

Description of *Ektaphelenchoides persicus* sp. n. (Nematoda: Ektaphelenchinae) from western Iran

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Abstract: *Ektaphelenchoides persicus* sp. n., recovered from a soil sample around the rhizosphere of grapevine in western Iran, is described and illustrated based on morphological and morphometric characters. The new species has a body length of 577-700 μm (in females) and 431-610 μm (in males). The cuticle is weakly annulated, with three lateral lines. Lip region offset. The stylet is 15-17 μm long without basal swellings. The excretory pore is located *circa* at one metacarpus diameter posterior to the metacarpus valve, and hemizonid is at 87-96 μm from the anterior end. The post-uterine sac is short, 6-10 μm long. Male tail bearing seven (1+2+2+2) caudal papillae, with a very long filiform terminus. Based on morphological and morphometric characters, the new species is close to *E. kelardashtensis*, *E. attenuata* and *E. musae*. It differs from the closest species *E. kelardashtensis* by its longer stylet (15-17 vs 13-16 μm), longer post uterine sac (6-10 vs 3-6 μm), more anterior location of excretory pore (just posterior to the base of metacarpus vs nerve ring level), longer spicules (14-15 vs 8-10 μm) and presence of P1 and P4 caudal papillae vs absence. Comparisons with other similar species of *Ektaphelenchoides* are also provided.

Key words: Ektaphelenchinae, grapevine, morphology, morphometric, new species, taxonomy

Introduction

The genus *Ektaphelenchoides* Baujard, 1984 was placed in the subfamily Ektaphelenchinae Paramonov, 1964 belonging to the family Aphelenchoididae Skarbilovich, 1947 (BAUJARD 1984). *Ektaphelenchoides* spp. have been isolated from different materials such as soil, bark of trees, galleries of bark beetles, insects and plant parts. The taxonomic study of Ektaphelenchinae in Iran has received great attention during the recent years. Most surveys were conducted on bark and wood samples and resulted in descriptions of six new species of the genus (ATIGHI et al. 2012, 2013a, 2013b, PEDRAM et al. 2012, ALIRAMAJI et al. 2014, 2015) (see ESMAEILI et al. 2014). Two species, *E. ruehmi* Yaghoubi, Pourjam, Atighi & Pedram, 2014 and *E. fuchsi* Esmaeili, Heydari, Pourjam & Atighi, 2014 were isolated from the rhizosphere of herbaceous plants (YAGHOUBI et al. 2014, ESMAEILI et al. 2014). In ad-

dition to these species, one previously described species of this genus, *E. compsi* Baujard, 1984, has been reported from Iran (RAFIEE et al. 2011). In order to extend the study of this genus in Iran, we conducted several samplings in western Iran during the summer of 2015. As a result, a species of *Ektaphelenchoides* from Gilan-e Gharb City, western Iran, was collected from the rhizosphere of grapevine. Detailed studies using light microscopy and morphological analyses indicated that this population belongs to a new species, which is described here as *E. persicus* sp. n.

Materials and Methods

Soil and root samples were collected from different fields in western Iran during August 2015. The tray method (WHITEHEAD & HEMMING 1965) was used to extract nematodes. The nematodes were handpicked

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under a stereomicroscope model Olympus SZH and heat-killed by adding boiling 4% formalin solution, then transferred to anhydrous glycerine and mounted in permanent slides according to DE GRISSE (1969). Permanent slides were prepared and studied using a stereomicroscope (Nikon E200). Photographs of live nematodes were taken by a digital camera attached to the same microscope. Drawings were made with the aid of a drawing tube.

Cross sections were made after examining the fixed individuals and selecting a suitable specimen. The chosen nematode was put on a drop of pure glycerin on an inverted cover of a 6 cm Petri dish and sliced perpendicular to the longitudinal axis using a common razor. Each cross section was transferred to a small drop of melted glycerin-gel (DE GRISSE 1969) and covered with a cover slip before the drop congealed. The cover slip was then positioned with point pressure to reveal the full cross section and sealed with nail polish.

Results

Ektaphelenchoides persicus sp. n. (Figs 1-3)

Material examined: Twelve females and three males, in excellent state of preservation.

