Description of *Ektaphelenchoides poinari* sp. n. (Nematoda: Ektaphelenchinae) from Iran with a compendium of the valid species of the genus *Ektaphelenchoides* Baujard, 1984

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Summary. *Ektaphelenchoides poinari* sp. n. is described and illustrated based on morphological, morphometric and molecular data. The new species is characterised by females with 477-565 µm long body, offset lip region 7.5-9.5 µm wide, separated from the rest of the body by a shallow constriction, 18-23 µm long stylet lacking knobs at base, position of excretory pore at the level to slightly posterior of the metacorpus base, three incisures in lateral field, short post-uterine sac (PUS) (0.3-0.4 corresponding body width), posterior end of the body conical, males rare, spicules having rounded condylus, pointed rostrum and blunt end. The new species is close to *E. attenuata*, *E. kelardashtensis*, *E. musae*, *E. pini*, *E. sylvestris* and *E. winteri*, but differs from them by its shorter PUS, shape of posterior body end in females and males, position of vulva and excretory pore and molecular characters. *Ektaphelenchoides poinari* sp. n. was easily differentiated from other sequenced species by the partial small subunit rRNA gene (SSU), D2D3 expansion segment of the large subunit rRNA gene (LSU) and internal transcribed spacer 1 (ITS1). Phylogenetic analysis with these sequences suggests that *E. poinari* sp. n. is close to other *Ektaphelenchoides* species and *Deviburs aphelenchus*. A compendium for valid species based on morphological and morphometric characters is also given.

Key words: internal transcribed spacer 1, large subunit rRNA gene, molecular, morphology, morphometrics, new species, phylogeny, small subunit rRNA gene, taxonomy, Tehran.

Hunt (2008) listed the valid species of aphelenchid genera. The genus *Ektaphlenchoides* Baujard, 1984 currently contains ten valid species namely *E. andrassyi* Atighi, Pourjam, Pedram, Ye & Aliramaji, 2013, *E. attenuata* (Massey, 1974) Baujard, 1984, *E. compsi* Baujard, 1984, *E. hunti* Atighi, Pourjam, Pedam, Ye & Robbins, 2012, *E. kelardashtensis* Atighi, Pourjam, Pedram, Ye, Robbins & Namjou, 2013, *E. musae* Baujard, 1984, *E. pini* (Massey, 1966) Baujard, 1984, *E. ruehmi* Yaghoubi, Pourjam, Atighi & Pedram, 2014, *E. spondylis* Kanzaki, Giblin-Davis & Center 2009, *E. sylvestris* Pedram, Pourjam, Atighi, Ye & Houshmand, 2012 and *E. winteri* Hooper, 1995. A review of related hosts and locality of the above mentioned species is given in Atighi *et al*. (2012). There is little information on the biology of the genus. According to Hooper (1995), the adults and juveniles of *E. winteri* were firmly attached to their insect host and the contents of the nematode intestine were pink, the same colour as the haemolymph of the insect host. This observation was similar to those reported for some other aphelenchid genera, *e.g. Acugutturus* Hunt, 1980 and *Noctuidonema* Remillet & Silvain, 1988 (Hunt, 1993). Kanzaki *et al.* (2009) recovered *E. spondylis* from the body cavity of *Spondylis buprestoides*. The present authors recovered several species on bark of different trees in Iran (some were not reported). For most of them, rearing on fungus plates and fungus plates with some other rhabditid and aphelenchid nematodes was not successful. The only successful culture was for *E. sylvestris* (Pedram *et al*., 2012) on co-cultures with aphelenchid and rhabditid nematodes, although its predatory behaviour was not observed during occasional observations.

Recetly, Kanzaki (2014) showed predatory feeding habit of *E. spondylis* Kanzaki, Giblin-Davis & Center, 2009 on *Pseudodiplogasteroides* sp. The current information leads us to propose a wide range of host association types for the members of the genus. During nematode surveys in the north of Iran, a new species of *Ektaphelenchoides* was recovered from bark samples of a dead pine tree having galleries of bark beetles and was described in the present paper as *E. poinari* sp. n. This is the fifth species originally described from Iran.

