

Short Paper

Occurrence of *Paratylenchus labiosus* Anderson & Kimpinski, 1977 (Nematoda: Tylenchulidae) in Iran, with discussion on the validity of the species

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Abstract: During a survey on the identification of plant-parasitic nematodes associated with walnut in Sanandaj region (Kurdistan province, western Iran), a population of *Paratylenchus labiosus* was collected and identified morphologically. Description, illustrations and morphometrics of the species are given and comparisons are made between it and some related species. *Paratylenchus labiosus* comes more close to three species namely *P. similis*, *P. tateae* and *P. italiensis* in having certain diagnostic characters such as short stylet less than 18 µm long, concave-conoid head with prominent submedian lobes and tail ending to an acute to finely rounded terminus. However, *P. labiosus* differs from them in having a spermatheca full of sperm cells and presence of males. Different modes of reproduction (parthenogenesis or amphimixis) may be observed in different populations of some species in this genus, but somewhat unique reproduction behavior of *P. labiosus* could be considered as a diagnostic character for separation of it from the three closely related species.

Keywords: Description, plant-parasitic nematodes, reproduction, Sanandaj, walnut

Introduction

Based on a population collected from potato fields of Prince Edward Island in Canada, a bisexual species, *Paratylenchus labiosus* Anderson & Kimpinski, 1977, was described having diagnostic characters such as short stylet (15-17 µm), concave-conoid head with prominent submedian lobes, well-developed spermatheca full of sperm and tail ending to a finely rounded terminus. The authors noted that

this species most closely resembles *P. tateae* Wu & Townshend, 1973 based on head shape, prominence of the submedian lobes and general body dimensions, but furthermore, they discussed that these closely related species differ in mode of reproduction and are readily distinguishable by development of the spermatheca, presence or absence of sperm in gonad of females and the presence or absence of males in population. However, Brzeski (1995) synonymized *P. labiosus*, *P. tateae* and *P. italiensis* Raski, 1975 with *P. similis* Khan, Prasad & Mathur, 1967. The designation of the *P. similis* as the senior synonym of *P. labiosus* was accepted by Andrassy (2007). Other nematologists such as Raski (1991), Esser (1992) and Siddiqi (2000) did not follow this action. The

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aim of the present paper is describing of a population of *P. labiosus*, collected from Iran and discussing on the role of males in taxonomy of the genus *Paratylenchus*.

Materials and Methods

During sampling and identification of plant parasitic nematodes associated with walnut trees in Sanandaj region, Kurdistan province, western Iran, some populations of *Paratylenchus* were identified. Nematodes were extracted using the centrifugal-flotation method, killed, fixed and processed to anhydrous glycerin (De Grisse, 1969). Permanent slides were prepared and the specimens were studied using a light microscope equipped with a drawing tube. Finally, the species was identified using available identification keys (Raski, 1991, Esser, 1992, Brzeski, 1998) and the specimens of *P. labiosus* were compared with some populations of *P. similis* which were recently recovered and reported from the rhizospheres of walnut (Bahmani *et al.*, 2013) and turfgrass (two populations) and alfalfa (Ghaderi & Karegar, 2013).

Results

Description of the Iranian population of *Paratylenchus labiosus* Anderson & Kimpinski, 1977.

Measurements: (Table 1).

Female: (Figs. 1–2) Body small, ventrally curved, assuming C-shape after fixing. Body cuticle with distinct annuli, 1.2–1.5 μm in mid-body. Lateral field with four lines, 19–25% of the body width, central band as wide as outer bands. Head concave-conoid, with indistinct annuli and prominent submedian lobes. Cephalic framework weak. Stylet well-developed, with rounded basal knobs, slightly backward posteriorly. Pharyngeal dorsal gland opens at 5.5–6.9 μm behind stylet knobs. Pharynx criconematoid, with well-developed corpus, relatively slender isthmus and oval basal bulb, set off from intestine. Secretory-excretory pore at level with anterior half of isthmus and always anterior to nerve ring. Deirids not observed. Ovary outstretched, spermatheca

oval, containing rounded sperm cells in all individuals. Vulva with distinct and relatively large lateral flaps. Tail conoid, usually ventrally curved, with acute, subacute or finely rounded terminus. Phasmids not observed.