Measurements: see Table 1.

Description: Female. Body cylindrical, slightly ventrally curved when heat-killed. Cuticle less than 1 μm thick, finely annulated. Lateral field with three incisures. Lip region set off by a slight depression, *ca* 3.0-4.0 μm high and 7.0-8.0 μm wide. Stylet 15-17 μm long, without basal swelling, conus delicate and occupying *ca* two-fifth of its total length. Procorpus tubular, ending in a rectangular median bulb (metacarpus) with central valve plates. Nerve ring situated *ca.* one metacarpus diam. posterior to it. Pharyngeal glands well-developed, overlapping intestine dorsally, 2.5-4.0 times as long as body diameter at level of median bulb. Excretory pore located *ca* one metacarpus diameter posterior to valve. Hemizonid situated *ca* about 1.0-1.5 metacarpus diam. posterior to excretory pore. Ovary outstretched anteriorly, located at dorsal side of intestine. Developing oocytes mostly in two rows. Oviduct small, spermatheca oval to irregular, filled with rounded sperm. Crustaformeria obscure. Uterus thick-walled, vagina slightly sclerotised, straight. Vulva located posterior at *ca* 74% of the body length. Vulval lips distinct, vulval flap absent. Post-uterine sac short, *ca* 0.6-1.0 body diameter at vulva region long. Intestine apparently ending in a blind sac at *ca* one third to one half of distance from vulva to posterior end. Rectum or functional

anus absent. Posterior body end (tail) conical, ending to a pointed to filiform terminus.

Male. Body slender, arcuate to J-shaped after heat relaxation, posterior end more strongly ventrally curved. Anterior region similar to that of female. Testis single, expanded anteriorly without reflexion. Spicules separate, 14.0-15.0 μm long measured along the arc, *ca* 3.2 times of capitulum width, lamina/calomus smoothly and symmetrically curved, rostrum short, moderately developed with pointed tip. Condylus distinct with round tip. Gubernaculum absent. Tail conoid, with very long filiform terminus. Seven (comprising a single ventral and three subventral pairs) genital papillae present: single midventral precloacal papilla (P1), located *ca* 1.3 times cloacal body diam. anterior to cloacal aperture; first paired papillae (P2) subventral located at level of or just posterior to cloacal aperture; second post-cloacal paired papillae (P3) subventral located at *ca* 43% of tail length posterior to cloacal aperture; and third paired small papillae (P4) subventral located at *ca* 60% of tail length posterior to cloacal aperture.

Type locality and habitat: Cheshmeh-e Nezamei of Gilan-e Gharb City, Kermanshah Province, western Iran (GPS coordinates: N 33°59', E 46°12'; altitude 1248 m a. s. l.); soil around the roots of grapevine.

Type material: Holotype female (Slide AEP001), four paratype females and two paratype males (Slides AEP002 and AEP003) deposited in the Nematode Collection of the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Iran. Three paratype females deposited at the nematode collection of the Royal Belgian Institute of Natural Sciences, Brussels. Three paratype females and one paratype male deposited at the National Nematode Collection of the Department of Nematology, Iranian Research Institute of Plant Protection, Tehran, Iran.

Diagnosis: *Ektaphelenchoides persicus* sp. n. is characterised by: body length of 577-700 μm and 431-610 μm for the females and males, respectively; lip region set off from the body by a distinct constriction; cuticle weakly annulated, with three lines in the lateral fields; medium sized stylet (15-17 μm) without basal swellings; excretory pore located *ca* one metacarpus diameter posterior to metacarpus valve; post-uterine sac short, less than half body diameter at vulva region (6-10 μm); posterior body end (tail) filiform in females; spicules with sharply pointed rostrum and rounded condylus; conoid tail with a filiform part hooked at the end in males. The male genital papillae comprise a single precloacal papilla, one adcloacal and two post-cloacal pairs.

