Research Article

Survey on Nematodes Biodiversity in *Agaricus bisporus* Producing Composts in East Azarbaijan Province, Iran

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Abstract | Mushrooms are one of the newest resources in the human food basket. *Agaricus bisporus* (J.E. Lange) Imbach, 1946 is the most cultivated fungus for the purpose worldwide, which was grown in small and huge amounts and used cooked or uncooked. Growing beds of the mushroom because of providing good conditions of humidity, air condition, temperature, and food resources are very optimum and suitable places for the growth and living of different plant parasitic organs as well as diverse pests. Nematodes, like other microfauna members, prefer to live in such places. In order to study the nematode biodiversity in mushroom-producing composts, during 2023 several samplings were carried out in commercial and personal mushroom-growing beds. Nematode extraction, killing, fixation, preparing slides, and identification were done using ordinary methods in nematology and using references. In this text as a second part, three species, namely Cephaloboides curviacaudatus, Rhabditiella axei, and Mesorhabditis sp., are reported and described. Genus Cephaloboides is here introduced as a new member of the nematode fauna in Iran.

Received | June 08, 2025; **Accepted** | July 18, 2025; **Published** | September 02, 2025
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Citation | Jabbari, H., 2025. Survey on nematodes biodiversity in *Agaricus bisporus* producing composts in East Azarbaijan province, Iran. *Pakistan Journal of Nematology*, 43(2): 110-121.
DOI | https://dx.doi.org/10.17582/journal.pjn/2025/43.2.110.121
Keywords | *Cephaloboides*, Compost, Fauna, *Mesorhabditis,* New record, *Rhabditella*

Introduction

Fungi species are estimated to be at least 14,000 worldwide; among them 7,000 and 2,000 species are reported as edible and medicinal, respectively (Hawksworth, 1991). Since 1990, production of mushrooms has increased more than 25-fold. Mass production of mushrooms along with other foods has been common in human societies for many years by collecting them from humid environments and using them cooked or uncooked as food (Khabbaz and Moradali, 2000). Netherlands, France and China are the biggest exporters of the mushroom (Mottaghi, 2013). Members of the genera *Agaricus* (button mushrooms, portabellas and criminis), *Pleurotus* (oyster mushrooms), and *Volvariella* (straw mushrooms) are the most famous fleshy fruiting bodies of fungi and include as edible species (Gayakwad *et al*., 2020). Among these, *Agaricus bisporus* (J.E. Lange) Imbach, 1946, is the well-known and produced one, contributing more than 30 percent of all mushroom production, also known as white mushroom, champignon, or cultured mushroom in different regions worldwide. This fungus belongs to

genus *Agaricus*, family Agaricaceae, order Agaricales, suborder Agaricomycetidae, class Agaricomycetes, subphylum Basidiomycotina, and phylum Basidiomycota (Hibbett *et al*., 2007). Mushrooms as food are relatively rich in different minerals and vitamins. They are considered rich sources for phosphorus, iron, calcium, and different vitamins, including A, B, D, E and K. At least 2.4% of the fresh weight of mushrooms contains sugars, and compared to some vegetables such as carrots, it is richer. 0.1 to 0.3 percent of the fresh weight of edible mushrooms is fat (Haghighi *et al*., 2013). Mushroom, like other fruits and vegetables, can be affected by various pests and pathogens during production, storage and sailing. For example, different insects (common mushroom fly, gall fly) and mites (fungus mite, mushroom mite) are reported from fungi at the growing-to-eating period. Various diseases are also reported that are caused by non-living factors such hardening of the blades and living factors like fungal molds, viral disease such as mummy, and bacterial disease such as brown spot disease. Nematode is another living factor that can affect production of mushrooms, which here we grouped as a disease factor. Members of different genera like *Aphelenchoides* and *Ditylenchus* can infect the mushroom culture substrates (Haghighi *et al*., 2013; Khabbaz and Moradali, 2000). The interaction between nematodes and fungi is a good example for a bilateral relationship between two members from two different kingdoms. A group of fungi known as nematophagous since the main food resource of them is fungi. They do this by making adhesive knobs (in *Dactylaria candida*), rings (in *Arthrobotrys brochopaga*), hyphae (in *Peniophorella praetermissum*), and other ways, like being endoparisite (in *Catenaria*) or producing some chemical with a poisoning effect on nematodes (in *Pleurotus*) (Karakas, 2020; Siddiqui and Aziz, 2024). The capacity to digest nematodes' cuticle and penetrate it by producing some enzymes like chitinase, collagenase, and protease is another capacity of fungi for invasion of nematodes (Soares *et al*., 2023). The degree of parasitic effects of fungi on nematodes is such that it makes them as one of the biological control factors on them. There are plenty of reports related to control of *Meloidogyne incognita* and *Heterodera glycines* by *Clonostachys rosea* and *Hirsutella minnesotensis* fungi, respectively (Soares *et al*., 2023). On the other hand, the species belonging to *Tylencholaimellus*, *Pseudhalenchus*, *Nothotylenchus*, *Leptonchus, Hexatylus*, *Ecphyadophora*, *Doryllium*, *Dorylaimellus*, *Ditylenchus*, *Diphtherophora* and *Deladenus* have a mushroom-eating habit, and fungal hyphae are their main source of food (Yeates *et al*., 1993). All kinds of mononchids, different species from the genera *Aphelenchoides*, *Caenorhabditis*, *Rhabditis*, *Acrobeloides*, *Diplogaster*, *Panagrolaimus*, *Cephalobus* and *Ditylenchus myceliophagus*, *D. destructor*, *Deladenus* sp., *Aphelenchus avenae*, have been identified and reported from the culture medium of edible mushrooms that are predatory, saprophytic, or parasitic with their fungal host (Clark, 1964; Singh and Sharma, 2016; Rijal *et al*., 2021). Symptoms of nematode presence in fungi beds appear as a reduction in amount and quality, dwarfing, mycelium yellowing, the production of abnormal knots on the gills, and mushroom destruction (Okigbo and Anuagasi, 2021; Ningombi and Kapoor, 2023). Despite nematodes direct effect on cultural beds, they affect indirectly by acting as vectors for different bacteria and fungi and introducing them to composts as fungi-growing beds (Ahmad *et al*., 2021). Since the nematodes can inter fungal hyphae using the mechanochemical force of stylet, this side of their penetration will act as a very safe channel for introducing other parasitic bacteria and fungi in mushrooms (Ningombi and Kapoor, 2023). The first step of fungi producing beds to contamination with nematodes can be by workers, water, instruments, or composts (Bellettini et al., 2018). Nematode presence is not always harmful because they act as decomposers and supply some useful macro- and micro- elements for fungi growth as well (Carrasco and Preston, 2020). On other hand, nematodes work as biological control elements on other pests like mites and flies (Rinker 2017). Cultivation of edible mushrooms in Iran was started almost 60 years ago (Khabbaz and Moradali, 2000). Although there are some studies in different countries in order to investigate the contamination of mushroom cultivation beds with nematodes (Nagesh and Reddy, 2000; Rinker, 2017; Singh and Sharma, 2016), such studies in Iran have not been done yet, so this is the first attempt. Before it as the first part of the research result, three species, *Diploscapter coronatus, Stomachorhabditis fastidiosa*, and *Panagrolaimus concolor*, were already reported (Jabbari, 2025- accepted paper), and here I am going to report ad describe three others.

