



**New report of three species of Dorylaimellinae Jarajpuri, 1964  
(Nematoda: Belonidiroidea) from Iran**

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**Abstract**

Re-description of *Dorylaimellus graminis* and new data (morphological, morphometrical, and molecular) for *D. globatus* and *D. (Axodorylaimellus) parvulus* are presented from Taf area, Khorramabad, Lorestan province, Iran. These three species are reported for the first time for Iranian nematode fauna. The population of *D. graminis* is characterized by its 0.8-1.0 mm long body, lip region offset by constriction, 6-8  $\mu\text{m}$  broad with sclerotized pieces around the vestibule, odontostyle 5-7  $\mu\text{m}$  long, neck region 234-290  $\mu\text{m}$  long, pharyngeal expansion occupying 55-57% of total neck length, female genital system didelphic-amphidelphic, vulva longitudinal ( $V = 54-57$ ), caudal region convex-conoid (33-36  $\mu\text{m}$ ,  $c = 23.0-30.4$ ,  $c' = 1.8-2.6$ ), spicules 21-25  $\mu\text{m}$  long, and four spaced ventromedian supplements with a hiatus. The Iranian populations of two other species herein studied don't have significant differences compared to previously described populations. For the first time, the phylogenetic positions of these species based on 28S rDNA (D2-D3 segment) in this study are presented. Also based on molecular analyses we suggest that the genera *Dorylaimellus* and *Axodorylaimellus* are synonymous.

**Keywords:** *Axodorylaimellus*, D2-D3 rDNA, *Dorylaimellus*, Lorestan, Taxonomy.

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## گزارش جدید سه گونه از *Dorylaimellinae* Jarajpuri, 1964 (*Dorylaimina*, *Belondiroidea*) از ایران

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### چکیده

در این مطالعه توصیف مجدد گونه *Dorylaimellus graminis* و داده‌های جدید (ریخت‌شناختی، ریخت‌سنجی و مولکولی) برای *D. globatus* و *Axodorylaimellus (Dorylaimellus) parvulus* از منطقه تاف شهرستان خرم آباد در ایران ارائه شده است. این سه گونه برای اولین بار برای فون نمادهای ایران گزارش می‌شوند. جمعیت *D. graminis* با طول بدن ۰/۸ تا ۱/۰ میلی‌متر، ناحیه لیبی با فرورفتگی نسبت به بدن و عرض ۶ تا ۸ میکرومتر با قطعات اسکروتیزه در اطراف منفذ دهان، ادونتواستایل ۵ تا ۷ میکرومتر، ناحیه مری به طول ۲۳۴ تا ۲۹۰ میکرومتر، بخش فراخ ۵۵ تا ۵۷ درصد از کل طول مری، دستگاه تولید مثلی ماده دوتایی و متقابل، شکاف تناسلی طولی (۵۷-۵۴٪)، ناحیه دمی مخروطی محدب (۳۳ تا ۳۶ میکرومتر،  $c = ۲۳/۰ - ۳۰/۴$  و  $c' = ۱/۸ - ۲/۶$ )، آلت نرینه به طول ۲۱ تا ۲۵ میکرومتر، چهار پاپیل جفت‌گیری در سطح شکمی و هیاتوس مشخص می‌شود. مشخصات جمعیت‌های ایرانی دو گونه دیگر که در اینجا مطالعه شده‌اند تفاوت معنی‌داری با جمعیت‌هایی که قبلاً توصیف شده‌اند، ندارند. در این مطالعه برای اولین بار موقعیت تبارزایی این گونه‌ها بر اساس ناحیه D2-D3 از ژن 28S rDNA ارائه شده است. هم‌چنین براساس مطالعه و تجزیه و تحلیل مولکولی، سینونیم بودن دو جنس *Axodorylaimellus* و *Dorylaimellus* پیشنهاد می‌گردد.