Male: (Figs. 1–2) Body slightly ventrally curved. Head conoid, submedian lobes protuberant, but reduced compared with those of female. Stylet absent. Pharynx and corpus apparatus valve not observed. Tail conoid, with finely rounded terminus. Spicules gently curved ventrally, 20.6 μm long, gubernaculum simple, 4.5 μm long. Penial tube well-developed, bursa absent.

Habitat and locality: This species was originally described from a potato field in Prince Edward Island, Canada (Anderson & Kimpinski, 1977). In present study, it was collected from a walnut orchard in Neshur village, at distance of 35 km in Sanandaj-Kamarian road, Kurdistan province, western Iran. It is reported for the first time from Iran.

Discussion

Plant-parasitic nematodes of genus *Paratylenchus* are associated with different grasses and woody plants (Čermák & Renčo, 2010). The occurrence and importance of the plant-parasitic nematodes, including *P. labiosus*, associated with walnut in Sanandaj region has recently been discussed (Bahmani *et al.*, 2013). During present study, a population of *P. labiosus* was recovered in low numbers. Unfortunately, further samplings with the aim to collect live material for performing biological or molecular phylogenetic studies were not successful.

In having a short stylet (< 18 μm), four lateral lines and presence of vulval flaps, the recovered population comes more close to the species given in Table 2. However, the morphological characters and the ranges of morphometric data fit well with the corresponding data for *P. labiosus* (Anderson & Kimpinski, 1977), except smaller range for “b” ratio (3.1–3.9 vs. 4.0–4.6) and position of secretory-excretory pore (at level with the anterior half of isthmus vs usually near junction of isthmus and basal bulb, or sometimes more posterior).

Table 1 Morphometrics of *Paratylenchus labiosus* from Iran and its comparison with original description (data are in format average \pm S. D. (range); measurements are in μm).

Character\Source	Present study		Anderson & Kimpinski, 1977	
	Female	Male	Female	Male
n	8	1	14	4
L	333 \pm 18.3 (309-363)	362	379 (329-400)	364 (327-402)
a	22.8 \pm 2.1 (19.9-25.0)	29.2	26 (23-29)	30 (25-34)
b	3.5 \pm 0.2 (3.1-3.9)	-	4.3 (4.0-4.6)	-
c	12.2 \pm 1.5 (9.9-14.9)	11.2	11 (9-12)	12 (11-13)
c'	3.0 \pm 0.3 (2.7-3.4)	3.5	3.5 (3.1-4.2)	-
V	81.0 \pm 1.2 (79.0-82.6)	-	81 (79-84)	-
Stylet	16.7 \pm 0.9 (15.5-17.5)	-	16 (15-17)	-
Conus	9.9 \pm 0.6 (8.9-10.7)	-	10 (9-11)	-
m%	59.6 \pm 1.5 (57.4-61.1)	-	61 (58-67)	-
Pharynx	94.6 \pm 7.0 (80-102)	-	88 (78-93)	-
Median bulb	53.5 \pm 2.9 (48.5-57.0)	-	-	-
MB	56.6 \pm 1.9 (54.5-60.6)	-	-	-
S. E. pore	66.5 \pm 3.1 (61.0-70.3)	77.2	74 (67-80)	-
Nerve ring	71.6 \pm 3.1 (66.7-77.1)	79	-	63 (59-67)
Head-vulva	270 \pm 16.6 (249-296)	-	-	-
Head-anus	306 \pm 18.7 (283-339)	330	-	-
Vulva-anus	35.5 \pm 3.3 (31.4-42.7)	-	-	-
Tail length	27.6 \pm 3.1 (24.3-33.6)	32.4	32 (27-38)	-
Body width	14.7 \pm 0.9 (13.7-16.1)	12.4	15 (13-17)	12 (12-13)
Vulval body width	12.4 \pm 0.8 (11.5-13.5)	-	-	-
Anal body width	9.1 \pm 0.4 (8.7-10.0)	9.3	-	-
St/L%	5.0 \pm 0.4 (4.3-5.6)	-	-	-
S. E. pore/L%	20.0 \pm 0.7 (19.1-21.0)	21.3	-	-
Spicule	-	20.6	-	21-23
Gubernaculum	-	4.5	-	3.8-4.4

