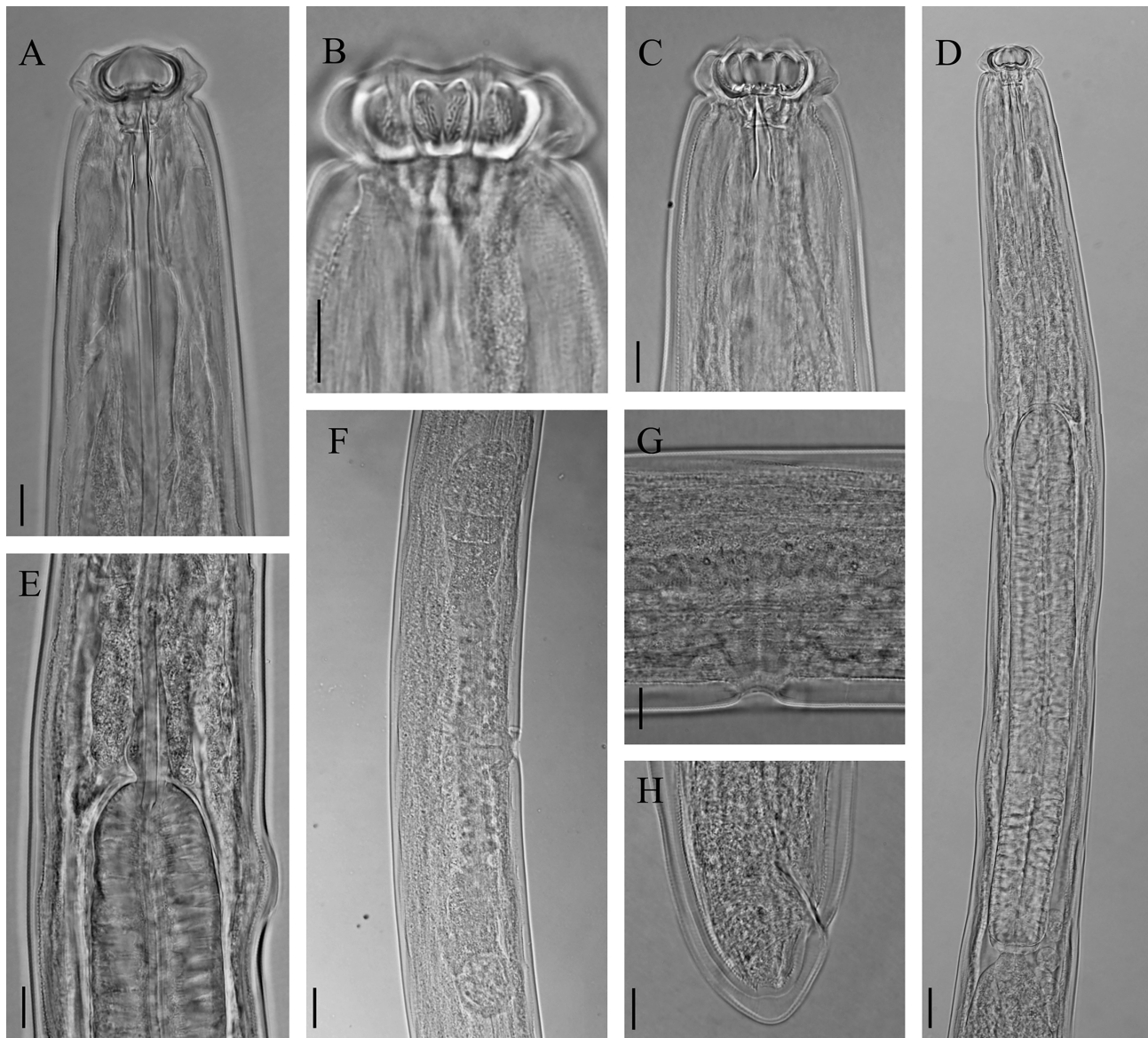
short, conoid, dorsally more convex, its tip widely rounded.