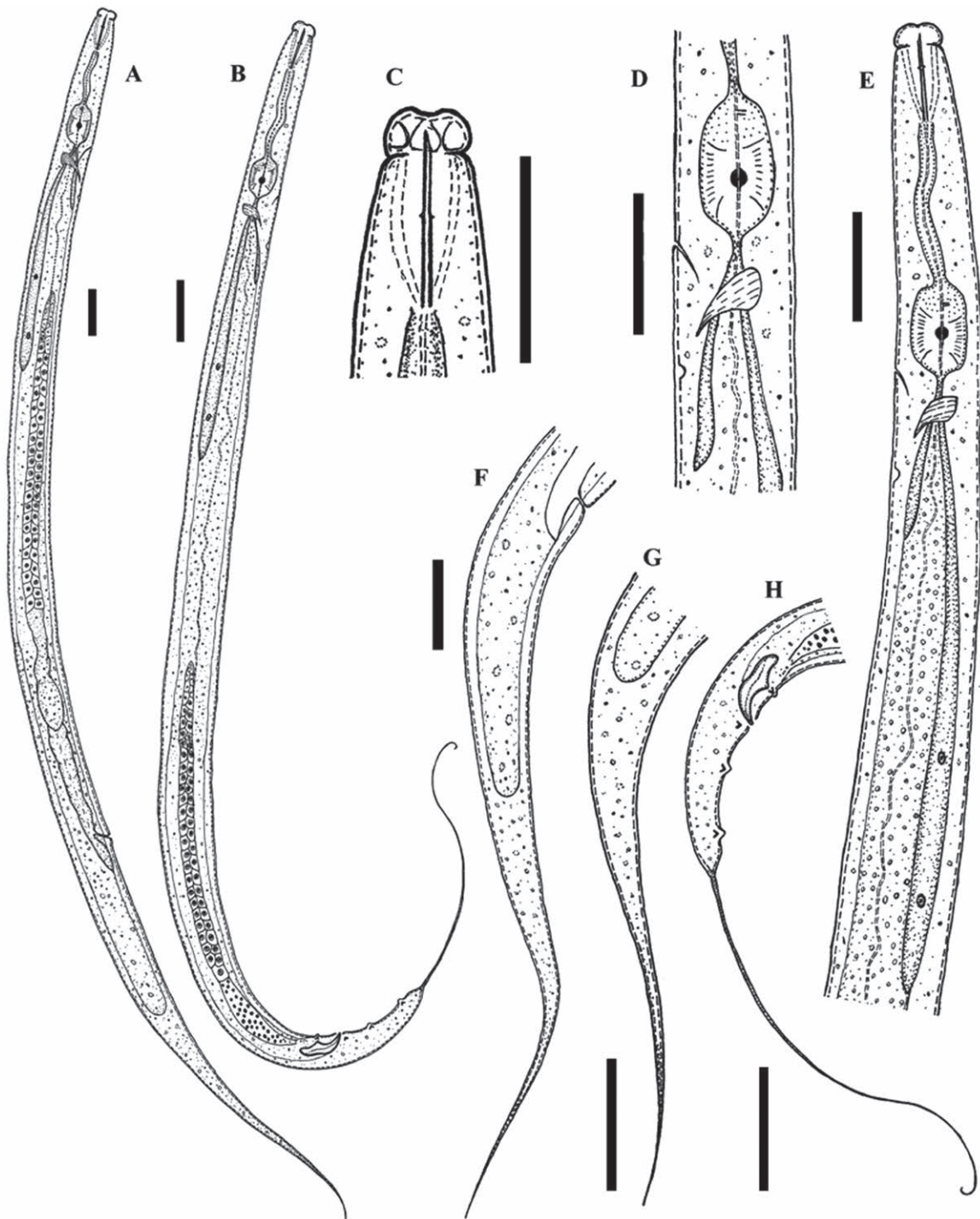


Fig. 1. *Ektaphelenchoides persicus* sp. n. A. Female, entire body. B. Male, entire body. C. Female, anterior region. D. Female, metacarpus region. E. Female, anterior part of body. F. Female, vulval region and lateral field. G. Female, posterior part of body. H. Male, posterior part of body. Scale bars 20 μ m.