کلیدواژه‌ها: *Axodorylaimellus*، *Dorylaimellus*، D2-D3 rDNA، تاکسونومی، لرستان

دبیر تخصصی: دکتر صدیقه عظیمی

## Introduction

Different ideas regarding the taxonomy of Dorylaimellinae have been suggested by several nematologists. Most researchers include a single valid genus, *Dorylaimellus*, for this subfamily. Jairajpuri & Ahmad (1980) listed nine and, later on (1992), 14 subgenera for this genus, and Ahmad & Naz (2010a, b, 2012) accepted the subgeneric grouping. Siddiqi (1983) differentiated 12 genera within the Dorylaimellinae. Andrassy (2009) divided the Dorylaimellinae into four genera *Axodorylaimellus* Jairajpuri & Ahmad, 1980, *Dorylaimellus* Cobb, 1913, *Ibadamus* Siddiqi, 1983 and *Mesodorylaimellus* Jairajpuri & Ahmad, 1980. While Peña-Santiago (2006) and Jimenez-Guirado et al. (2007) did not agree with including any subgenera or genera in the subfamily and regarded *Dorylaimellus* as the single genus in the subfamily Dorylaimellinae and prefer to accept the genus as a relatively homogenous taxon.

In a recent nematological survey conducted on identifying the belonderids in the Taf area, Khorramabad, Lorestan province, Iran, three known species of the genus *Dorylaimellus* were collected. New data and the phylogenetic position of the species based on the sequences of the D2-D3 segments of 28S rDNA are presented in this paper.

## Materials and Methods

Soil samples were collected from natural vegetation areas in the Taf region, Khorramabad, Lorestan province, Iran. Nematodes were extracted from the soil samples using the Brown & Boag (1988) modified method. Specimens for light microscopic examination were killed, fixed and transferred to dehydrated glycerin according to De Grisse's (1969) method and mounted on permanent glass slides. Nematodes were measured using an Olympus BX31 light microscope equipped with a Dino-eye eyepiece camera and Dino Capture version 2.0 software. Photographs were captured by a DP50 digital camera

attached to the Olympus BX41 light microscope.

## DNA extraction, PCR and sequencing

For DNA extraction, a single nematode was crushed in small pieces on a clean slide in a drop of worm lysis buffer (WLB). The pieces were transferred to an eppendorf tube containing 25.65  $\mu$ l ddH<sub>2</sub>O, 2.85  $\mu$ l 10X PCR buffer and 1.5  $\mu$ l proteinase K (600  $\mu$ g/ml). For 30 min, the tubes were stored in -80°C and immediately incubated at 65°C (1 h), and finally at 95°C (15 min). Amplification of the DNA and PCR reactions were performed using forward primer D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TCGGAAGGAACCAGCTACTA-3') of 28S rDNA gene (Nunn, 1992). The PCR cycles of amplified fragments were carried out according to Archidona-Yuste et al., (2016). The purified PCR products were sequenced using the same primers with an Applied Biosystems® 3730/3730xl DNA Analyzer in South Korea. The sequences obtained in this study were submitted to the GenBank database under accession numbers OR600370 (*Dorylaimellus globatus*), OR600368 (*Dorylaimellus graminis*) and OR594283 for *Dorylaimellus* (*Axodorylaimellus*) *parvulus*.

## Phylogenetic Analyses

The 28S rDNA gene sequences of previously deposited Belonidiridae Thorne, 1939 representatives were obtained from the GenBank database and used for phylogenetic reconstruction. The sequences were aligned using MEGA6 software (Tamura et al., 2013). The appropriate model of DNA evolution was obtained using ModelTest 2.3 (Nylander, 2004) with the Akaike information criterion (AIC). Phylogenetic analysis was performed using Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Posterior probabilities (PP) are given on appropriate clades. The phylogenetic tree was visualized using the program FigTree v1.4.3.

## Results and Discussion

### *Dorylaimellus graminis* Kruger 1965 (Jairajpuri & Ahmad 1980), (Fig. 1).

Measurements See Table 1.

#### Description

##### Adult

Slender nematodes of medium size, 0.8-1.0 mm long. Habitus after fixation curved ventrad to C shaped. Outer cuticle layer thin throughout the body and with fine transverse striations, 1.0-1.5  $\mu\text{m}$  thick at mid body and 2.5-3.0  $\mu\text{m}$  on tail. Lateral chord occupying 38-41% of the mid-body width, without glandular bodies. Lateral pores obscure. Lip region offset by constriction, with sclerotized pieces around the vestibule, 2.0-2.5 times as wide as high and about 21-25% of the body diameter at neck base. Lips moderately separate, without perioral disc. Amphid fovea cup-shaped, its opening occupying 57-60% of the lip region width. Odontostyle straight, 0.7-0.9 times lip region width. Odontophore flanged, 2.0-2.5 times the odontostyle length. Guiding ring simple, 5.0-5.5  $\mu\text{m}$  from anterior end. Nerve ring at 23-27% of neck length or 268-290  $\mu\text{m}$  from anterior end. Anterior part of the pharynx weakly muscular with a spindle-shaped swelling shortly behind the base of the odontophore flanges. Pharyngeal basal bulb cylindrical, 13-14 times as long as wide, occupying 55-57% of the total neck length, twisted in a spiral muscular sheath. Pharyngeal gland nuclei obscure. Cardia rounded conoid, 1.0-1.5 times as long as wide. Tail slightly convex-conoid, ventrally almost straight with a rounded terminus, hyaline part about 3.0-3.9  $\mu\text{m}$ .