Materials and Methods

Sampling

Sampling was carried out during the year 2023 in different personal and commercial fungi production

places. The samples were taken randomly and with complete separation of them from each other so that the possibility of mixing the samples was zero. The sampling was carried out at all stages of fungi production, from starting to last terminal (span to final harvesting) times. In addition, 24 samples were taken; all were kept at 4˚C until extraction as the next step in the research.

Extraction

Since the nature of samples (hyphae and compost) is completely different from soil, for achieving the best results and extracting as much as possible of all stages of nematodes, I use a modified sieve and centrifuge (Jenkins, 1964) and a sieve (Whitehead and Hemming, 1965) as inactive and active methods for nematode extraction, which are common in nematology for all samples.

Killing, fixation, preparation of permanent slide, genera and species identification

All were carried out based on De Grisse (1969). The identification of mounted nematodes, in anhydrous glycerin on glass slides carried out using morphological and morphometrical features, and after comparing with past reports, the final decision was made. Imaging of the species was done by connecting a camera to the Hund light optical microscope. Photoshop and Corel Draw 11 softwars are also used for preparing the image plates and drawings.

Results and Discussions

Cephaloboides curviacaudatus (Schneider, 1866) Zullini, 1982

Syn: = *Odontorhabditis musicola* Timm, 1959

= *Flagicaudoides pawani* Khan, Singh and Baird, 1999

Measurement: Table 1

Figure: Figures 1, 2 and 3

Female

Body cylindrical, medium sized, less than a mm long, more or less slender (a = 17.6–19.5). Upon fixation, the habitus very slightly curved ventrad. Lateral chords look like a band about 25–34% of body width at vulval region; just the outer ones are prominent. Cuticle smooth with not clear annulation, longitudinal line very faint. Lips amalgamated, rounded, with any protruded papillae, continuous with body, 28-34% of body diameter at vulva and 2.0-2.5 times as broad as high. Buccal cavity about two times the body width

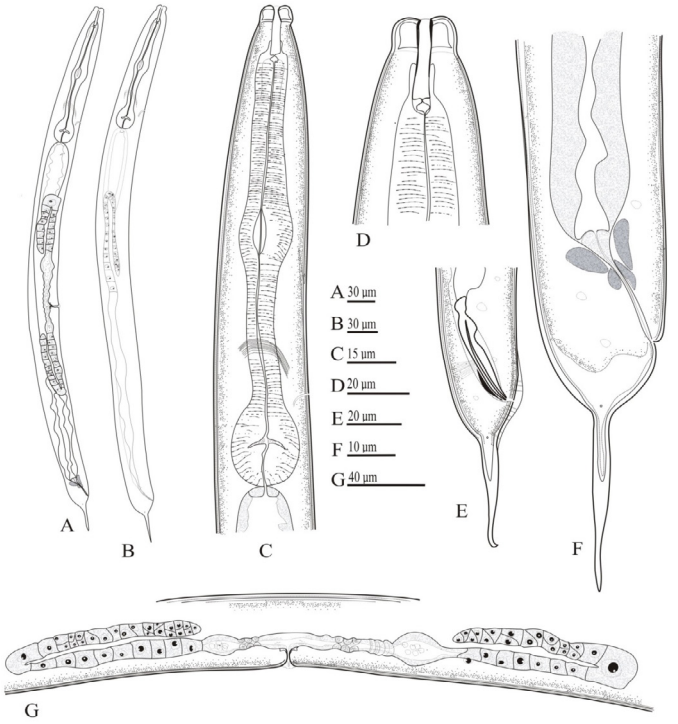

Figure 1: *Cephaloboides curvicaudatus. A: Entire female body, B: Entire male body, C: Pharynx and anterior body region, D: Head and buccal cavity, E: Male posterior region F: Female posterior region, G: Female genital system.*

Figure 2: *Cepaloboides curvicaudatus. A: Female entire body, B: Pharynx and anterior part of intestine, C: Pharynx, E: Head and buccal cavity, E: Pharynx terminal bulb, F: Vulva and parts of genital branches, G and H: Vulva and vagina, I: Anterior genital branch terminal part, J: Posterior genital branch terminal part, K: Lateral field, L: Tail and terminal part. (Scale bars: A: 70, B, C: 30, D:15 E, F: 30, G, H, I, J, K, 15, L: 25 µm).*