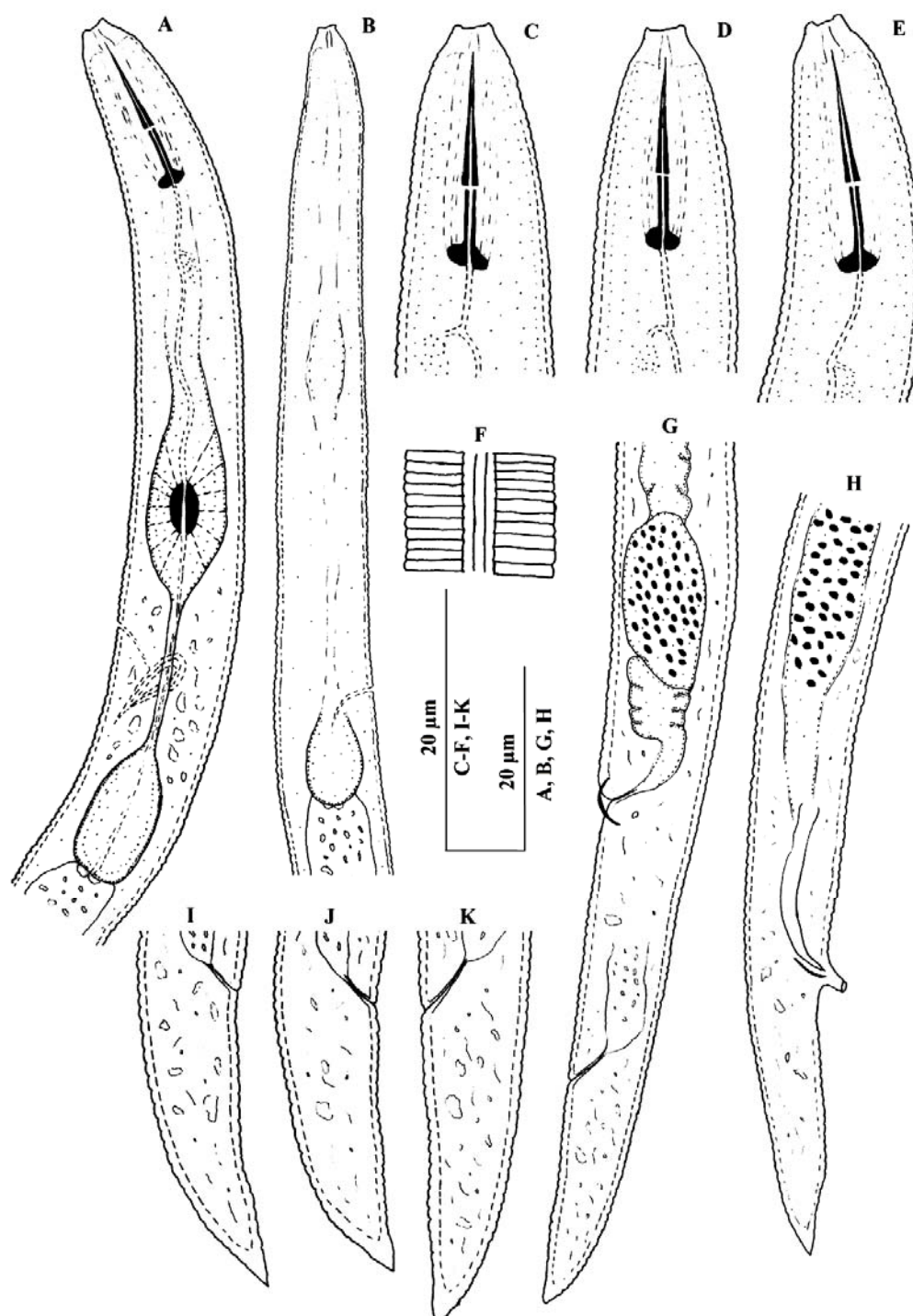


Figure 1 *Paratylenchus labiosus* from Iran. A: Female anterior end; B: Male anterior end; C-E: Female head and stylet; F: Lateral field; G: Female posterior end and part of reproductive system; H: Male posterior end; I-K: Female tail terminus variation.