##### Female

Reproductive system didelphic-amphidelphic, anterior and posterior genital branch equally developed, (142-196 and 116-200  $\mu\text{m}$ , respectively), ovaries reflexed, 36-48  $\mu\text{m}$  (anterior) and 37-42  $\mu\text{m}$  (posterior); anterior and posterior oviduct, 56-67 and 50-63  $\mu\text{m}$ , respectively, anterior and posterior uterus length 16-27 and 21-29  $\mu\text{m}$ , respectively, vulva a longitudinal slit,

vagina extending inwards 14-16  $\mu\text{m}$  or 65-73% of body diameter, *pars proximalis* 6.0-8.0  $\times$  3.5-4.5  $\mu\text{m}$  and somewhat hemispheroid, *pars refringens* absent, and *pars distalis* 3.0-3.5  $\mu\text{m}$  in length. Prerectum 5.0-6.5 and rectum 1.0-1.5 times anal body widths long.

##### Male

Similar to female in general morphology except the posterior region being more curved ventrad. Adcloacal supplements, located at 4.0-4.8  $\mu\text{m}$  anterior to cloacal aperture, four ventromedian pairs arranged in two groups. Spicules dorylaimoid, strongly curved at middle, 3.0-3.5 times as long as wide. Prerectum 4.5-5.5 times cloacal body width long.

##### Distribution

Taf region, Korramabad, Lorestan province, Iran (GPS coordinates: N 33°17'45.6", E 48°22'51.1"), from the rhizosphere of astragalus (*Astragalus gossypinus* L.).

##### Remarks

*Dorylaimellus graminis* has been recorded only from South Africa (Kruger, 1965; Jordaan & Heyns, 1984). Its occurrence in Iran, therefore, represents its third record. Iranian specimens are nearly identical to South African populations have very minor differences in *b* (3.5-3.7 vs 4.1-4.4) and *c'* (1.8-2.6 vs 1.2-1.7) values.

### *Dorylaimellus (Axodorylaimellus) parvulus* Thorne, 1939, (Fig. 2).

Measurements: See Table 1.

##### Distribution

Chub Tarash village, Korramabad, Lorestan province, Iran (GPS coordinates: N 33°21'03.2", E 48°24'51.1"), from the rhizosphere of walnut (*Juglans regia* L.) trees.

##### Remarks

This species is widely spread in Europe, including Belgium, Hungary, Spain and South Africa (Andrássy, 2009). The Iranian population herein studied does not differ from those previously described (Peralta & Pena Santiago, 2000; Andrássy, 2009) in any remarkable feature, either morphological or morphometric data.

**Table 1. Morphometrics of the Iranian populations of *Dorylaimellus* (*Axodorylaimellus*) *parvulus* Thorne 1939, *Dorylaimellus globatus* Yeates 1970 and *Dorylaimellus graminis* Kruger 1965 (Jairajpuri & Ahmad 1980). All measurements are in  $\mu\text{m}$  and in the form: mean  $\pm$  s.d. (range).**