**Male.** Not found.

**Locality.** In this study, it was recovered from sandy soils at Caspian seashore in Noshahr city from the rhizosphere of *Cynodon dactylon* (L.) Pers. and was reported for the first time from Iran (GPS coordinates: N 36°38'6.225", E 51°33'52.236").

**Materials.** Three slides including the specimens of this population are deposited in the Nematode Collection of Tarbiat Modares University, Tehran, Iran under the accession codes: TM5120-TM5122.

**Remarks.** *Carcharolaimus banaticus* was described from Banat, former Yugoslavia (Krnjaic & Loof, 1975). The studied population represents Iran as a new geographical area for the genus, and matches the type population morphologically. However, body length of the Iranian specimens is longer than that of the type specimens (1.8-2.0 vs 1.23-1.76 mm) (Krnjaic & Loof, 1975) and specimens from other populations (1.8-2.0 vs 1.49 mm in Italian population and 1.21-1.66 mm in Spanish population) (Vinciguerra & Zullini, 1980; Peña-Santiago & Liébanas, 1994).



**Fig. 1.** Photomicrographs of Iranian population of *Carcharolaimus banaticus* Krnjaic & Loof, 1975 (Noshahr population, female). A: Anterior body region; B: Small denticles at stomatal walls; C: Larger denticles at base of stoma; D: Pharynx; E: Constriction at junction of anterior part of pharynx with the pharyngeal bulb; F: Genital branches; G: Vulval region; H: Posterior body region. Scale bars: A-C, E, G, H = 10  $\mu$ m, D and F = 20  $\mu$ m.

**Table 1.** Morphometrics of Iranian populations of *Carcharolaimus banaticus* Krnjaic & Loof, 1975 and *Aetholaimus rotundicauda* (de Man, 1880) Coomans & Loof, 1978. All measurements except L are in  $\mu\text{m}$  and in the form: mean  $\pm$  SD (range).

Species	<i>Carcharolaimus banaticus</i>	<i>Aetholaimus rotundicauda</i>
Characters	Noshahr population	Kaleibar population
	Females	Females
n	4	7
L (mm)	1.8 $\pm$ 0.1 (1.8-2.0)	1.4 $\pm$ 0.1 (1.3-1.6)
a	29.0 $\pm$ 4.9 (24.8-36.0)	37.8 $\pm$ 3.5 (31.3-42.6)
b	4.2 $\pm$ 0.5 (3.7-4.8)	3.5 $\pm$ 0.1 (3.2-3.5)
c	73.6 $\pm$ 9.9 (61.6-85.8)	65.3 $\pm$ 6.5 (56.2-72.3)
c'	0.8 $\pm$ 0.1 (0.7-0.8)	0.9 $\pm$ 0.1 (0.8-1.1)
V%	51.5 $\pm$ 0.5 (51-52)	49.2 $\pm$ 2.6 (44.6-53.4)
Anterior end to vulva	920 $\pm$ 55 (855-977)	700.8 $\pm$ 15.0 (687.5-732.5)
Lip region diameter	33 $\pm$ 4 (29.3-34.0)	14.9 $\pm$ 0.5 (14.0-15.5)
Odontostyle/tooth length	21.0 $\pm$ 0.7 (20.3-21.8)	10.4 $\pm$ 0.7 (9.5-11.5)
Odontophore length	27.5 $\pm$ 0.3 (27.5-28.0)	-
Stylet total length	50.0 $\pm$ 1.5 (48-50)	16.4 $\pm$ 1.1 (15-18)
Guiding ring from anterior end	19.6 $\pm$ 1.7 (18-22)	11.1 $\pm$ 0.7 (10-12)
Neck length	443.3 $\pm$ 40.0 (385.0-475.9)	426.5 $\pm$ 13.4 (409-447)
Pharyngeal expansion length	265.4 $\pm$ 28.8 (223.0-287.4)	252.9 $\pm$ 13.1 (232.0-267.5)
Pharyngeal expansion diameter	35.3 $\pm$ 3.4 (31.9-38.2)	21.4 $\pm$ 1.8 (20-24)
Diameter at pharynx base	60 $\pm$ 11 (49.5-70.5)	36.3 $\pm$ 1.7 (34-38)
Diameter at mid-body	65.1 $\pm$ 10.5 (51.0-74.2)	37.0 $\pm$ 2.2 (35-40)
Diameter at anus	33.9 $\pm$ 3.3 (29-36)	23.4 $\pm$ 1.7 (21.5-26.0)
Prerectum length	60.0 $\pm$ 8.6 (50.8-69.6)	33.7 $\pm$ 4.8 (30.0-43.5)
Rectum length	24.0 $\pm$ 3.3 (20.5-27.0)	21.2 $\pm$ 2.3 (18-25)
Tail length	25.3 $\pm$ 2.3 (23.0-28.4)	22.0 $\pm$ 1.7 (19.5-25.0)