Table 1: *Morphometric data of Iranian populations of Cephaloboides curvicaudatus, Rhabditella axei and Mesorhabditis sp. The data are written in ((avearage± sd (Minimum-maximum)) and in micrometer.*

	Cephaloboides currvicaudatus		***Rhabditiella axei***		***Mesorhabditis***
	♀ (n=11)	♂ (n=3)	♀ (n=12)	♂ (n=2)	♀ (n=8)
L	631.87±103.98 (505-750)	572.5±82.54 (483.2-685.2)	1000.33±94.06 (926-1125)	612.5,714.2	568.5±78.26 (495.0-687.5)
a	18.89±1.12 (17.6-20.2)	18.5±2.1 (18.1-19.5)	28.06±2.52 (24.7-30)	24.5, 26.2	24.98±4.41 (19.45-30.55)
b	4.13±0.58 (3.67-5.0)	4. 3±0.4 (3.25-4.9)	6.76±0.71 (6.0-.7.6)	4.2,4.4	4.37±0.50 (3.9-5.2)
c	14.82±2.8 (11.75-17.6)	13.42±1.7 (9.45-14.45)	5.46±0.41 (5.1-6.0)	4.9,5.2	10.87±1.1 (9.6-12.5)
c'	2.45±0.70 (1.7-3.4)	2.7±0.70 (2.2-3.1)	8.45±1.8 (7.0-10.8)	5.2,5.7	3.94±0.8 (3.0-5.0)
%V	51.2±4.54 (46.66-55.74)	-	49.08±1.33 (47.3-50.0)	-	77.72±12.1 (75.75-81.09)
Spicule length	-	42.2±5.4 (40.3-45.2)	-	37.5,40.3	-
Pharynx	153.33±13.45 (137.5-167.5)	150.33±11.45 (130.2-159.5)	150.0±28.95 (125.0-187.5)	145.8,162.3	130.2±5.25 (125.3-137.5)
Body width at Mid body	33.75± 7.5 (28.2-42.5)	32.45± 6.5 (29.2-42.5)	35.83±2.45 (32.5-37.5)	25,27.2	21.5±2.85 (17.5-25.0)
Anus	22.5±2.5 (20.3-25.6)	20.8±1.8 (18.1-22.2)	22.5±3.67 (17.5-25.0)	24.1,24.3	13.5±1.2 (12.5-15.1)
Nerve ring – Anterior end	135.2±11.5 (121.2-143.4)	123.4±11.5 (109.1-133.6)	100.6±10.1 (94.2-119.1)	90.6 , 98.1	80±11.21 (73.58-83.4)
Excretory pore - Anterior end	155±14.2 (148.8-172.3)	129.3±11.4 (118.3-132.1)	98.4±7.2 (94.2-99.7)	92.2 , 97.6	85.62±7.3 (82.1-89.4)
Stoma length	25.3±2.7 (20.1-29.4)	23.6±2.7 (18.1-26.4)	13.1±1.3 (11.5-17.3)	11.1,12.3	16.5±1.2 (15.1-17.5)
Ovory length	141.02.13±5.3 (155.5-162.7)	-	30.52±7.1 (27.3-34.4)	-	134.4±13.7 (128.9-139.7)
G1	285±11.51 (277.6-292.3)	-	201.3±22.3 (197.5-205.2)	-	385±11.51 (377.6-392.3)
G2	245±17.5 (235.6-266 .3)	-	301.6±24.2 (292.5-307.3)	-	-
PUS length	-	-	-	-	21.5±2.85 (17.5-25.0)-
Vagina/ Body width at Mid body	0.28±0.02 (0.26-0.32)	-	0.4±0.03 (0.38-0.44)	-	0.33±0.5 (0.2-0.4)
Rectum length	12.5±0.31 (11.2-13.7)	31.1±1.9 (25.6-34.2)	36.2±4.3 (34.9-38.1)	34.9-38.1	23.5±1.51 (20.4-27.2)
Phasmid	9.33±1.2 (8.7-9.5)	8.1±2.2(7.6-9.2)	-	-	19.4±0.51 (15.7-23.3)
Phasmid/Body width at Anus	0.41±0.3 (0.37-0.46)	0.38±0.2 (0.35-0.43)	-	-	1.43±0.21 (1.25-1.5)
Tail length	54.2±100.0 (43.5-67.5)	56.2.2±100.0 (51.7-60.5)	183.3±6.13 (175.0-187.5)	125.4,138.3	52.5±6.87 (45.2-62.5)

at the anterior region, cylindrical, with relatively thick walls, especially at beginning parts; metastegostom symmetric, isotopic and isomorphic; each outgrowth of that provided with a spur-like denticle or a small wart. Collar region prominent, started about half buccal cavity length; denticle and glottoid hard to see. Pharynx consisting of a slender and wide procarpus, 40-48 µm, muscular and vulvated median bulb (15.8-

16.6×20.4-25.7) µm, cylindrical isthmus 42-51 µm in length, and well-developed terminal bulb with 17-22 × 24.7-27.3 µm diameter. Excretory-secretory pore 20 µm behind nerve ring. Cardia consisting of two rounded cells. Genital system didelphic-amphidelphic, both branches developed equally and well, dorsally reflexed at distal part, never reaching or surpassing oviduct-uterus junction, anterior one 200 µm and posterior 185 µm long, respectively situated at right and left side of intestine, oocytes arranged in one or two rows at different parts of ovary, vagina extending inwards 8 µm or less than one half (20%) of body diameter at the region, vulva equatorial, transverse slit with normal and not protruding from body counter lips, small circular valval flap in entrance of genital system. Length of rectum less than anal body diameter (0.87%). The anus is located at a distance of 231 µm from vulva on average. Tail copula-shaped, with a long spike about two body diameters in length at the anal region. Phasmid prominent, located at starting point of spike.

