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Three new species of free living nematodes for nematode fauna of Iran

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Abstract

Three unrecorded species of free-living nematodes, namely *Chronogaster typica*, *Eutobrilus nothus* and *Thornenema baldum* were collected from the rhizosphere of rice (*Oryza sativa* L.) in Guilan province of Iran, during 2014-2016. The Iranian population of *Chronogaster typica* is characterized by having 950-1350 µm long body, specified crystalloid bodies and vacuolated lateral glandular bodies (VLGB), two well-developed lines in lateral fields and 125 to 169 µm long tail with a blunt ventral mucro at terminus without any spines. The Iranian population of *Eutobrilus nothus*, is distinguished by having 1250-1700 µm long body, funnel shaped buccal cavity, a small prominent tooth in each overlapping pockets, the six echinate ampulla and a longer and thicker thorn in the center. The Iranian population of *Thornenema baldum*, is characterized by short (800-1010 µm) body length, distinct post-labial sclerotizations, set off and narrower lip region than rest of the body and presence of bulges exactly under the lip region in the tissues.

Keywords: Descriptions, free living nematodes, morphometric, morphology, *Oryza sativa*

1. Introduction

Chronogaster Cobb, 1913^[12], as the type and only genus of the family Chronogasteridae Gagarin, 1975^[18], with 48 valid species Holovachov & De Ley^[22], the *chronogaster* species are distributed all over the world, they apparently prefer the warm zones, a lot of them occurs in the tropical and subtropical of earth, they found in freshwater, thermal springs, moist soils, fungal mats, and salty habitats including caves, they have great physiological plasticity and tolerance to high level of oxygen fluctuation Abebe *et al*^[2], Andrassy^[8], Hodda *et al*^[21]. Brief recognition of the genus are as follow: having developed cephalic setae, short and fine; tubular stoma, more or less; esophagus with terminus bulb equipped with longitudinal serrate valve, long post bulbar extension; an annulated cuticle, whether or not longitudinal lines and spines; female gonad monodelphic-prodelphic, tuboid supplements and long tail without spinneret.

The genus *Eutobrilus* tsalolikhin, 1981^[35] belongs to the family Tobrilidae Tsalolikhin, 1981^[35], the majority of species are aquatic and live in aquatic ecosystems but less proportion of them inhabit moist soil, moss, brackish water and others wet terrestrial habitats and they are particularly common in water bodies where oxygen is limited or absent (Nuss^[36], Jacobs^[24]). *Eutobrilus* is the largest genus in the family due to number of the species. So far, 23 nominal species have been described under the genus Gagarin & Naumova^[20], Andrassy^[7]. *Eutobrilus* showed some remarkable morphological features as follows: stoma structure with overlapping pockets or almost so, teeth fairly near each other (2-8 apart), cephalic setae mostly long (usually 40-60% of head diameters), simple vagina, well developed echinate and protruding supplements in males.

First time, Andrassy^[7] offered the genus *Thornenema* for some species of the genus *Dorylaimus* Dujardin, 1845^[16] and clearly distinguishes them from *Dorylaimus* with being different in the structure of lip region, female reproductive system, tail shape and absence of male. At present, *Thornenema* Andrassy, 1959^[7] is the richest genus in the family thomenematidae Siddiqi, 1969^[27], with 25 valid species, including terricolous and semi-aquatic free living nematodes. In summary, the most distinctive feature of the *Thornenema* are: small to medium sized body, the labial region with a sclerotized framework, mono-opisthodelphic female genital organ, range of vulva position (31-50%); 3-9 supplements in number with far spaced arrangements in male and different tail in sexes. Recently some soil and aerial parts-related nematodes of plants are reported from Northern Iran (Aliramaji *et al*^[4], Atighi *et al*^[5], Mobasser *et al*^[26], Pedram *et al*^[27], Soleymanzadeh *et al*^[32]).