| Characters*                | <i>D. parvulus</i>            | <i>D. globatus</i>                | <i>D. graminis</i>             |                                |
|----------------------------|-------------------------------|-----------------------------------|--------------------------------|--------------------------------|
|                            | Female                        | Female                            | Female                         | Male                           |
| N                          | 6                             | 6                                 | 3                              | 3                              |
| L                          | 522.9 $\pm$ 22.0<br>(495-549) | 1253.6 $\pm$ 56.3<br>(1178-1311)  | 912.1 $\pm$ 93.6<br>(816-1034) | 1053 $\pm$ 34.7<br>(1013-1073) |
| A                          | 28.7 $\pm$ 2.0<br>(27-32)     | 42.6 $\pm$ 1.9<br>(41-45)         | 41.9 $\pm$ 3.0<br>(37-45)      | 45.8 $\pm$ 1.4<br>(45-48)      |
| B                          | 2.6 $\pm$ 0.3<br>(2-3)        | 3.2 $\pm$ 0.1<br>(3.1-3.3)        | 3.6 $\pm$ 0.1<br>(3.5-3.7)     | 3.7 $\pm$ 0.0<br>(3.6-3.8)     |
| C                          | 31.3 $\pm$ 3.7<br>(26.5-37.0) | 19.0 $\pm$ 0.7<br>(18-20)         | 27.5 $\pm$ 3.3<br>(23-32)      | 29.5 $\pm$ 3.6<br>(26-33)      |
| c'                         | 1.3 $\pm$ 0.1<br>(1.1-1.4)    | 3.8 $\pm$ 0.1<br>(3.8-4.0)        | 2.2 $\pm$ 0.2<br>(1.8-2.6)     | 2.1 $\pm$ 0.3<br>(1.8-2.4)     |
| V                          | 58.8 $\pm$ 0.4<br>(58-60)     | 56.7 $\pm$ 1.1<br>(55-58)         | 55.6 $\pm$ 0.8<br>(54-57)      | -                              |
| G1                         | 10.6 $\pm$ 3.0<br>(7.5-14.5)  | 13.5 $\pm$ 2.3<br>(10-16)         | 17.8 $\pm$ 3.4<br>(15-24)      | -                              |
| G2                         | 10.8 $\pm$ 2.3<br>(9-14)      | 14.0 $\pm$ 3.2<br>(11-19)         | 17.1 $\pm$ 4.7<br>(11-24)      | -                              |
| Lip region diameter        | 5.1 $\pm$ 0.2<br>(5-6)        | 6.2 $\pm$ 0.1<br>(6.0-6.5)        | 6.5 $\pm$ 0.6<br>(6-8)         | 7.6 $\pm$ 0.2<br>(7-8)         |
| Amphid aperture            | 3.4 $\pm$ 0.3<br>(3.2-3.8)    | 2.9 $\pm$ 0.9<br>(2-4)            | 3.6 $\pm$ 0.2<br>(3-4)         | 2.9 $\pm$ 0.0<br>(2.5-3.0)     |
| Odontostyle length         | 4.9 $\pm$ 0.3<br>(4.5-5.5)    | 6.1 $\pm$ 0.1<br>(6.0-6.5)        | 5.5 $\pm$ 0.6<br>(5-7)         | 6.6 $\pm$ 0.5<br>(6-7)         |
| Odontophore length         | 8.2 $\pm$ 1.0<br>(7-10)       | 11.6 $\pm$ 0.3<br>(11-12)         | 11.5 $\pm$ 0.5<br>(10.5-12.0)  | 11.4 $\pm$ 0.6<br>(10.5-12.0)  |
| Guiding ring from ant.     | 4.2 $\pm$ 0.2<br>(4.0-4.5)    | 5.6 $\pm$ 0.1<br>(5.5-6.0)        | 5.4 $\pm$ 0.2<br>(5-6)         | 5.0 $\pm$ 0.0<br>(5-5)         |
| Neck length                | 199 $\pm$ 22.6<br>(183-235)   | 382.5 $\pm$ 15.4<br>(365-400)     | 271.0 $\pm$ 19.6<br>(234-288)  | 280.1 $\pm$ 10.6<br>(268-290)  |
| Pharyngeal bulb length     | 107.1 $\pm$ 16.7<br>(96-135)  | 236.7 $\pm$ 14.2<br>(220.7-254.5) | 159.3 $\pm$ 14.9<br>(138-182)  | 158.6 $\pm$ 13.9<br>(142-168)  |
| Body diameter at mid-body  | 18.2 $\pm$ 0.6<br>(17-19)     | 29.4 $\pm$ 1.7<br>(28-33)         | 22.1 $\pm$ 1.3<br>(20.5-25.0)  | 23.0 $\pm$ 1.4<br>(21-24)      |
| Body diameter at neck base | 18.1 $\pm$ 0.3<br>(18-19)     | 30.5 $\pm$ 1.8<br>(27-32)         | 21.8 $\pm$ 1.3<br>(20-24)      | 22.9 $\pm$ 1.7<br>(21-24)      |
| Body diameter at anus      | 12.9 $\pm$ 0.4<br>(12.5-13.5) | 16.5 $\pm$ 0.4<br>(15.5-17.0)     | 13.7 $\pm$ 0.7<br>(13-15)      | 17.1 $\pm$ 0.6<br>(16.5-18.0)  |
| Pre-rectum length          | 48.5 $\pm$ 2.6<br>(46-52)     | 106.7 $\pm$ 10.1<br>(98-121)      | 76.1 $\pm$ 9.1<br>(68-90)      | -                              |
| Tail Length                | 16.8 $\pm$ 1.7<br>(15-19)     | 64.9 $\pm$ 2.9<br>(61-67)         | 33.9 $\pm$ 1.1<br>(33-36)      | 35.9 $\pm$ 4.5<br>(32-41)      |
| Spicule's length           | -                             | -                                 | -                              | 22.8 $\pm$ 2.1<br>(21-25)      |