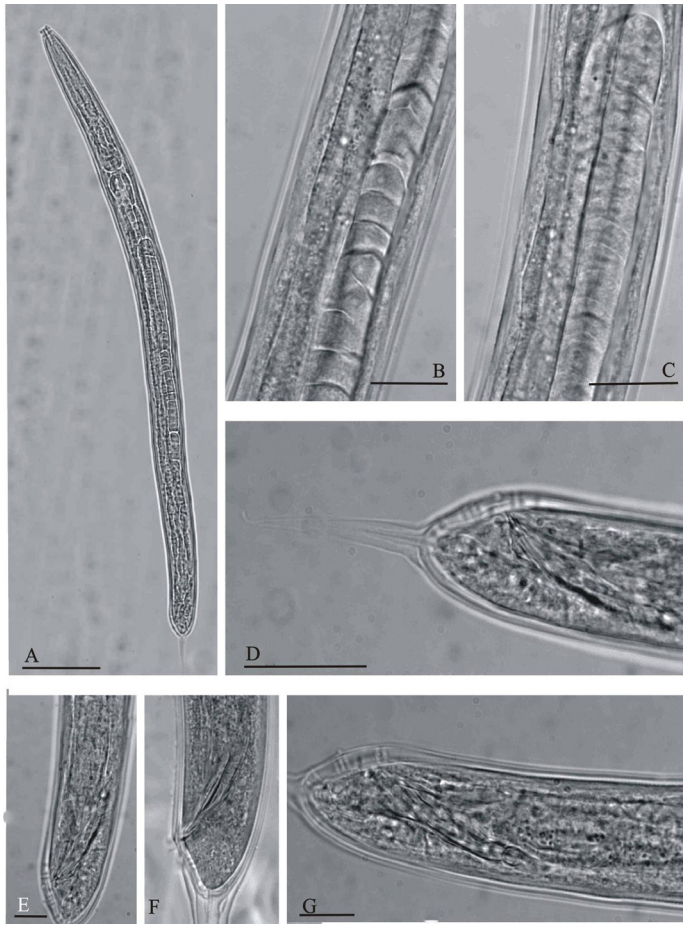

Figure 3: *Cephaloboides curvicaudatus. A; Male entire body, B and C: Parts of testis, E. F and G: Tail, Spicule, Gubernaculum and posterior part of male. (Scale bars. A: 70, B, C: 15, D: 20, E, F, G: 10 µm).*

Male

In general, morphology is similar to females, with one testis, distally and ventrally reflexed, with arrows of spermatocytes located at the right side of the intestine. Spicules long, strongly circularized, cylindrical, curved slightly, head of it more or less spherical, main part of it with a thorn-like structure in most parts of it, at dorsal part simple but with an edge-like structure in 1/3 of its length at the ventral side. Gubernaculum well development, at the distal part wider than the end part. Bursa present, leptoderan. Three pairs preanal, with six pairs postanal genital papillae, grouped as 1+2/1+3+2+p. Phasmid visible, located at starting point of spike at the tail, like females. The tail is similar in both sexes.

Diagnosis and relationship

Identification is going mainly on the basis of Tahseen *et al.* (2017) but Massey (1974), Sudhaus and Fitch (2001), Sudhaus (2011, 2023) and Andrassy (1983) also used. The species is characterized by body length, a cuticle that consists of very fine striation longitudinal lines, the presence of denticles in the stoma, and cylindroid spicules with rounded distal part in males. The Iranian population compared to the main description has acceptable overlapping in all mentioned morphometric data. Compared with Tahseen *et al.* (2017), population body length is less (600-700 µm *vs.* 970-1282 µm), the *a* index is more (17.6-19.5 *vs.* 13.6-16.6), and the gubernaculum is fairly shorter (12.6-13 µm *vs.* 13-22 µm). Most morphologics are similar between these two populations (current and Tahseen *et al.*, 2017) the only difference is that the manubrium in Iranian population is completely rounded and the cephalic papillae are not visible under a microscope. The population differs from *C. dimorphus* by the shape and size of the the papillae (well-arisen *vs.* not so prominant) and from *C. paraciliatus, C. musicola, C. anisospiculus* and *C. parapapillosus* by the shape and size of the spicule. *C. curvicaudatus* also differs from *C. armatus* by gubernaculum length and genital papillae arrangement (12-22 µm, Gp1 very close to Gp2 and Gp3 *vs.* 25 µm, Gp1 very far from Gp2 and Gp3). *C. curvicaudatus* was first reported by Schnider (1966) from wet soil with organic matter; in sewage, sludge, compost, and dung in Berlin, Germany. This is the first report of the nematode from Bostan Abad region, East Azarbaijan province, Iran.

Note: Genus *Cephaloboides* has 7 valid species. Tahseen

et al., (2017) described *Cephaloboides anisospiculus*, explained about genus, and gave a list of species with related information for them. Here I supposed an identification key for species as follow. Since morphometric data in most cases has a very wide range in one species and consequently overlapping between species, the key is arranged based on morphological characters.

Identification key for genus *Cephaloboides* Rahm (1928).