***Aetholaimus rotundicauda* (de Man, 1880)  
Coomans & Loof, 1978  
(Fig. 2)**

**Measurements.** See Table 1.

**Female.** Body long and slender, very gradually narrowing towards anterior end. Ventrally curved to more or less spiral after heat fixation. Cuticle marked with fine transverse striae, more distinct at anterior and posterior ends, 1.0-1.3  $\mu\text{m}$  thick at anterior end, 1.4-1.6  $\mu\text{m}$  at mid-body and 1.5-2.0  $\mu\text{m}$  at anus. Lip region set off from the rest of the body by a shallow constriction, 2.20-2.54 times wider than high. Amphidial fovea cup-shaped, its slit 6.5-7.5  $\mu\text{m}$  wide. Cheilostom spacious, its walls sclerotised. Guiding ring single. Stoma armed with a needle-like mural tooth. Pharynx dorylaimoid, consists of a narrow anterior, and an expanded posterior part. Pharyngeal dorsal gland nucleus located at 62.0-64.7% of the pharynx. Cardia

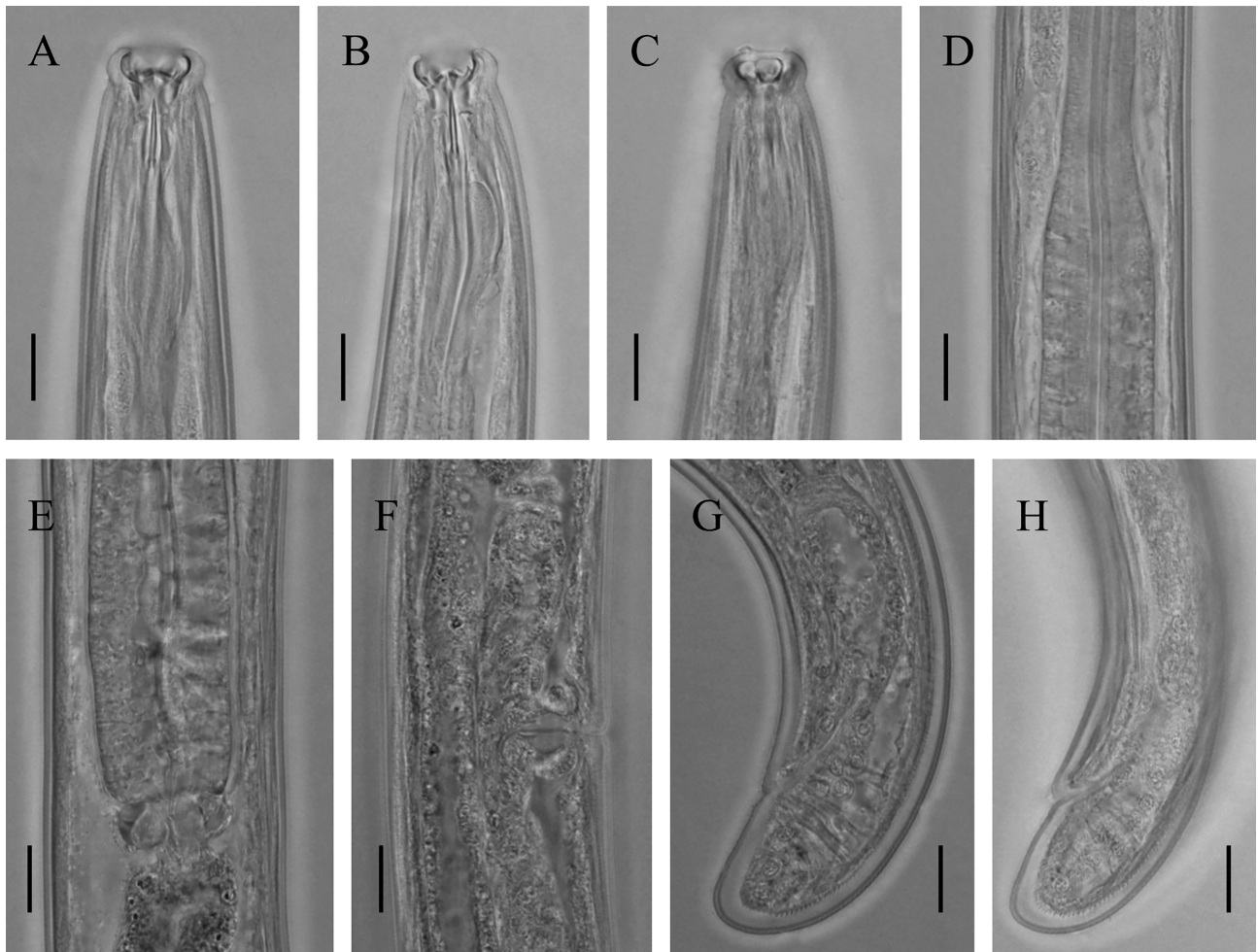
hemispherical, 7-10 $\times$ 14-19  $\mu\text{m}$  sized, bearing three knob-like glands. Reproductive system didelphic-amphidelphic with reflexed branches, the anterior branch 157.0-182.5  $\mu\text{m}$  and the posterior branch 152.5-231.0  $\mu\text{m}$  long, uterus tubular, 25-45  $\mu\text{m}$  long, and vulva a transverse slit. Tail rounded to rounded-conoid, dorsally convex and ventrally almost flat.

**Male.** Not found.

**Locality.** The Iranian population was recovered from grasslands in Kaleibar city, East Azarbaijan province, northwestern Iran (GPS coordinates: N 38°50'6.341", E 46°59'1.291").

**Materials.** Three slides including the specimens of this population are deposited in Nematode Collection of Tarbiat Modares University, Tehran, Iran under the accession codes: TM5123-TM5125.

**Remarks.** *Aetholaimus rotundicauda* was originally recovered in the sand dune region near Scheveningen, The Netherlands, and described as *Dorylaimus rotundicauda* de Man, 1880. It was