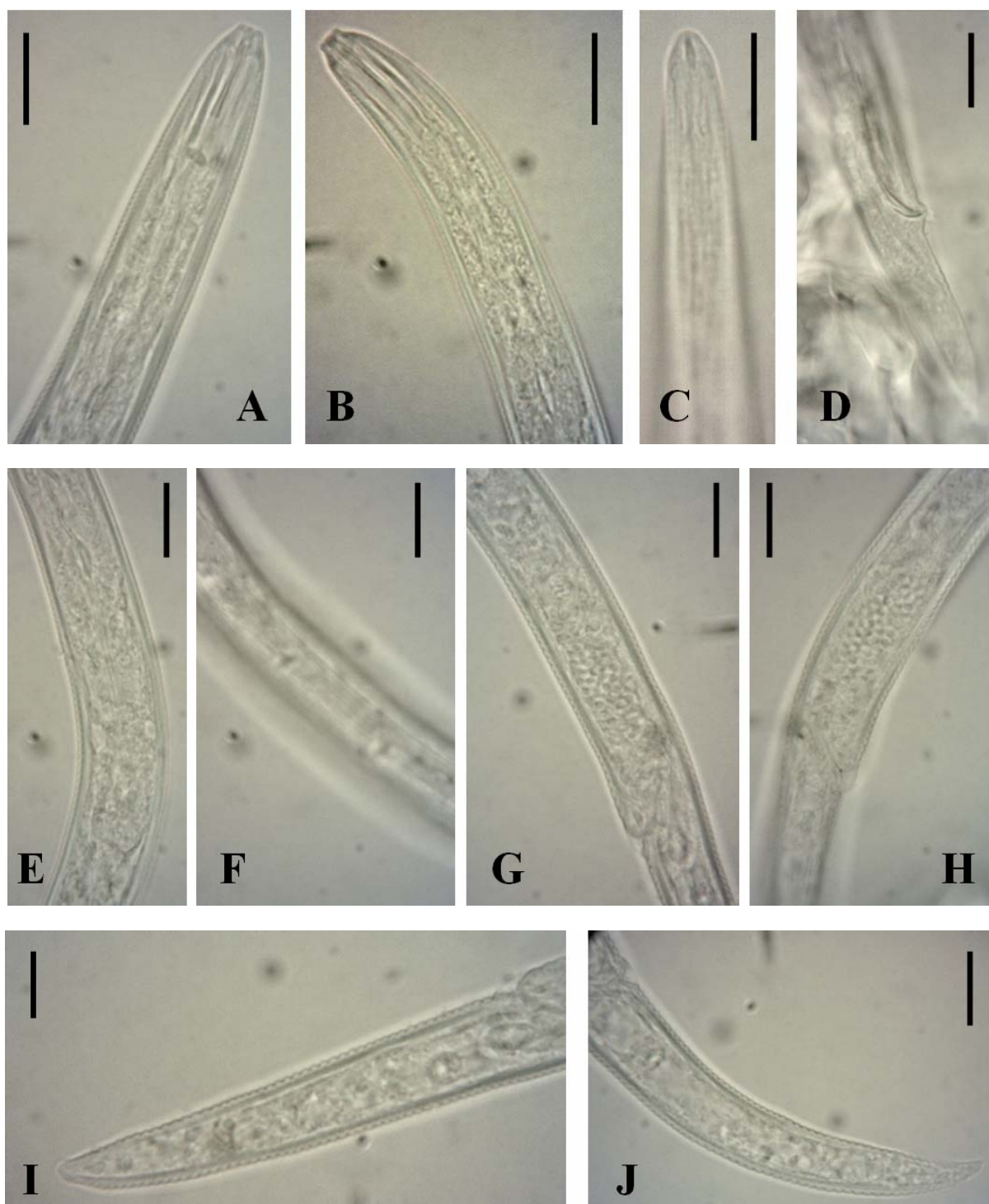


Figure 2 *Paratylenchus labiosus* from Iran. A & B: Female anterior end; C: Male anterior end; D: Male posterior end; E: Female pharyngeal region; F: Female lateral field; G & H: Part of female reproductive system; I & J: Female posterior end (All scale bars 10 μm).

Table 2 Comparison of *Paratylenchus labiosus* with the most closely related species (measurements are in μm).

Species\characters †	A	B	C	D	Stylet	L	V
<i>P. vexans</i>	1	1	3	3	15-18	290-380	84-87
<i>P. amundseni</i>	1	2	1	2	17-19	320-370	76-80
<i>P. goldeni</i>	1	2	3	2,3	14-19	350-430	78-81
<i>P. microdorus</i>	1	2	3	1,2,3	13-19	250-450	79-86
<i>P. minutus</i>	1	2	3	2,4	16-21	200-290	79-84
<i>P. recisus</i>	1	2	3	2	15-17	270-390	78-83
<i>P. variabilis</i>	1	2	3	3	13-16	250-350	82-87
<i>P. veruculatus</i>	1	3	2	1,2,4	11-15	230-350	80-86
<i>P. breviculus</i>	2	1	3	3	15-17	190-230	84-86
<i>P. colbrani</i>	2	2	3	3	15-18	240-350	76-83
<i>P. serricaudatus</i>	2	2	3	2,5	15-20	180-260	80-86
<i>P. pernoxius</i>	3	3	3	2,3	13-16	280-380	80-86
<i>P. similis</i>	3	1	3	2,3	11-19	220-410	78-88
<i>P. labiosus</i> ‡	3	2	2	2	15-18	310-400	79-84

† A: head from lateral view; A1: without submedian lobes; A2: with small submedian lobes; A3: with prominent submedian lobes. B: males and their stylet; B1: males unknown; B2: without stylet; B3: with degenerated stylet. C: J4 stylet; C1: absent; C2: present; C3: data not available. D: female tail terminus shape; D1: acute or pointed; D2: subacute to finely rounded; D3: bluntly rounded; D4: digitate or indented; D5: serrated.

‡ data from present study and those of Anderson & Kimpinski, 1977.

P. similis sensu Brzeski (1995) includes four species namely *P. similis*, *P. tateae*, *P. italiensis* and *P. labiosus*. There are no distinct differences between *P. tateae* and *P. italiensis*, and these two species have the overlapping range of the stylet length (15-17 vs. 12-17 μm). The range of stylet length in original description of *P. similis* (Khan *et al.*, 1967) is 11-12 μm , but Raski, 1975 while studying two female paratypes found they have a 16 μm long stylet. However, there is not a general consensus on synonymy of *P. labiosus* with *P.*

similis. This synonymy was proposed by Brzeski (1995), an action that was not followed with Raski (1991), Esser (1992) and Siddiqi (2000). Andr ssy (2007) has accepted this synonymization.