1. Lips with arised and well developed papillae, ventral triangular process of spicule weak……*C. dimorphus*
Lips without such papillae, ventral triangular process of spicule strong, moderate or absent…..2
2. Spicule with horn like structure at dorsal side, ventral triangular process of spicule absent ……*C. paraciliatus*
Spicule normal, ventral triangular process of spicule moderate or strong…….3
3. Spike in female and male looks Filiform……4
Spike not filiform or only filiform in females ……5
4. Spicule 42-81 and gubernaculum 23 micrometer, capitulum hood like…..*C. musicola*
Spicule 26-35 and gubernaculum 10-15 micrometer, capitulum rounded or ellipsoid……..*C. parapapillosus*
5. Spicules similar to each other, length 30 or more, capitulum rectangular……..6
Spicules not similar to each other, 19-29 micrometer, capitulum bilobed or hook like.*C. anisospiculus*
6. Gubernaculum 12-22 micrometer, Gp1 very close to Gp2 and Gp3…….*C. curvicaudatus*
Gubernaculum 25 micrometer, Gp1 very far from Gp2 and Gp3…..*C. armatus*

Rhabditella axei (Cobbold, 1884) Chitwood, 1933
Syn: *Rhabditis axei* Cobbold, 1884
=*Rhabditis jagdishi* (Sultan, Chhabra and Kaul, 1985)
Measurement: Table 1
Figure: Figures 4, 5 and 6

Female

Medium-size nematodes, body length a bit more or less than a millimeter, open C shape after fixation. Cuticle smooth, with very fine striations, one less than a micrometer in width. Stoma opening surrounded with amalgamated lips, which are continuous with rest of the body, with no papilla and protruding on the lips. Stoma long and narrow, tubular; cheilostome short with no glottoides structures or denticle at base; collar about 20% of stoma distal part. The pharynx consists of a tubular procarpus that is about 20% of its length, a well-developed valvate median bulb that is 20×30 µm in diameter, an isthmus that is 20% of pharynx length, and a well-developed basal bulb with a grinder structure that is 35×20 µm in diameter. Nerve ring at 75% of isthmus length, excretory-secretory pore almost at the level of the nerve ring at the ventral side. Lateral lines four, two outer ones more visible and prominent. Cardia looks like two globular structures, with the length as half of the width. Reproductive system paired, anterior one at right and posterior one at left side of intestine. The ovary reflexed once, the anterior one was too long and sometimes passed the vulval region, longer than the posterior one, about 30-32% of the body length, and the posterior one less than 20% of the body length. Oocysts arranged in two or three rows at the distal part in both branches; no sphincter is visible at different parts of them; uterus tubular; vagina straight, about 25% of body width at the region; vulva at mid-body region; lips protruding with a prominent flap present at the region. Anus distinct, rectum straight, more than body width at anus in length, phasmid not visible. Tail fairly long and filiform.

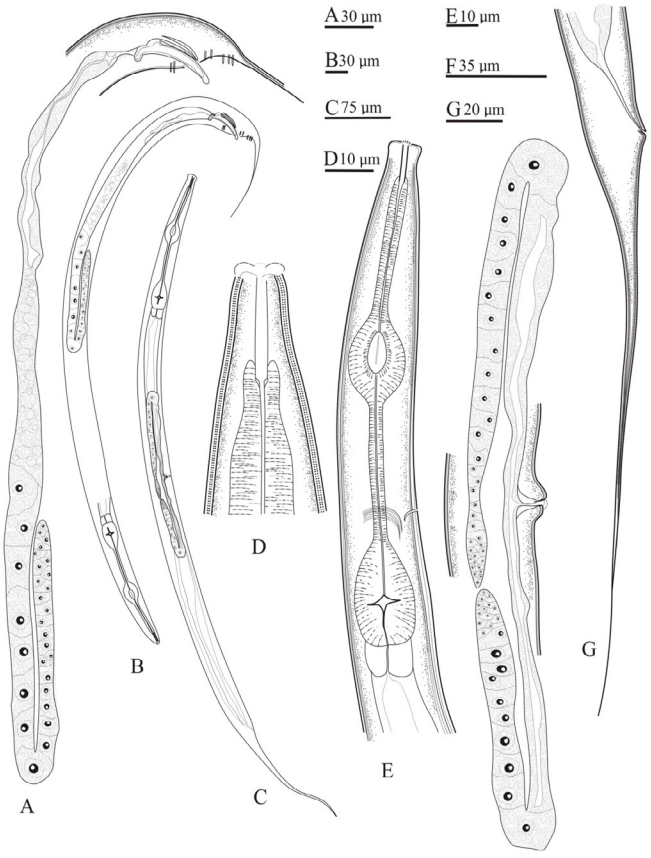

Figure 4: *Rhabditella axei. A: Male reproductive system, B: Male entire body, C: Female entire body, D: Anterior region, E: Pharynx, F: Female genital system, G: Female tail.*

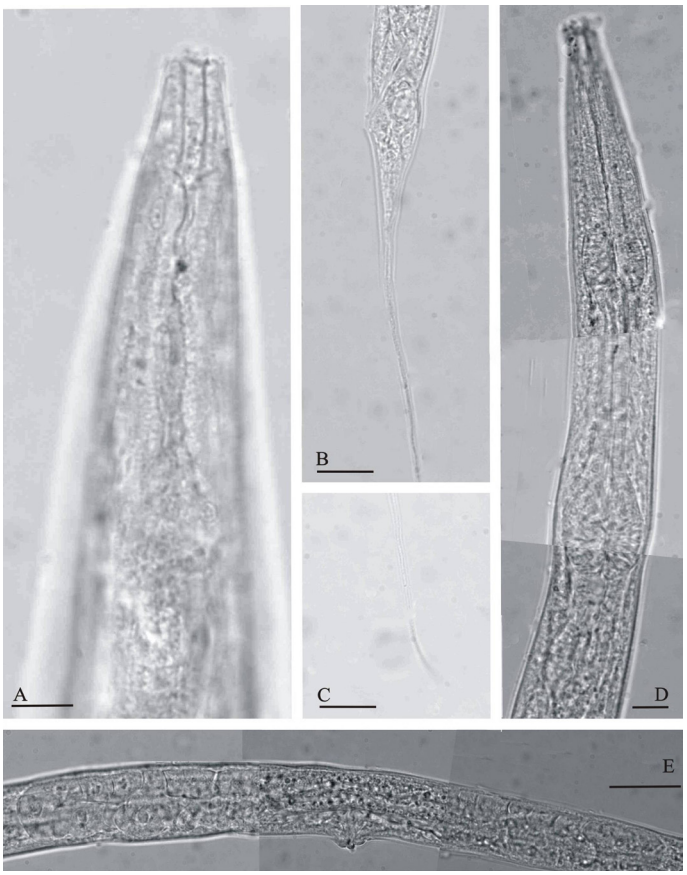

Figure 5: *Rhabditella axei. A: Anterior region, B: Tail, C: Distal part of tail, D: Pharynx. E: Female genital system. (Scale bars: A:, B, C: 20, D: 7, E: 35 µm)*