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2. Material and methods

Soil samples were collected from the rice fields in Guilan Province of Iran, during 2014–2016. Nematodes were extracted by using sieve and centrifugation techniques according to De Grisse [13], then the collected specimens were killed and fixed following by Hooper [23] and mounted on permanent glass slides for further studies. Observations were made under a Nikon E600 light microscope and drawings and morphometric data were performed with an Olympus BX41 light microscope equipped with a drawing tube. Species were identified due to their morphology and morphometric characters, valid identification keys and compared to the original and reliable descriptions (Bajaj & Bhatti [10], Khan & Nanjappa [25], Andr assy [7], Andr assy [8], Baqri and jairajpuri [9], Gagarin [18], Siddiqi [31], Raski & Maggenti [28], Carbonell & Coomans [11], Williams [38], Thorne [34], De Man [14], De Coninck [14] and etc.).

3. Results and discussion

Free-living nematodes have very important and beneficial roles in the decomposition of organic material, the recycling of nutrients in soil, important environmental indicators, predators of damaging nematodes, etc. nevertheless, this group as one of the most important groups of nematoda are poorly understood and their benefit significantly have been less considered Shahabi *et al* [29]. In this paper, three species belonging to this group namely: *Chronogaster typica* (De Man, 1921 [15]) De Coninck, 1935 [14], *Eutobrilus nothus* Gagarin, 1989 [19] and *Thornenema baldum* (Thorne, 1939 [34]) Andr assy, 1959 [7] are identified.

3.1 *Chronogaster typica* (De Man, 1921) De Coninck, 1935

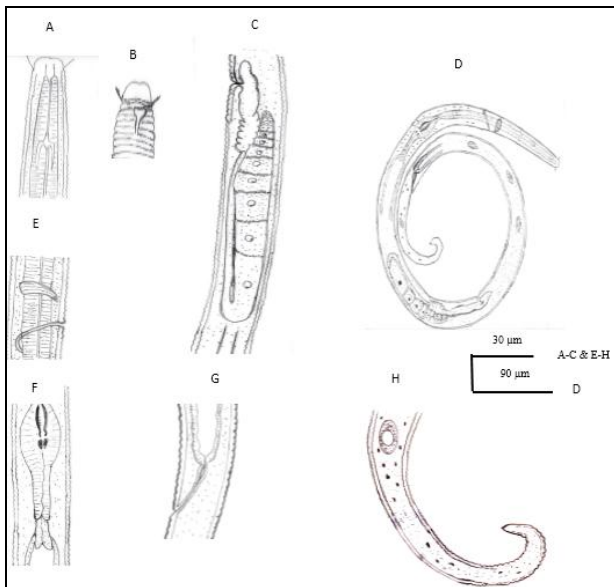


Fig 1: *Chronogaster typica*, (Female). Head region (A & B), reproductive system and Lateral fields (C), entire body (D), oesophagus region (E & F), and posterior region and tail (G & H).

3.2 Female: Body long and slender, ventrally curved in spiral shape upon fixation, overall cylindrical tapering towards both extremities but more pronounced in tail region. Labial capsule truncate, non-annulated, continuous with adjacent body, with two sub median cephalic setae which originating forward, labial papillae indistinct, the end-on view of lips showing rounded and amalgamated. Cuticle with well-defined transverse annuli (except for the lip region and tail terminus), annuli width variable: about 0.9 μm close to anterior end, 1.4

μm close to midbody, 1.1 μm at the posterior end. Stoma almost cylindroid except for short conoid posteriorly narrowing, elongate (approximately one-fourth as long as the cylindroid part long) and separated from esophagus lumen by constriction, radial tubules originating 18–24 μm from base of stoma. Amphid funnel shaped with narrow transverse oval aperture about 2.1 μm across, posterior extending somewhat to the middle of the stoma, boundary smooth located about one to two annuls under the lip region. Two lateral lines well expressed. Body without longitudinal rows. Small and oval shaped crystalloid bodies occurring throughout the body cavity but more significant at esophagus region. Vacuolated lateral glandular bodies (VLGB) specified. Esophagus slender, basal bulb ovate 14–16 \times 19–24 μm , contains denticulate valve with longitudinal rows, bulbar extension long 22–26 μm . Nerve ring located at 81 to 91 μm from front end. Excretory pore observed about 7–10 μm behind nerve ring. Female genital apparatus is monodelphic-prodelphic. Vulva with a short transverse slit, not prominent, located near mid-body, vagina about three to four annuls body extending inward, its length almost 3–5.4 μm , ovary reflexed back near to vulva, post-vulval uterine sac length is almost one-third as long as body width in that area. Rectum about 1.5 time's anal body width long. Tail length approximately 8–9 anal diameter of body in length, tail elongate conoid, tapering regularly from anus toward the end, tip terminus non-annulated with single ventral mucro without spines, caudal glands absent. Male: Not observed.