Relationships: The general characteristics of this species fit well with that of the genus *Ektaphelenchoides*. The new species is close to *E. attenuata* (Massey, 1974) Baujard, 1984, *E. musae* Baujard, 1984 and *E. kelardashtensis* Atighi, Pourjam, Pedram, Ye, Robbins & Namjou, 2013 in morphology and morphometrics. It differs from the closest species, *E. kelardashtensis*, by its longer sty-

let (15-17 vs 13-16 μ m), longer post-uterine sac (6-10 vs 3-6 μ m), more anterior position of excretory pore (located just posterior to base of metacarpus vs nerve ring level), longer spicules (14-15 vs 8-10 μ m) and the presence of P1 and P4 caudal papillae vs absence. The new species differs from *E. attenuata* by its smaller body length (577-700 vs 959 μ m), somewhat lower a ratio (33-42 vs 42), shorter post-uterine

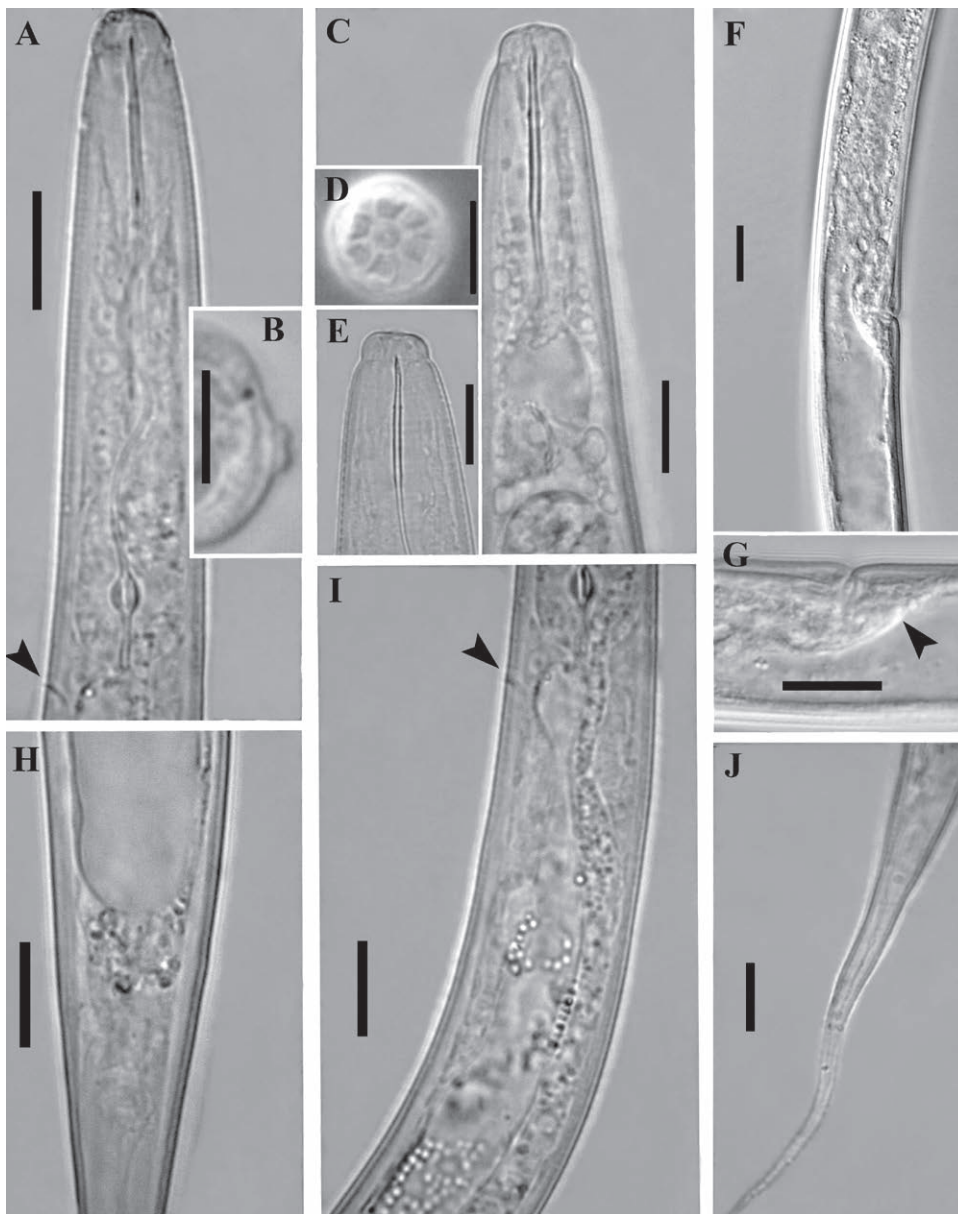


Fig. 2. *Ektaphelenchoides persicus* sp. n., female. A. Anterior region of pharynx showing excretory pore (arrowhead). B. Cross section showing lateral field. C. Anterior end. D. Lip region, en-face view. E. Stylet region. F. Vulval region. G. Vulval region and post-vulval uterine sac (arrowhead). H. End of intestine region. I. Pharyngeal gland region and excretory pore (arrowhead). J. Posterior end. Scale bars 10 μ m.

sac (6-10 vs 23-28 μ m), slightly vs clearly offset lip region, shape of the tail (conical with slight transition to filiform shape vs conical with sharp transition to filiform shape) and the presence of seven male caudal papilla vs four. The new species can be distinguished from *E. musae*, by its shorter stylet (15-17 vs 18.5-22.0 μ m), oocytes in ovary arranged in single row vs 2 or 3 rows, somewhat shorter post-uterine sac (6-10 vs 9-19 μ m), more posterior location of vulva (V = 67.2-88.0 vs 64-69) and the presence of male individuals in the population (vs absence).

Remarks: Nematode culture of this species in fungi culture (*Botryotinia fuckeliana*) was successful

but PCR/DNA sequencing on ribosomal DNA was not successful after numerous attempts; thus, no phylogenetic relationships can be examined in this study.

Etymology: The species name refers to Persia, the Latin name of Iran, the country of origin.

Discussion

Diagnostic characteristics of the genus *Ektaphelenchoides* are given by BAUJAR (1984) and HUNT (1993). However *Ektaphelenchoides* spp. show a high morphological plasticity, making their identification and delimitation problematic.