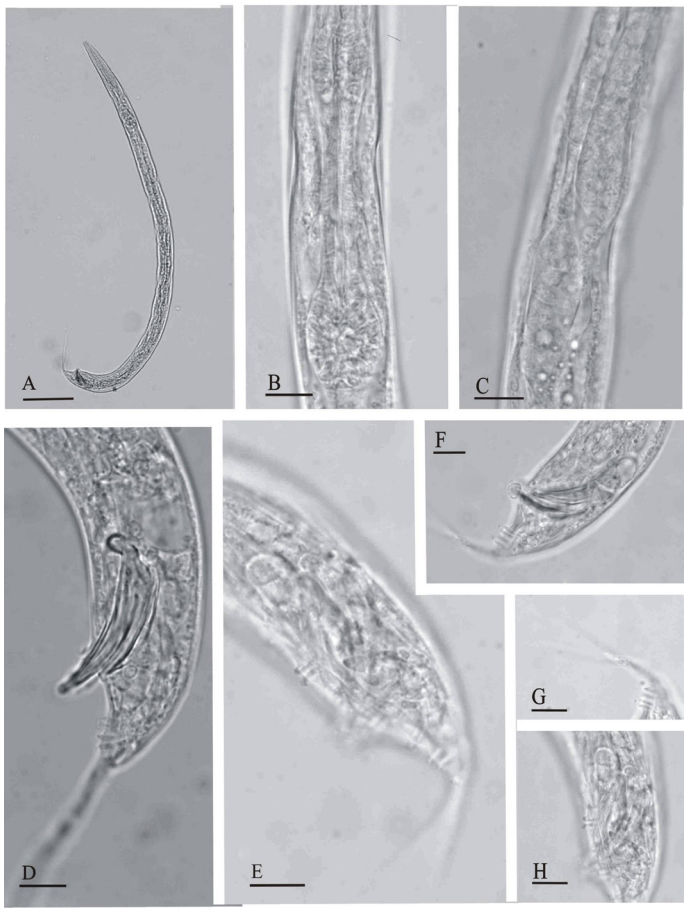

Figure 6: *Rhabditella axei. A: Male entire body, B: Posterior part of pharynx, C: Terminal part of testis, D: Spicule, gunbernaculum, genital papilaae, E: Genital papilaae, F: Posterior region, G: Tail tip and post cloacal genital papille, H: Precloacal genital papillae. (Scale bars: A: 80, B, C, D, E, F: 12 µm).*

Male

Posterior region more curved compared to females. With one testis, reflexed. Spicule and gubernaculum easy to see and prominent; bursa leptoderan; seven genital papillae are present with a (2/2-3) arrangement, two precloacal and five postcloacal; the postcloacals make two groups that are very close to each other. Tail long, about eight times as wide as body at anal region, filiform.

Diagnosis and relationship

Identification of the nematode carried on based on the key supported by Kiontke (1999). *R. axei* compared to the other six members of the genus is unique and easy to identify because of its very long and prominent gubernaculum. In Iran, *R. axei* is already reported by Shokoohi and Abolafia (2011) and Meamar *et al.* (2007) in association with mushroom compost and AIDS patients, respectively. Compared to Kiontke (1999), there are no main differences, but the buccal cavity in the understudy population is shorter (11.5-17.5 µm *vs.* 19.5-26.4 µm), and the tail is also very short (175-187 *vs.* 235-363) compared with Memar *et al.* (2007). The amounts of *a*, *b*, and *c* indexes and the position of the vulva are similar between the understudy population and Sciandra *et al.* (2024) reports. On the other hand, the maximum body width in the Iranian population is less (32-37.5 µm *vs.* 42.5-92.6 µm), the esophagus is short (125-187 µm *vs.* 19.8-242.8 µm), the stoma is short (11.5-17.5 µm *vs.* 23.4-34.4 µm), and the tail is shorter (175-187 µm *vs.* 221.5-434.4 µm) in the Iranian population, too. Among all these differences between populations, the variation in tail length looks very high, but since this character is not included as a differentiated character between species in the given identification key by Kiontke (1999), and looks are not critical in making separation between species, and on other hand, even between female and males of a species, there is a huge variation in tail length (for example 221.5-434.4 µm in female and 177.1-332.8 µm in males (Sciandra *et al.*, 2024) and 235.4-363.0 µm in female and 154.0-228.8 µm in males (Memar *et al.*, 2007), this difference can be ignored. *R. axei* was first described by Cobbold in

1884 in London, England, in a diseased hoof "seedy toe," of a horse and is mostly found in dung and compost, cosmopolitan (Sudhaus, 2011), but it was subsequently re-described twice by Chitwood (1933) and Goodey (1963). In this research, the nematode was reported from Shabestar region.