3.3 Habitat and locality: This species observed in some samples in rice fields in Astara country, during September 2014. Astara is the northern city of Guilan province and located on western margin of the Caspian Sea which situated at 142 km north-west of Rasht (capital of Guilan province) with this geographical coordinates: 38° 25' 44.7" North, 48° 52' 19.2" East. Astara, covering an area of 344 square kilometers, rice is cultivated as main crop in this region. *C. typica* is a new geographical record for Iran.

3.4 Discussion of *chronogaster typica*

Iranian population of *Chronogaster typica* is in both morphological and morphometric comply with the data given by Andr assy [8] and Bajaj & Bhatti [10] (see Table 1). *Chronogaster typica* is characterized by having slightly medium-sized and slender body, crystalloids and vacuolated lateral glandular bodies, short post vulva sac, two distinct lateral fields without incisures, long tail with a ventrally bent mucro on the tail terminus without any spines. *Chronogaster typica*, comes close to three known species within the genus namely: *C. neotypica* Tahseen & Seddiqi, 2003 [33], *C. carolinensis* Abebe, Ferebee, Taylor, Mundo-Ocampo, Mekete & De Ley, 2013 [1] and *C. ethiopica* Abebe & Coomans, 1996 [3]. Comparison with *C. neotypica*: *C. typica*, is most similar to *C. neotypica* in containing one ventral mucro without additional spines and the presence of crystalloid bodies, but can be distinguished by presence of lateral lines and VLGB (vs. absence), longer distance of radial tubules from stoma (vs. 13–22 μm) and the longer tail (vs. 128 μm). Comparison with *C. carolinensis*: *C. typica*, is also in having one ventral mucro without additional spines, presence of crystalloid bodies and VLGB, comes close to *C. carolinensis* but can be easily distinguished by the presence of two longitudinal lines (vs. absence), shorter stoma (vs. 9–10 μm), and fewer esophagus length (vs. 246 – 285 μm). Comparison with *C. ethiopica*: *C. typica*, is also in containing

both crystalloid bodies and VLGB and tail terminus with only one blunt ventral mucro closet to *C. ethiopica*, however, the most distinctive characteristics between them is two

longitudinal lateral lines in *C. typica* (vs. lack of longitudinal lines) and lesser values for the ratios c'(vs. 15-17.8).

Table1: Morphometric characters for the females of *Chronogaster typica* population in the form: \pm means standard deviation range (all measurements in μm).

Population Characters	Present study Female	Andrássy ^[8] Female	Bajaj & Bhatti ^[10] Female	Abebe <i>et al</i> ^[1] Female
n	6	6	5	-
L	1100 \pm 245(950-1350)	1080-1440	900-1100	1080-1440
a	52 \pm 6.5(44-58)	48-69	57-60	40-69
b	5.1 \pm 0.6(4.4-5.6)	4.7-5.4	4.7-5.5	4.7-6.0
c	7.3 \pm 1.1(6.8-8.2)	6.3-8.7	7.1-7.5	5.8-7.8
c'	11.2 \pm 1.5(9.5-12.5)	10-12	12-13	12-14
V	47.5 \pm 4.8(44-52)	45-51	46-49	45-51
Ph L	235 \pm 10 (221-242)	229-266	-	-
CSL(cephalic setae length)	6.4 \pm 0.8(6.1-7.3)	9-11	-	8-11.5
Amph-ABE	5.3 \pm 0.4 (4.5-6.2)	-	-	-
Stoma L	7.5 \pm 0.5 (6.1-8.1)	8-9	-	-
Stoma W	2.9 \pm 0.4 (2.2-3.3)	-	-	-
Bulbar extension	24 \pm 2.5 (23-26)	21-28	-	-
Anterior genital branch length	142 \pm 20.5(121-164)	-	-	-
Posterior uterine sac length	9.2 \pm 3.2 (6.5-12.1)	-	-	-
Diam. At mid-body	23 \pm 3.2(21-26)	20-22)(-	-
Diam. At anus-body	17.5 \pm 1.5(14-19)	-	-	-
Tail	145 \pm 21(125-169)	165-171	-	130-200
RL	28 \pm 2.5 (25-31.5)	-	-	-