MATERIALS AND METHODS

Several wood samples were collected from different regions in the north of Iran during 2010 to 2012. Bark samples from a dead European red pine (*Pinus sylvestris* L.), showing bark beetles galleries, were collected from the city of Tehran and yielded an aphelenchid nematode, belonging to *Ektaphelenchoides*. The nematode was also recovered from the soil around the trunk of the host tree. The second population with few individuals was recovered from decaying dung samples collected from the city of Shahr-e-Rey. They were extracted from the wood samples by soaking a small amount of wood in water for 48 h and were hand picked under a Nikon stereomicroscope, model SMZ1000. To extract nematodes from soil and dung, the tray method (Whitehead & Hemming, 1965) was used. The nematodes were heat-killed by adding boiling 4% formalin solution and then transferred to the anhydrous glycerin and mounted on permanent slides according to De Grisse (1969). Permanent slides were made and examined under a Nikon Eclipse E600 light microscope. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope powered with differential interference contrast (DIC).

Ten nematodes were picked into distilled water and their identity was confirmed with light microscopy before being placed into 50 µl AE buffer (10mM Tris-Cl, 0.5mM EDTA; pH 9.0) and crushed with a pipette tip. DNA samples were stored at –20°C until used as a PCR template. Primers for SSU amplification were forward primer 18S965 (5'-GGCGATCAGATACCGCCCTAGTT-3') and reverse primer 18S1573R (5'-TACAAAGGGCAGGGACGTAAT-3') (Mullin *et al*., 2005), forward primer SSUF07 (5'-AAAGATTAAGCCATGCATG-3') and reverse primer SSUR26 (5'-CATTCTTGGCAAATGCTTTCG-3') (Floyd *et al*., 2002), and forward primer 18SnF (5'-TGGATAACTGTGGTAATTCTAGAGC-3') and reverse primer 18SnR (5'-TTACGACTTTTGCCCGGTTC-3') (Kanzaki & Futai, 2002). Primers for ITS1 amplification were forward primer rDNA2 (5'-TTGATTACGTTCCCTGCCCTTT-3') (Vrain *et al*., 1992) and reverse primer rDNA1.58S (5'-ACGAGCCGAGTGATCCACCG-3') (Cherry *et al*, 1997). Primers for 28S D2D3 amplification were forward primer D2a (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3b (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). The 25 µl PCR was performed using Apex *Taq* Red Master Mix DNA polymerase (Genesee Scientific Corporation, San Diego, CA, USA), according to the manufacturer's protocol. The thermal cycler program for PCR was as follows: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 2 min. A final extension was performed at 72°C for 10 min (Ye *et al*., 2007). PCR products were cleaned using ExoSap-IT (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's protocol and were sequenced by Genomic Sciences Laboratory in North Carolina State University using an Applied Biosystems 3730 XL DNA Analyzer (Life Technologies, Carlsbad, CA). The resulted DNA sequences were compared with other nematode species in GenBank using the BLAST homology search program. The most similar sequences were downloaded for phylogenetic analysis. The DNA sequences were aligned by Clustal W (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The model of base substitution in the SSU and LSU sets were evaluated using MODELTEST version 3.07 (Posada & Crandall, 1998). The Akaike-supported model, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) running the chain for 1,000,000 generations and setting the 'burn in' at 1,000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority-rule.

DESCRIPTION

Ektaphelenchoides poinari sp. n.

(Figs 1-3)

Measurements. Table 1.

Female. Body cylindrical, tapering gradually to both ends, open J-shaped when heat-killed. Cuticle finely annulated. Lateral field with three incisures

Table 1. Morphometrics of *Ektaphelenchoides poinari* sp. n. All measurements in μm and in the form: mean ± s.d. (range).