Note: Despite the nematode, we should notice that the existence of *R. axei* is high and reported from different regions associated with various kinds of rotting substrate, compost and dung (Kinotke, 1999). Although the nematode feeds mainly on present bacteria in decaying organic matter, soil, and other substrates, the nematode can colonize in some species of snails. The nematode known as the free-living, pseudoparasitic, necromenic, and parasitic nematode depends on the host. Despite other hosts, *R. axei* has also been detected in flies, reed mace, birds and mammals (please note the first report and description of nematode, which was mentioned above), including humans, (Sciandra *et al.*, 2024; Hague, 1963; Kinotke, 1999). About the nematode host range, the most important point is that the human is one of them! The First time 1950 *R. axei* was reported from two patients in China with urinary infection (Feng and Li, 1950). In 1967 and after reports of the nematode's presence in another case of urinary infection in a Zimbabwean woman by Goldsmid, the nematode discussed as a medical case. Later there are other reports of nematode from the human gut and urinary system, especially in patients with poor immune system and/or less access to clean water (Shao *et al.*, 2003; Meamar *et al.*, 2007; Shuo *et al.*, 2018). Although researchers note that the relationship between the nematode and human looks to be a case of pseudoparasitism, which means the parasite is usually introduced to host's body with food by any relationship with the host and parasites (Pierre *et al.*, 1995), introducing the nematode by fungi in large amount can be harmful, at least in the long term. Another important subject about *R. axei* is the nematode has interactions with different insect hosts, which may give the nematode the ability to be used as biological control for pests. Hague (1963) reported that the presence of this nematode in a colony of *Stomoxys calcitrans* in the laboratory caused reduction and delay the in the emergence of the fly.

Mesorhabditis sp.
Measurement: Table 1
Figure: Figures 7 and 8

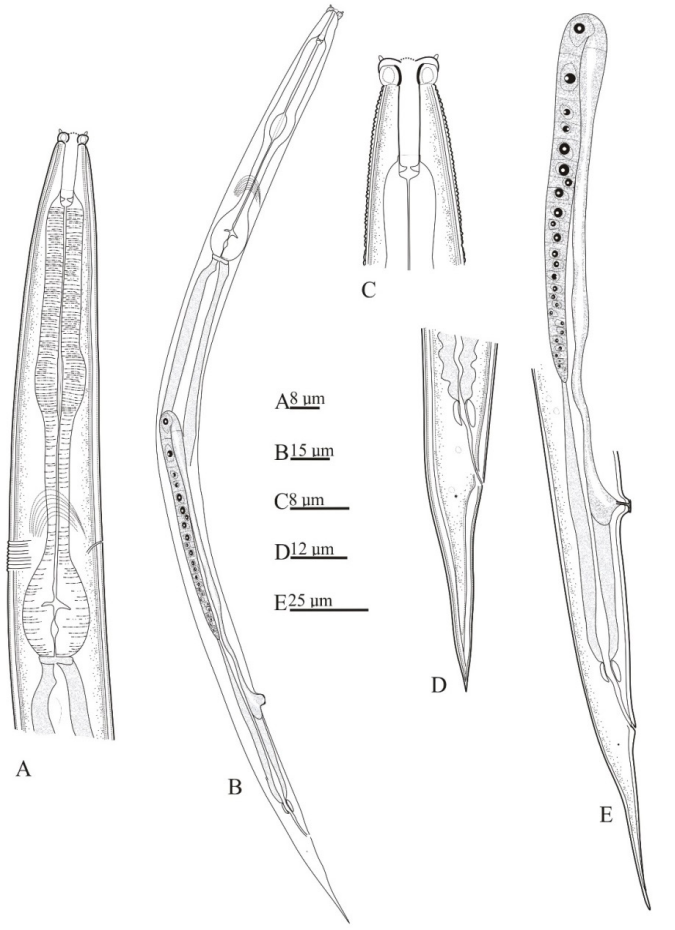

Figure 7: *Mesorhabditis sp. A. Pharynx, B: Female entire body, C: Head, buccal cavity and anterior region, D: Tail, E: Genital system and posterior part of body. A: Pharynx, B: Entire body, C: Head and buccal cavity D: Tail and posterior regions, E: Genital system, vulva-anus, rectum and distal part of intestine and Tail.*

Female
Small nematodes, with a body length less than 1 millimeter, after fixation look like an open C. Cuticle smooth and with very fine striations. Lateral fields as 25% of body width at vulval region, with four prominent lines, two inner ones very faint and two outer ones more visible. Lips six in number, completely separated from each other, offset with the rest of the body, head width twice its height, and under light microscope, a papillae on each lip visible. Stoma tubular, 3.4-4 times as wide, simple, with no glottoide structures or denticle at base, collar present just at distal part of it, cheilostom a short tube with cuticularized walls, gymnostom covering larger part of the stoma, which is followed by stegostom and metastegostom. The Pharynx is about 20% of the entire body length, well developed, consisting of a tubular procarpus as one-fourth of the esophagus and 31 µm in average length, followed by a well-developed and