\* L = body length; a = body length/maximum body diameter; b = body length/neck length; c = body length/tail length; c' = tail length/body diameter at anus; V = distance of vulva-anterior end/body length  $\times$  100; G1 = anterior genital branch length/body length  $\times$  100; G2 = posterior genital branch length/body length  $\times$  100.

***Dorylaimellus globatus* Yeates, 1970, (Fig. 3).**

Measurements: See Table 1.

**Distribution**

Taf region, Korramabad, Lorestan province, Iran (GPS coordinates: N 33°17'44.6", E 48°22'50.1"), from the

rhizosphere of apricot (*Prunus armeniaca* L.) trees.

**Remarks**

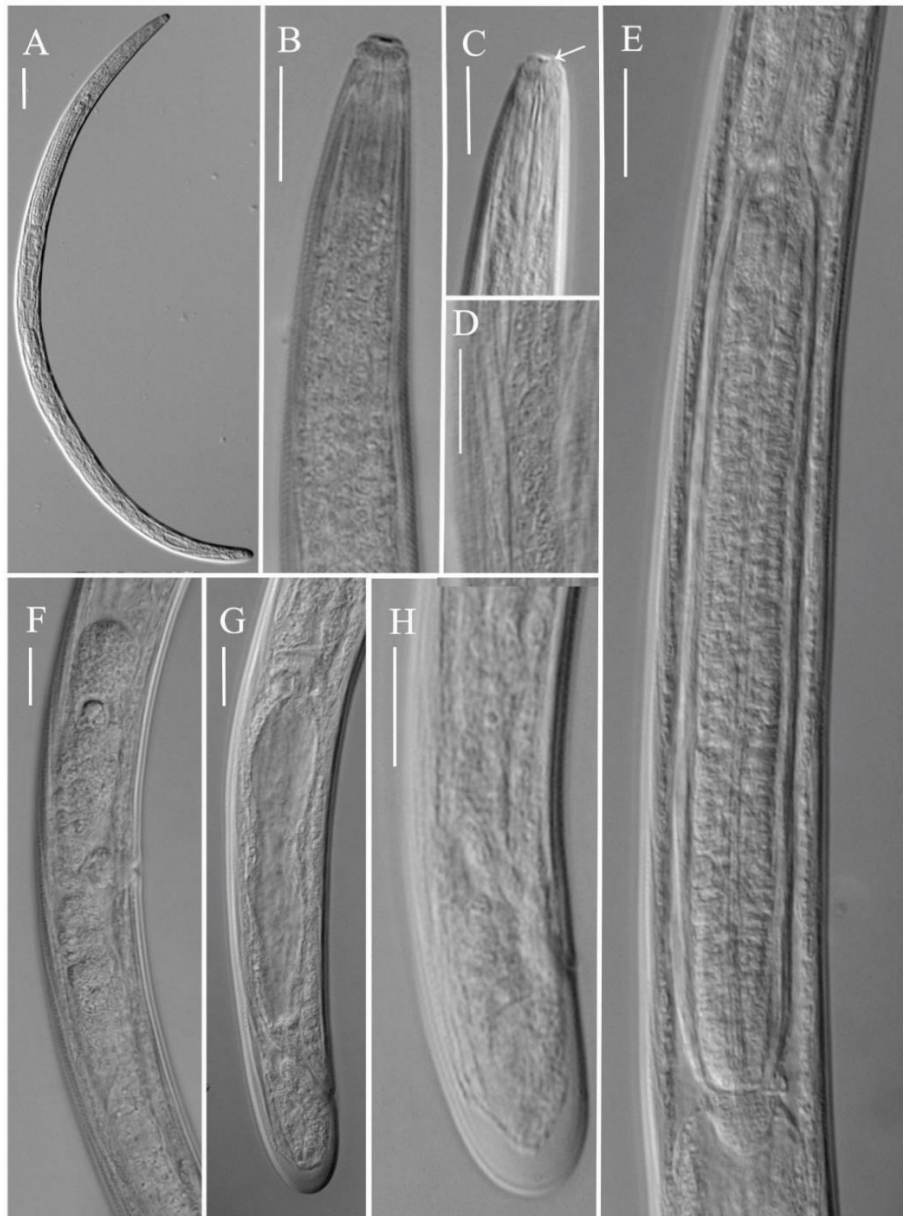
The species was originally described in England by Yeates (1970) from Buckthorn Carr. The description and measurements of