3.5 *Eutobrilus nothus* Gagarin, 1989

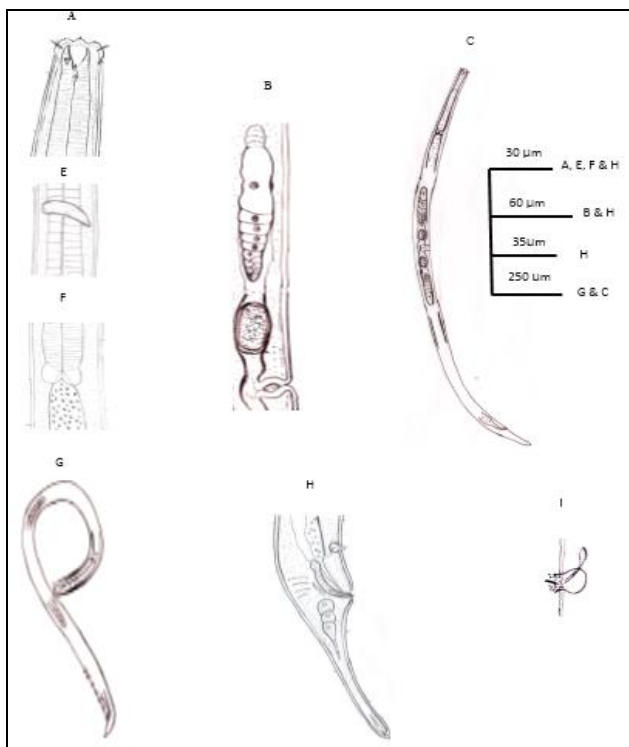


Fig 2: *Eutobrilus nothus*. Female, Part of Pharynx (E & F), head region (A), Part of reproductive system (B), entire body (C). Male, entire body (G), posterior and tail region (H) and supplement (I).

3.6 Female: Body medium sized, less straight, most arcuate ventrally after fixation, cylindrical shaped, tapering toward both extremities, gradually tapering anteriorly to bluntly lip region, more tapering dorsally to elongate conoid tail. Lip region wide, almost flat at apex, continuous with body contour. Six outer labial sensillae papilliform, cephalic setae thin and non-articulated. Cuticle layers distinct; smooth and finely annulated between male's supplements with LM, about

1.5 μm thick at mid-body. Cheilostoma wide, with sclerotized lining, occupying more than one-third of lip region diameter, buccal cavity funnel-like, 18-23 μm long, with two overlapping adjacent pockets, by one small prominent tooth in each pocket which about 6-8 μm apart from each other. Amphid distinct, forming a cup shaped, situated at posterior of buccal cavity. Body scattered faintly setae, numerous and more at tail region. Pharynx totally muscular, cylindrical shape which gradually expanding towards the base, about 240-270 μm long, cardia oval glands large, about 19-24 μm wide and 10-12 μm long. Nerve ring roughly located at 40-45% of pharyngeal length. Female reproductive system didelphic-amphidelphic, ovaries reflexed and approximately two ovaries equal in length. Vaginal length about 40-45% of corresponding body diameter, with muscular thick wall, vulva transverse slit, situated about middle of the body. Vulval lips simple, lacking protruding structures, uterus containing an intrauterine rectangular-shaped egg, measuring 70-84 \times 38-45 μm . Tail elongate conoid, tail length about 4-5 times of the body diameter at anus, a little ventrally arcuate in some materials, regularly tapering to a rounded terminus, three caudal glands cells distinct and opening via spinneret pore at the end of tail.