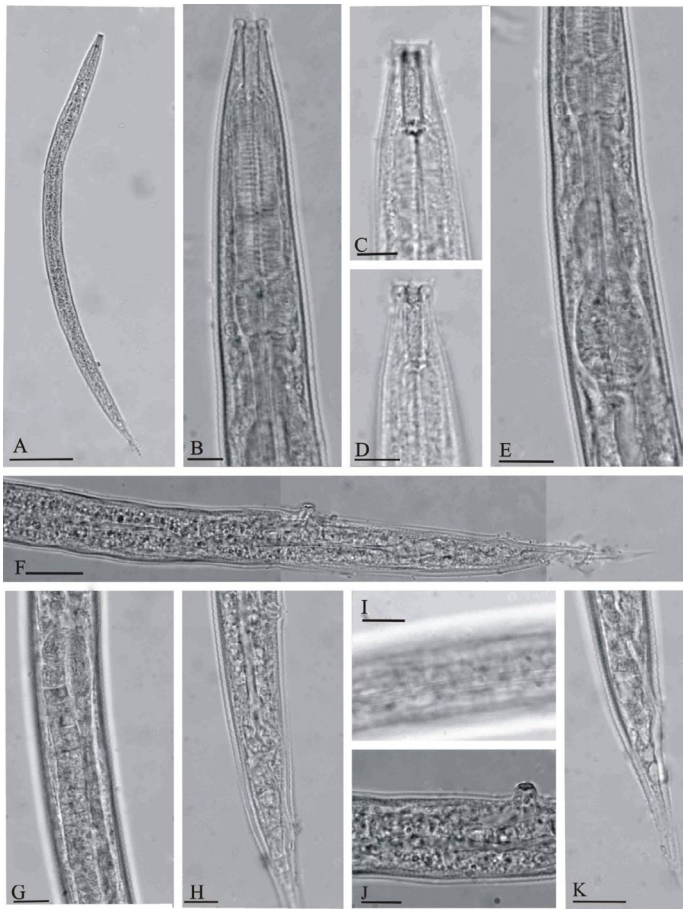

Figure 8: *Mesorhabditis sp. A: Female entire body, B: C and D: Head and buccal cavity, parts of pharynx, E: Posterior part of pharynx, F: Genital system, tail and posterior regions, G: Part of genital branch, H: Vulva-anus, rectum and distal part of intestine, I: Lateral field, J: Vulva and vagina, K: Tail. (Scale bars: A: 75, B, C, D, E:: 10, F: 20, G, H, I, J: 10, K: 15 µm).*

valvate median bulb, with 17×15 µm in diameter; the isthmus length is only some micrometers more than procarpus, 30-35 µm; the basal bulb is pyriform, 28×20 µm; and the cuticulized grinder structure is well developed. The nerve ring is situated at the second half of the isthmus and 60-65% of the pharynx length, and the excretory-secretory pore prominent, almost or very close to nerve ring. Cardia view is simple and as a flat structure, not conical and not lobed. Reproductive system single, prodelphic; ovary reflexed dorsally, oocysts arranged at two at distal part, then at one row; any sphincter visible in entire genital branch; spermatheca also not distinguishable; uterus tubular; vagina straight; 12-14 % of body width at vulval region depth; vulva distinct; at one-third of body length, vulval lips highly protruding from body counter; post-uterine sac present, very short, less than body width at reproductive system opening; in most of nematodes, plug structure with dark color under light microscope noticeable at vulva, which may be due to mating situation. Anus with normal lips, situated at 60 (58-75) µm from the vulva, rectum length more than body width at the anus, with no associated glands. Tail conical, 3-5 times as long as anal body width length, pointed at tip; phasmid visible, situated at about anal body width distance from anus.

Male

Not founded.

Diagnosis and relationship

Mesorhabditis belonged to species (39 species) divided into two main groups namely the *Monhystera*-group and the *Spiculigera*-group, based on morphology of the pharynx, mode of reproduction, distance between vulva and anus, tail shape, presence or absence of males and frequency of that, bursa shape, and spicule length (Sudhaus, 2011, 2023). The Iranian population of *Mesorhabditis* belongs to the *Spiculigera*-group because of tail and tail tip shape (conical with pointed tip). Distance between vulva and anus (less than tail length) is another characteristic of this group's members. Bashir *et al.* (2022), by reporting on a new isolate of *Mesorhabditis monhystera* re-evaluated *Monhystera*-group members using molecular data and scanning electron microscopic observations and provided pictorial key for all members based on female anterior and posterior region (lateral view) and the male tail region (lateral and ventral view). In this key, at least in most of species, the distance between vulva and anus (V-A) is more than the tail length, which is reported as less than the tail length in Sauhaus (2011) despite it being one of the main characters for nematode identification in Sauhaus (2011). It seems that the (V-A) distance should be reviewed and corrected based on literatures and references. By this, the Iranian population is more similar to four species, namely *Mesorhabditis belari*, *Mesorhabditis paucipapillata*, *Mesorhabditis signifera* and *Mesorhabditis sambhanensis*. Although based on Bashir *et al.* (2022) drawings, the population looks more similar to *M. paucipapillatus* out of the above four, it does not fit with any of them completely. The nematodes is distinguished from *M. signifera* by having a fairly strong metacarpus (*vs.* a weak metacarpus), shape (not bulb-shape *vs.* bulb-shape), and diameter of cardia (length less than half width diameter *vs.* length more than half diameter in width). It differs from *M. sambhanensis* by not having a globose median bulb (*vs.* globous and 16×16 µm), excretory-secretory

pore position (far from nerve ring *vs.* immediately after that), and a distinct phasmid (*vs.* not visible). Unfortunately, there is no available description for two other species (*M. belari*, *M. paucipapillata*). Tail shape is the most noticeable difference between *M. belari* and the under-study population from Iran (conoid with pointed tip *vs.* needle-like in ¼ of distal part). The ratio of the distance between the vulva-anus to the tail length in *M. belari* is 1.25, which is 1.13 in *M. paucipapillata* and 1.27 in under-study specimens. With all these differences, the author prefers to report the population as *Mesorhabditis* sp. from Bostan Abad.

Conclusions

By absence of fungus-eating nematodes despite of complete access to hyphae, a comprehensive investigation of the reason should be studied. Not optimum temperature, availability of more water, high percent of humidity, applying some nematicide in different steps of compost preparing, and … can be some of the reasons. *R. axei* as a psudoparisite in human is very dominant in some mushroom-producing compost centers. More study about any possible interaction of the nematode and humans health must be studied in details by related scientists.

Acknowledgment

The author would like to show her deep thanks to workers at mushroom production places, colleagues in relevant departments at the Ministry of Agriculture, Islamic Republic of Iran, East Azarbaijan branch and friends who helped to prepare some unavailable articles and made them useable and available.

Novelty Statement

1. Reporting 3 species belonged to free living and bacterivorous nematodes from mushroom producing beds in Iran, as second part of the research results.
2. Providing an identification key to species belonged to Cephaloboides.
3. Highlighting some potential harms from contaminated mushrooms with Rhabditella axei for human health.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The author declare that there is no conflict of interest regarding the publication of this article.

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