Character	Original population from city of Tehran			Shahr-e-Rey population
	Female		Male	Female
	Holotype	Paratypes	Paratype	
n	–	15	1	4
L	506	517.0 ± 25.4 (477-565)	527	513.0±76.5 (410-595)
a	29.8	28.5 ± 1.4 (25.1-31.1)	32.9	29.7±4.0 (25-35)
b	6.1	7.4 ± 0.6 (6.1-8.4)	8	8.0±0.7 (7.0-8.5)
b′	2.9	3.0 ± 0.2 (2.5-3.4)	3.4	3.2±0.5 (2.7-3.8)
c	–	–	15.5	–
c′	–	–	2.0	–
T or V	78.9	79.3 ± 0.7 (77.8-80.4)	63.9	77.8±1.0 (76.5-78.7)
Head height	3.5	4.0 ± 0.5 (3.5-5.0)	4	3.8±0.5 (3-4)
Head width	7.5	8.0 ± 0.6 (7.5-9.5)	8	8.5±1.3 (7-10)
Stylet	18	20.0 ± 1.3 (18-23)	20	20.3±1.5 (19-22)
Stylet conus	8	8.0 ± 0.7 (7-9)	8	6.3±0.5 (6-7)
m	44.4	39.9 ± 3.2 (33.3-46.2)	40	31.0±4.3 (27.3-36.8)
Median bulb from anterior end	62	59.5 ± 2.9 (56-67)	56	56.5±5.0 (49-60)
Excretory pore from anterior end	67	68.0 ± 2.2 (65-72)	70	64.5±3.0 (63-69)
Hemizonid from anterior end	90	88.0 ± 2.4 (83-93)	88	79.5±6.0 (74-86)
Pharynx base from anterior end	83	70.5 ± 4.7 (65-83)	66	64±4 (59-69)
Nerve ring from anterior end	82	71.0 ± 3.6 (65-78)	68	84±6 (78-91)
Median bulb width	10	11.0 ± 0.5 (10-12)	10.5	11±1 (10-12)
Median bulb length	17	18 ± 1 (16-19)	17	15.5±1.5 (13-17)
Pharyngeal overlapping	92	102.0 ± 8.6 (89-117)	90	96±11 (88-111)
Maximum body width	17	18.0 ± 1.1 (17-20)	16	18.6±1.1(17.5-20)
Vulval body width (VBW)	16	17.0 ± 0.9 (15.5-19)	–	17.3±1.3 (16-19)
Body width at MB	14.5	15.0 ± 0.8 (14-17)	15	15.5±0.5 (16-19)
PUS	7	7.0 ± 1.1 (5-8)	–	8.0±0.8 (7-9)
Vulva – body end	107	107.0± 7.4 (96-121)	–	114±15 (94-127)
Testis or ovary length	241	246 ± 25 (187-293)	337	231±23 (197-250)
Anal (cloacal) body width	–	–	12	–
Tail	–	–	34	–
Spicule length (arc line)	–	–	15	–
Capitulum	–	–	6.5	–

(Figs 1P, 3C). Head set off from body contour by a shallow constriction, width *ca* 2 times height. Stylet without basal knobs, conus *ca* 44.5% total stylet length. Procorpus cylindrical, 1.6-2.2 times longer than stylet, connected to a muscular, rectangular metacorpus with the granular part *ca* 30% of its length and the posterior part weakly muscular. Pharyngo-intestinal junction one metacorpal valve width posterior to metacorpus. Pharyngeal glands well developed, overlapping intestine dorsally *ca* 1.4-1.7 times the distance from anterior end to the base of median bulb or *ca* 5.8-8.3 times body width at median bulb level. Nerve ring surrounding pharyngeal glands and intestine at *ca* 3.7-4.9 metacorpus length posterior to the base of metacorpus. Excretory pore with slight variation in position, *i.e.* at the level of the metacorpus base to slightly posterior. Hemizonid 17.6-25.2 times metacorpus length from anterior end. Reproductive system monodelphic-prodelphic, gonad outstretched, occupying 38-54% of the body length, located at left of intestine. Oocytes mostly in two rows. Oviduct small, spermatheca oval to irregular, filled with rounded sperm (Figs 1I & J, 2E). Crustaformeria