Male: males presented as much as females, similar to females in general morphology, except sexual organs. Male's reproductive system diorchic with opposed outstretched testis. Supplementary organs approximately identical at size, well developed, echinate with protruding shoulders, having a thorns in the center and many small thorns surrounding, the contents of ampulla concentrated in the top part, arranged in to variable spaces distances between supplements; S₁-S₂: 25.5 \pm 3; S₂-S₃, 24.2 \pm 2.5; S₃-S₄, 30.3 \pm 1.2; S₄-S₅, 32.5 \pm 2.1; S₅-S₆, 34.5 \pm 2.2; S₆-anus, 44.5 \pm 3.

Short and ventrally curved spicules. Gubernaculum thick with special shape (almost is composed of two parts: a parallel part with spicules axis and a curved section to the spicules axis). Male's tail shorter than females, about 3-4 times anal body diameter, but similar in shape in both sexes.

3.7 Habitat and locality: Recovered in some specimens around the paddy in Bandare-Anzali country, during September 2015. Bandare-Anzali, is located at the southern coast of the Caspian Sea in Guilan province, Iran; It covers an area about 275 square kilometers with GPS coordinates: 37° 27' 50.0724" North, and 49° 28' 47.5032" East. *E. nothus* as new geographical record of *Eutobrilus* species from Iran is described.

3.8 Discussion of *Eutobrilus nothus*

Iranian population of *E. nothus* is in morphological and morphometric agreement with the original description by Gagarin [19] (Table 2). This species is characterized by medium body length; high lips; buccal cavity with two subventral overlapping pockets; a comparatively small teeth in each pocket; short and slightly curved spicules; short, bifurcate shaped and heavily cuticularized gubernaculum; presence of 6-8 echinate supplements with a longer and

thicker thorns in center and numerous small thorns in around; conical shaped and comparatively long tail; well-developed caudal glands and spinneret; absence of crystalloids and cervical setae. *E. nothus* is different from a close species, *E. gracileformis* (Altherr and Delamare Deboutteville, 1972) Tsalolikhin, 1981 [35] by a shorter body (vs. 1680-2200 μm), shorter spicule length (vs. 45-46), and less numbers of supplements (vs. 6-9) and other morphometric ratios. Also *E. nothus* is similar to *E. obesus* Gagarin and Naumova, 2012 [20] in shape of supplements in males (ampulla contents concentrated at ampullae basis). but it differs in having shorter body length (vs. 2110–2750 μm), shorter tail length (vs. 314), shorter spicules length (vs. 58–67 μm), less number of precloacal supplements (vs. 6-8) and in shape of the gubernaculum (vs. without bifurcate shape) and tail (vs. tail consists of two parts which the distal cylindrical part about two times of proximal conical part long).

Table 2: Morphometric characters for the females of *Eutobrilus nothus* population in the form: \pm means standard deviation range (all measurements in μm).

Population Characters	Present study		Gagarin [19]	
	Female	Male	Female	Male
n	8	6	14	6
L	1520 \pm 195 (1250-1700)	1450 \pm 200(1300-1630)	1580(1330-1830)	1540(1390-1680)
a	26.5 \pm 4.5 (22-32)	25.5 \pm 2.5 (25-28)	25.6(21.9-30.9)	35.1(30.8-41.6)
b	5.5 \pm 0.75 (4.5-6.1)	5.2 \pm 0.25 (5.3-5.5)	4.9(4.6-5.1)	5.2(4.9-5.5)
c	11 \pm 1.5 (9.1-12.7)	14.5 \pm 1.4 (12.5-15.2)	11.3(9.9-12.3)	15.7(12.7-16.7)
c'	4.5 \pm 0.65 (3.2-5.1)	4.1 \pm 0.3 (3.6-4.5)	-	-
V	48 \pm 2.4 (45.5-51)	-	50.2(46.6-54.3)	-
Tail	141 \pm 12 (136-154)	116.5 \pm 5.1 (110-121)	115-150	85-123
Cephalic setae length	5.1 \pm 1.5(3.5-7.5)	-	2.5-8.2	-
Anterior tooth-ABE	16.2 \pm 1.5 (15-17.2)	-	-	-
Posterior tooth-ABE	23 \pm 1.5 (22-25)	-	-	-
Anterior genital branch length	298 \pm 4.5 (290-305)	-	-	-
Posterior genital branch length	308 \pm 5.5 (301-315)	-	-	-
Spicules	-	38 \pm 3.5 (34-42)	-	35-43
Gubernaculum	-	16.5 \pm 1.8 (15-18)	-	-
RL	19.2 \pm 1.2(18.1-22)	-	-	-
Length of supplements row	-	196.2 \pm 6.2(191-211)	-	-