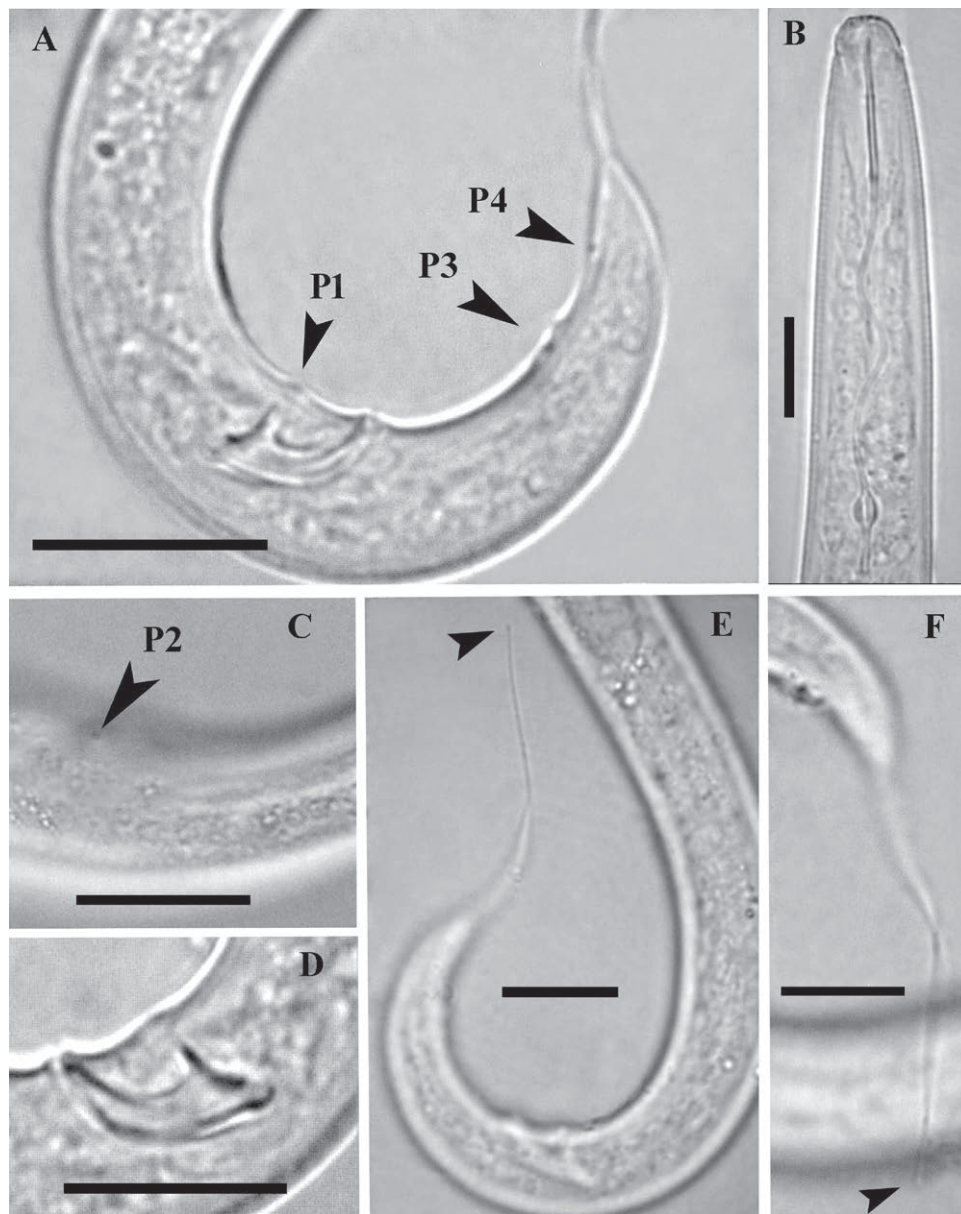


Fig. 3. *Ektaphelenchoides persicus* sp. n., male. A. Posterior part of body and papillae arrangement (arrowhead). B. Anterior body. C. Cloacal region showing P2 cloacal papilla (arrowhead). D. Showing spicules in detail. E, F. Posterior body region including 'tail' (arrowheads showing tail tip). All scale bars 10 μ m, except A 15 μ m.

Unfortunately, we could not get the useful molecular data (*i.e.* D2/D3 28S and 18S rDNA) to characterise the population studied.

Morphologically, females of *Etpahlenchoides persicus* sp. n. could be separated from *Ektaphelenchus* Fuchs, 1937 by having a long filiform tail vs bluntly short tail and six equal-sized lips vs narrow lateral sectors. Compared to *Devibursaphelenchus* Kakuliya, 1967, it could be separated by its six equal-sized lips vs having narrow lateral sectors, the absence vs presence of bursa on the male tail tip. We examined two males from the ventral view of the tail end for presence of a bursal flap, but none

was detected. Based on these characters (the lack of an anus and rectum in females, a long filiform tail and absence of bursa), this population is considered as a member of *Ektaphelenchoides*. In our previous work, we suggested that transferring *E. fuchsi* and *E. pini* (Massey, 1966) Baujard, 1984 to the genus *Devibursaphelenchus* may "solve one aspect of paraphyly" of the genus *Ektaphelenchoides* (see ESMAEILI et al. 2014). Currently, based on derived morphological traits, we cannot justify the absence of bursal flap as an apomorphic (autapomorphic) trait for the genus *Devibursaphelenchus* and change the generic definition. Such a structure has not been re-