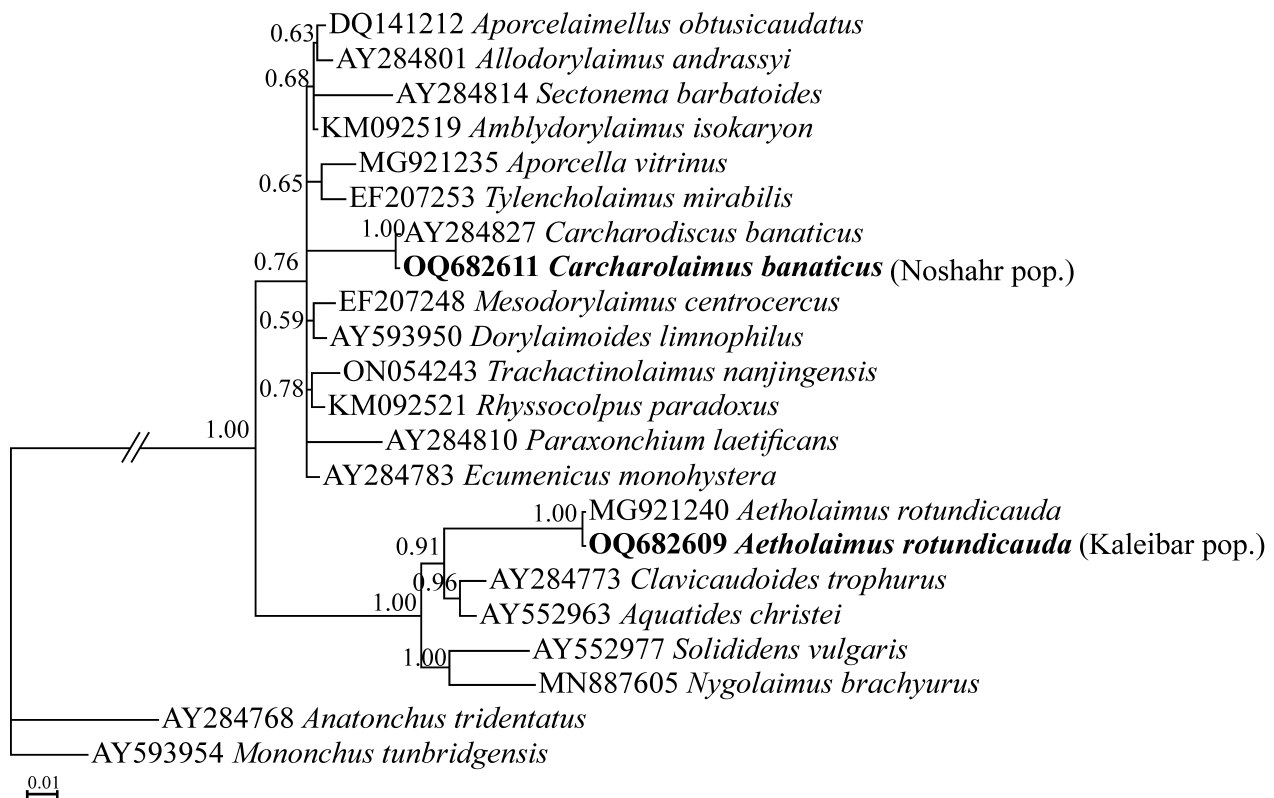


**Fig. 2.** Photomicrographs of Iranian population of *Aetholaimus rotundicauda* (de Man, 1880) Coomans & Loof, 1978. (Kaleibar population, female). A-C: Anterior body region in different focus; D: Anterior part of pharyngeal expansion; E: Pharyngo-intestinal junction (showing three knob-like cardiac glands); F: Vulval region; G: Posterior body region with prerectum; H: Posterior body region with anus. Scale bars = 10  $\mu$ m.

transferred to other genera: *Actinolaimus rotundicauda* (de Man, 1880) Steiner, 1916 and *Carcharolaimus rotundicauda* (de Man, 1880) Thorne, 1939, respectively (Steiner, 1916; Thorne, 1939). Finally, having a mural tooth and three cardiac glands, it has been shown to belong to *Aetholaimus*. This species was later recovered and redescribed from dunes near Heemskerk, The Netherlands and Airolo, Switzerland (Coomans & Loof, 1978). Its recovery in the present study extends the geographical distribution areas of the genus to Iran. The present data of the Iranian population of *A. rotundicauda* are in accordance with the data given in original description; however, the mean female body length is rather shorter (1.4 vs 1.7 mm). In comparison with the data presented by Coomans and Loof (1978), the Iranian population has longer prerectum (30.0-43.5 vs 14-33  $\mu$ m) and relatively shorter rectum (18-25 vs 20-32  $\mu$ m).