Thornenema baldum (Thorne, 1939) Andr ssy, 1959

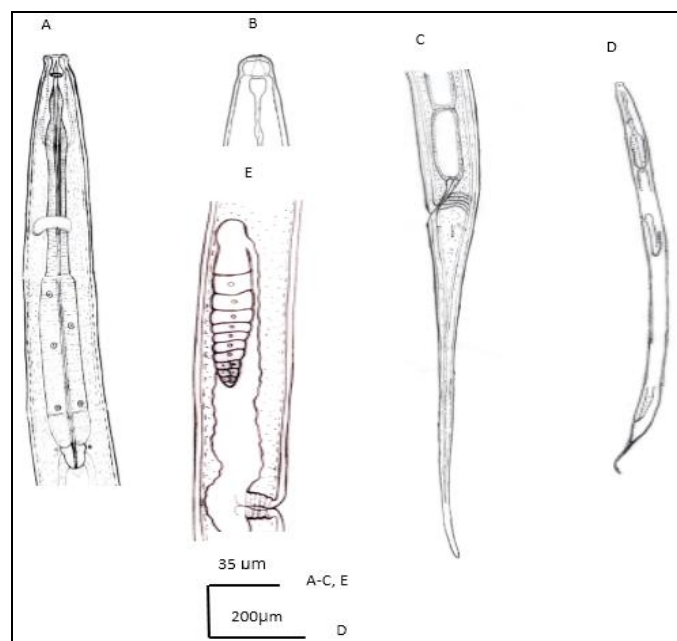


Fig 3: *Thornenema baldum* (Female). Anterior end and head region (A & B), reproductive system (F), posterior and tail region (C) and entire body (E).

3.9 Female: body cylindrical, ventrally weakly curved when relaxed, tapering to both end. Cuticle consisting of two layers (a smooth outer layer and a thin layer with faint striations at inner with LM), thickness about 1.5-2.5 μm at mid-body. Lips region amalgamated, narrower than adjacent body, truncated with flattened anteriorly, with distinct labial papilla, obviously offset by constriction, having bulges under lip region in the tissues, post-labial framework more sclerotized than labial framework. Amphid cup-shaped, its aperture occupying one-third or somewhat more as wide as lip region diameter. Odontostyle short and slender, odontophore rod like, 1.4-1.7 times longer than odontostylet, guiding ring simple. Pharynx consisting of a slender tube which gradually expanded in basal portion, anterior slender part about 1.1-1.3 times of the basal expanded cylindrical portion length. Nerve ring located at 40- 45% of total neck length from the anterior end. Cardia cylindrical with rounded end. Female genital system monodelphic–opisthodelphic, the posterior branch is well developed, ovary reflexed with a single row oocytes except at the tip, anterior uterine sac less half than the corresponding body diameter long. Vulva a transverse slit, lacking pars refringens, vagina extending inwards about 15-18 μm , occupying about half of corresponding body width, uterus simple, oviduct–uterus junction not clearly separated. Prerectum about 2-3 times and rectum nearly 1-1.5 times anal body width long. Tail long, almost 8-9 times anal body width long, consists of two parts; at proximal slightly convex-conoid and distal elongate filiform which gradually tapering to a minute rounded tip, proximal portion about one-third or some less as long as distal portion. Two pairs of caudal pore are distinct in some materials.

Male: not found.

3.10 Habitat and locality: specimens collected from some samples around the paddy in Khotbeh Sara village in Astara

country, Guilan province, northwestern Iran, with this geographical coordinates: 38°01'51"North, 48°54'39"East, during August 2014. *T. baldum* as new geographical species discovered for Iranian nematode fauna.