this understudy population fit highly with the original description (Yeates, 1970)

except in having slightly more  $c'$  (3.8-4.1 vs 2.9-3.8) value.



**Figure 1.** Iranian population of *Dorylaimellus graminis* Kruger 1965 (Jairajpuri & Ahmad, 1980). A-H & J, Female, I & K, Male. A, B: Anterior end; C: Lateral chord; D: Neck region; E: Vagina; F: Cardia; G: Anterior genital branch; H: Female tail; I: Male tail; J: Female entire body; K: Male entire body. Scale bars: A-I = 10  $\mu$ m; J, K = 30  $\mu$ m. The arrows in Figs. B, D, and I indicate sclerotized pieces around the oral opening, globular bodies and ventromedial supplements, respectively.



**Figure 2.** Iranian population of *Dorylaimellus* (*Axodorylaimellus*) *parvulus* Thorne 1939. Female. A: Entire body; B, C: Anterior end (the arrow indicates sclerotized pieces around the oral opening); D: Lateral chord; E: Part of neck region; F: Reproductive system; G, H: Posterior end. Scale bars: A = 30  $\mu$ m; B-H = 10  $\mu$ m.

### Molecular characterization and phylogeny

So far, no sequence data of 28S rDNA have been recorded for this subfamily. In this study, three sequences, OR600370 (*Dorylaimellus globatus*), OR600368 (*D. graminis*), and OR594283 (*D. parvulus*) of D2-D3 expansion segments of the 28S rDNA gene were obtained. In our nucleotide phylogenetic analysis, the species belonging to the subfamily Dorylaimellinae were found closely related to the members of the genus *Belondira* Thorne, 1939 of subfamily

Belondirinae Thorne, 1939 compared to other genera of the family Belondiridae, so that form a well-supported sister clade (1.00 BPP) with the species of the genus *Belondira*. On the other hand, in the phylogenetic tree (Fig. 4), the abovementioned clade is a sister clade including other genera from subfamily Belondirinae, *Axonchium* Cobb, 1920, *Syncheilaxonchium* Coomans & Nair, 1975, *Metaxonchium* Coomans & Nair, 1975 and *Axonchoides* Thorne, 1967 with a 0.99 BPP. *D. (Axodorylaimellus)*