**Molecular characterisation and phylogenetic relationships.** The partial SSU rDNA was amplified and sequenced for two species. The sequences were 934 bp long for *Carcharolaimus banaticus* (OQ682611) and 1479 bp long for *Aetholaimus rotundicauda* (OQ682609).

The BLAST search of newly generated SSU sequence of *C. banaticus* revealed it has 100% identity with the previously deposited sequence of the species in GenBank (AY284827). The BLAST search of newly generated SSU sequence of *A. rotundicauda* revealed it has 100% identity with other previously deposited sequence of the species in the database (MG921240). Figure 3 represents the SSU phylogenetic tree reconstructed with the newly generated sequences of the two Iranian populations. In this tree, the included species of dorylaimid taxa are separated into the two major clades, each including representatives of suborders Nygolaimina



**Fig. 3.** Bayesian phylogenetic tree reconstructed using SSU rDNA sequences of the two Iranian species, *Carcharolaimus banaticus* Krnjaic & Loof, 1975 and *Aetholaimus rotundicauda* (de Man, 1880) Coomans & Loof, 1978 under the GTR + G + I model (lnL = 3572.7937; freqA = 0.2807; freqC = 0.2017; freqG = 0.2557; freqT = 0.2620; rAC = 1.1279; rAG = 3.3573; rCG = 0.4502; rCT = 5.7199; rGT = 1.0000; R (f) = 1; Pinv (I) = 0.7009; alpha (G) = 0.1796; pinv (I+IG) = 0.5151; alpha (G+GI) = 0.6252). Bayesian posterior probabilities equal to, or more than 0.5 are given for appropriate clades. Newly obtained sequences are highlighted in bold.

and Dorylaimina. The monophyly of each of the aforementioned suborders has already been demonstrated (Peña-Santiago, 2021). The phylogeny of the order Dorylaimida has not been explored in recent years as many representatives of the order have not been sequenced, and a phylogenetic study needs to include several species from different subfamilies, families and higher taxa.

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Новые данные о хищных дорилаймидах (Nematoda: Dorylaimida) из Ирана.

**Резюме.** Два вида хищных дорилаймид были обнаружены в разных частях северного и северо-западного Ирана. Первый вид, *Carcharolaimus banaticus*, был обнаружен в городе Ношахр провинции Мазандаран и характеризуется самками длиной 1,8-2,0 мм и одонтостилем 20,3-21,8 мкм. Это первая находка этого вида в Иране. По сравнению с типовой популяцией из бывшей Югославии длина тела иранской популяции было несколько больше (1,75-1,86 против 1,23-1,76 мм). Второй вид, *Aetholaimus rotundicauda*, был обнаружен на лугах города Калейбар провинции Восточный Азербайджан и характеризуется самками длиной 1,3-1,6 мм и зубцами длиной 9,5-11,5 мкм. Это также первая находка этого вида в Иране. По сравнению с типовой популяцией из Нидерландов, тело нематоды несколько короче (в среднем = 1,4 против 1,7 мм). Обе находки в Иране представляют новую географическую зону распространения также для родов *Carcharolaimus* и *Aetholaimus*. Филогенетические взаимоотношения этих двух видов с другими избранными таксонами дорилаймид были изучены с использованием сиквенсов генов малых субъединичных последовательностей рибосомальной ДНК. На предполагаемом дереве представители двух подотрядов Dorylaimina и Nygolaimina образовали две отдельные клады. Иранская популяция *Carcharolaimus banaticus* образовала кладу с ранее секвенированным изолятом этого вида, а иранская популяция *Aetholaimus rotundicauda* также образовала кладу с ранее секвенированным изолятом этого вида.

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