On the importance of males in taxonomy of the genus *Paratylenchus*, Tarjan (1960) noted that occurrence of males cannot be used for distinguishing of two species. Brzeski & Szczygiel (1963) mentioned that this character is only usable for distinguishing of physiological races. Both Tarjan (1960) and Brzeski &

Szczygiel (1963), concluded that populations of a certain species lacking males would normally not be expected to exhibit clearly defined spermathecas, due to the absence of spermatozoa. They also pointed out that some populations of the same species existing in different environmental conditions, might possibly favour development of functional males, and reasonably yielding in females with occupied spermathecas. Brzeski (1995) mentioned that males may occur in certain populations of some species, and they may or may not be associated with sperm in spermatheca. He concluded that presence or absence of males and sperm in spermatheca is not considered as a specific character until it is experimentally shown that the flow of genes between mono- and bi-sexual populations is not possible.

In spite of the above mentioned discussions, we found that the importance of presence of males in populations of *P. labiosus* and their absence in populations of *P. tateae*, *P. italiensis* and *P. similis* somewhat differs from other cases observed in genus *Paratylenchus* (e.g. in *P. microdorus*, *P. nanus*, *P. veruculatus*, *P. arcuatus* and *P. obtusicaudatus*). All females of *P. labiosus* in Canadian and Iranian populations have distinct spermatheca and often contained sperm cells, but in most of reported populations of *P. tateae*, *P. italiensis* and *P. similis*, spermatheca could not be observed or was reduced, the case was also observed in four populations from Iran (Bahmani et al., 2013; Ghaderi & Karegar, 2013) and very rarely contained sperm (Brzeski, 1995). Furthermore, no male has been found for the above mentioned three species. On the other hand, bisexual populations with low to high proportion of males are reported for some other species, that were originally described as monosexual species. This case is observed for some species namely *P. microdorus* (Brzeski, 1995), *P. nanus* (Brzeski & Szczygiel, 1963; Brzeski, 1995), *P. arcuatus* (Brzeski et al., 1999; Gharakhani et al., 2007) and *P. obtusicaudatus* (a population from Iran, unpubl.). On the other hand, some species (like *P. veruculatus* Wu, 1962) are originally

described as bisexual species, but their monosexual populations are also known (Brzeski, 1995; Ghaderi & Karegar, 2013). However, such species have spermatheca that contain sperm in almost all females in monosexual populations. Other minor differences of *P. labiosus* with *P. similis* including more posterior position of the dorsal pharyngeal gland orifice from stylet knobs, larger and more prominent vulval flaps and longer tail that originally have been noted (Anderson & Kimpinski, 1977), were also seen in Iranian population. From the above explanations, it may be concluded that *P. labiosus* may be considered still as a valid species; nevertheless, some more ecological and biological studies are required for making better decisions on the validity of this species.

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گزارش *Paratylenchus labiosus* Anderson & Kimpinski, 1977 (Nematoda: Tylenchulidae)**از ایران و شواهدی مبنی بر اعتبار آن**جبرئیل بهمنی^{۱*}، شاپور باروتی^۲ و رضا قادری^۳

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چکیده: طی شناسایی نماتدهای انگل گیاهی مرتبط با درختان و نهالستان‌های گردو در شهرستان سنندج (استان کردستان، غرب ایران)، جمعیتی از گونه *Paratylenchus labiosus* جمع‌آوری و تشخیص داده شد. شرح کامل، ترسیم‌ها، داده‌های ریخت‌سنجی و مقایسه آن با گونه‌های مشابه ارائه شده است. این گونه از نظر داشتن صفاتی همچون استایلیت کوتاه زیر ۱۸ میکرومتر، سر مخروطی مقعر با برآمدگی‌های چهارگانه مشخص و دم با انتهای تیز (acute) تا گرد باریک (finely rounded) به گونه‌های *P. similis*، *P. tateae* و *P. italiensis* بسیار نزدیک است ولی با داشتن کیسه ذخیره پر از اسپرم و وجود نماتدهای نر از آنها متفاوت است. هر دو نوع تولید مثل جنسی و بکرزایی در تعدادی دیگر از گونه‌های این جنس مشهود است، ولی حالت منحصر به فرد این ویژگی در *P. labiosus* شاید دلیلی بر اعتبار این گونه باشد.

واژگان کلیدی: شرح کامل، نماتدهای انگل گیاهی، تولید مثل، سنندج، گردو