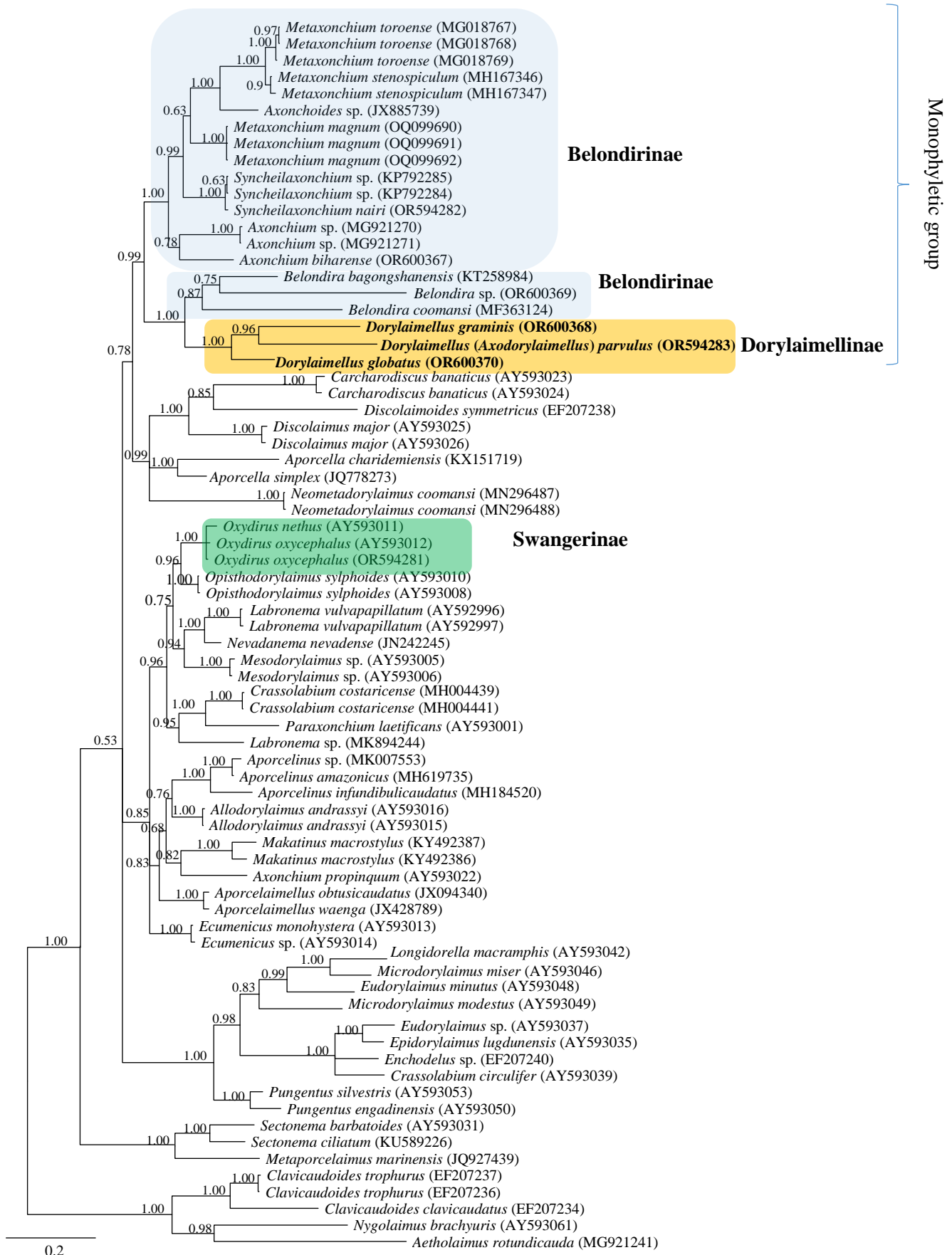
*parvulus* (OR594283) in the tree (Fig. 4) is located adjacent to the two species of *Dorylaimellus* with a well-supported posterior probability (96% BPP). According to Andr assy (2009), *Axodorylaimellus* is similar to *Dorylaimellus* in having lateral glandular organs, circumoral sclerotized pieces, and the conspicuously flanged odontophore but differs from the latter in having a perioral disc, the transverse vulva, and the

cylindroid or clavate tail with very thick terminal cuticle. Therefore, according to the position of these species in the inferred phylogenetic tree and morphological characteristics, these two genera are also considered synonyms. Meanwhile, as mentioned in the introduction, some other researchers have already agreed with placing a single genus (*Dorylaimellus*) in the subfamily Dorylaimellinae.



**Figure 3.** Iranian population of *Dorylaimellus globatus* Yeates, 1970. Female. A body; B, C: Anterior end; D: Lateral chord; E: Part of neck region (the arrow indicates globular bodies); F: Reproductive system; G: Cardia; H: Vagina; I: Posterior end. Scale bars: A = 30  $\mu$ m; B-I= 10  $\mu$ m.





**Figure 4.** Bayesian inference tree from three known species of *Dorylaimellus* based on sequences of the 28S rDNA region. Bayesian posterior probabilities equal to, or more than 0.5 are given for appropriate clades. Newly obtained sequences are in bold letters.

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