Table 1. Morphometric data of *Ektaphelenchoides persicus* sp. n. All measurements are in μm and in the form: mean \pm s.d. (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
N	–	12	3
L	693	646 \pm 39.2 (577-700)	524 \pm 89 (431-610)
A	31.5	32.9 \pm 1.5 (30.3-35.1)	30 \pm 4.9 (26.9-35.9)
B	10.3	10.2 \pm 1.0 (9.0-12.6)	7.1 \pm 0.5 (6.6-7.6)
B'	4.3	4.3 \pm 0.5 (5.6-7.2)	4.0 \pm 0.7 (3.4-4.7)
C	–	–	4.5 \pm 0.7 (3.7-4.9)
C'	–	–	11.7 \pm 0.4 (11.4-12.2)
V or T	68.7	70.5 \pm 6.1 (67.2-88)	40 \pm 4 (40.2-47.6)
Lip region height	3.0	3.4 \pm 0.5 (3.0-4.0)	3.3 \pm 0.6 (3-4)
Lip region width	8.0	7.4 \pm 0.4 (7.0-8.0)	6.8 \pm 0.3 (6.5-7.0)
Stylet length	16	16.4 \pm 0.7 (15-17)	15.3 \pm 1.2 (14-16)
Conus length	7	7.0 \pm 0.5 (6.0-8.0)	6.2 \pm 0.8 (5.5-7.0)
M ¹	43	42.5 \pm 2.8 (37.5-47.1)	40.2 \pm 3.2 (37.5-43.8)
MB ²	74	83.6 \pm 4.9 (74.6-93.1)	81.8 \pm 4 (76.9-85)
Body diameter at median bulb	16	15.5 \pm 0.8 (14-17)	12.5 \pm 0.5 (12-13)
Body width	22	19.6 \pm 1.3 (18-22)	17.3 \pm 1.5 (16.0-19.0)
Nerve ring from anterior end	76	71.6 \pm 4.9 (65-77)	73.3 \pm 9 (64-82)
Median bulb width	11	10.8 \pm 0.6 (10-12)	9.3 \pm 2.1 (7-11)
Median bulb length	17	17.5 \pm 0.7 (17-19)	14.7 \pm 0.6 (14-15)
Excretory pore from anterior end	78	77.4 \pm 4.2 (72-84)	66.3 \pm 8 (58-74)
Hemizonid from anterior end	125	109 \pm 19.7 (87-125)	79.7 \pm 7.5 (72-87)
Pharynx length	67	64.1 \pm 6.3 (50-70)	73.3 \pm 7.6 (65-80)
Overlapping	95	94.5 \pm 15 (75-116)	78.8 \pm 14.3 (65-93)
Ovary length or Testis	343	299 \pm 29.3 (250-343)	223 \pm 20 (250-245)
Post-uterine sac	9	7.9 \pm 1.3 (6.0-10.0)	–
Anal (cloacal) body diameter	–	–	10 \pm 1 (9.0-11.0)
Tail length	–	–	117 \pm 7.5 (110-125)
Spicules length (arc line)	–	–	14.3 \pm 0.6 (14.0-15.0)
Capitulum width	–	–	4.7 \pm 0.6 (4.0-5.0)

¹ Length of conus as percentage of total stylet length.

² Distance between anterior end of body and centre of metacarpus as percentage of pharyngeal length.

ported for males of *Ektaphelenchoides*. The presence of a bursa might have evolved independently for ap-helenchoidid taxa (KANZAKI et al. 2011). It is known that *Ektaphelenchoides* and *Devibursaphelenchus* have predatory feeding (GU et al. 2010, PEDRAM et al. 2012, KANZAKI 2014) and both genera also lack a functional rectum. Such characters might have evolved from a recent common ancestor.

Ektaphelenchoides persicus sp. n. also shares some morphological characters with *Bursaphelenchus* Fuchs, 1937, e.g., the presence of a P1 pre-cloacal papilla in males (Fig. 3A), spicule morphology and cephalic region set-off with body counter. The new species can be separated of *Bursaphelenchus* spp. by lack of an anus and rectum in females, a long filiform tail and absence of bursa. Based on current data, P1 was observed among the genus (including the new species), except for two species (*E. kelardashtensis* and *E. poinari* Aliramaji,

Pourjam, Atighi, Ye, Roshan-Bakhsh & Pedram, 2014), in which it was not detected. In addition, by having the given characters, *E. persicus* sp. n. could be separated from members of family Seinuridae Husain & Khan, 1967 by lacking a functional anus.

Acknowledgements: The authors thank the University of Tehran for financial supports and Mrs Fatemeh Ansari for her help with sampling. Authors deeply thank Dr. Weimin Ye (North Carolina Department of Agriculture and Consumer Services, Raleigh, USA) and Dr. Kerrie Davies (University of Adelaide, Australia) for critical review of the manuscript, suggestions and comments. Dr Weimin Ye also kindly edited the text of the manuscript.

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Received: 10.07.2018
Accepted: 22.08.2018