3.11 Discussion of *Thornenema baldum*

This species is characterized by having short sized body, structure of lip region (offset, cylindroid-truncated in shape, strongly sclerotized at post labial region and amalgamated lips), the presence of bulges beneath the lip region, with short posterior uterine sac (less than half body width in that region). Baqri and Jairajpuri [9] synonymized *T. baldum* with *T. paradoxum* described by Siddiqi [30], based on morphological and morphometric characteristics, our material comply with the description given by Baqri & Jairajpuri [9] in both the measurements and morphological characters (Table 3). Only one species of the genus, *T. mauritanum* (Williams, 1959 [37]) Baqri & Jairajpuri, 1967 [9] have been reported from Iran by Fadaei-Tehrani & Coomans [17] yet. *T. baldum* closely resembles *T. mauritanum* but differs in having some characters including a rather smaller body (vs. 1.2- 2.1 μm), structure of lip region (vs. rounded and continues or slightly offset from the body contour and post-labial framework not clearly sclerotized), the presence of bulges exactly under the lip region (vs. lacking of this structure), presence of anterior uterine sac (vs. absence of anterior uterine sac) and by other morphometric ratios.

In current study, three rare nematode species referred to above reported as new species of free living nematodes for Iran's nematode fauna so far. It occurs in rice fields in Guilan province of Iran. Morphological and morphometric descriptions of three mentioned species and their comparisons with other reports are described. Overall, no significant variation in morphometric and morphologic data was observed compared with published original descriptions.

Table 3: Morphometric characters for the females of *Thornenema baldum* population in the form: \pm means standard deviation range (all measurements are in μm).

Population	Present study	Carbonell & Coomans [11]	Thorne [34]	Williames [38]	Baqri & Jairajpuri [9]
Characters	Female	Female	Female	Female	Female
n	6	6	1	5	10
L	901 \pm 200(800-1010)	1000 (900-1100)	1000	1000(970-1040)	1010(990-1040)
a	30.1 \pm 2.5(26-33)	33.6(32.5-34.4)	31	30(28-32)	30(29-31)
b	4.5 \pm 0.6(3.8-5.3)	4.9(4.7-5.2)	4.7	5.1(4.7-5.4)	5.1(4.7-5.6)
c	6.7 \pm 0.9 (6.4-7.8)	6.9(6.4-7.2)	6	7(7-8)	9(7-10)
c'	7.5 \pm 0.85(6.1-8.3)	8.1(8-8.5)	-	-	-
V	35 \pm 2.4(32.1-37.5)	33.1(31-36.3)	3.3	32-38)(34	30-37)(34
Odontostyle	11.5 \pm 1.9(10.5-13.2)	12(10-13.5)	-	12-14	11-13
Odontophore	18.1 \pm 1.6(16.5-20)	17.5(16.5-18.5)	-	-	17-19
Guiding ring from ant. end	7.3 \pm 0.6(6.5-8.1)	8	-	-	7-8
Nerve ring from ant. end	92.2 \pm 3.4 (88-96)	94.0(91-96)	-	-	80-90
Ph L	210 \pm 5.5 (200-215)	215(205.5-219.5)	-	-	-
DO	61.8 \pm 2.2(60-64.3)	65.1(64-67.9)	-	-	-
DN	63.5 \pm 2.8(61-65)	65.7(64.6-67.9)	-	-	-
S ₁ N ₁	72.5 \pm 3.4(71-75)	75.2(73.8-76.8)	-	-	-
S ₁ N ₂	77 \pm 5.5(75-78)	77.8(77.0-78.8)	-	-	-
S ₂ N	84 \pm 3.2(83-88)	86.4(85.8-87.7)	-	-	-
S ₂ O	85 \pm 4.1(83-87)	88.4(78.2-87.7)	-	-	-
Anterior uterine sac length	10.3 \pm 1.5 (8.5-11.2)	9-12			
Posterior genital branch length	16.5 \pm 155 (145.2-174)	134.7(109-172)			
Tail	152 \pm 26.4(131-177)	154	-	-	-
RL	24.1 \pm 1.8(22-27)	26.7(25.5-29.5)	-	-	21-26
Prerectum	41.1 \pm 6.5 (36-49)	38(30-44)			35-48

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