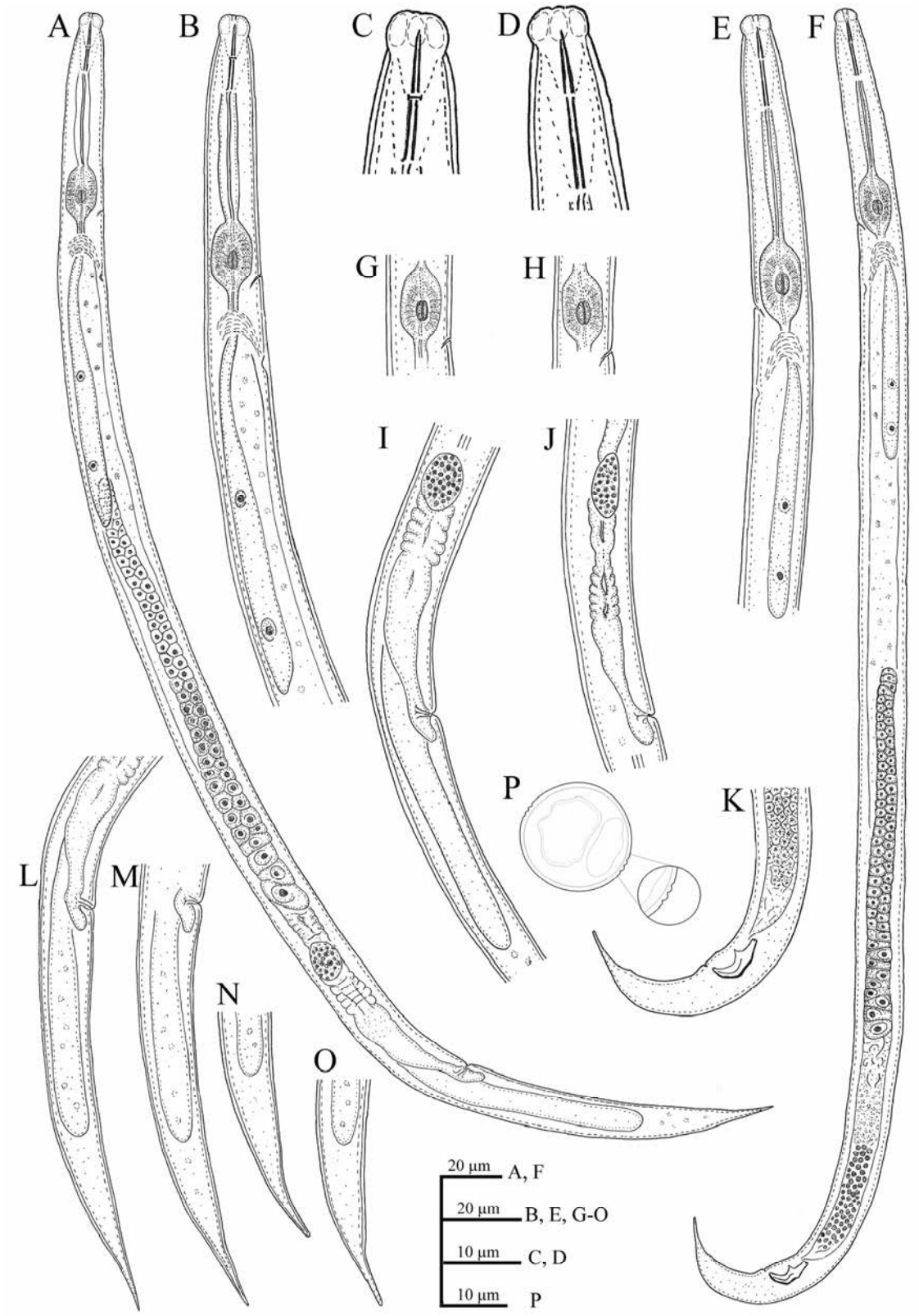

Fig. 1. *Ektaphelenchoides poinari* sp. n. A: Female entire body; B: Female pharyngeal region; C: Female anterior end; D: Male anterior end; E: Male pharyngeal region; F: Male entire body; G & H: Variation in position of excretory pore; I & J: Part of female reproductive tract; K: Male tail region; L-O: Variation in female posterior body end; P: Lateral field with three incisures.

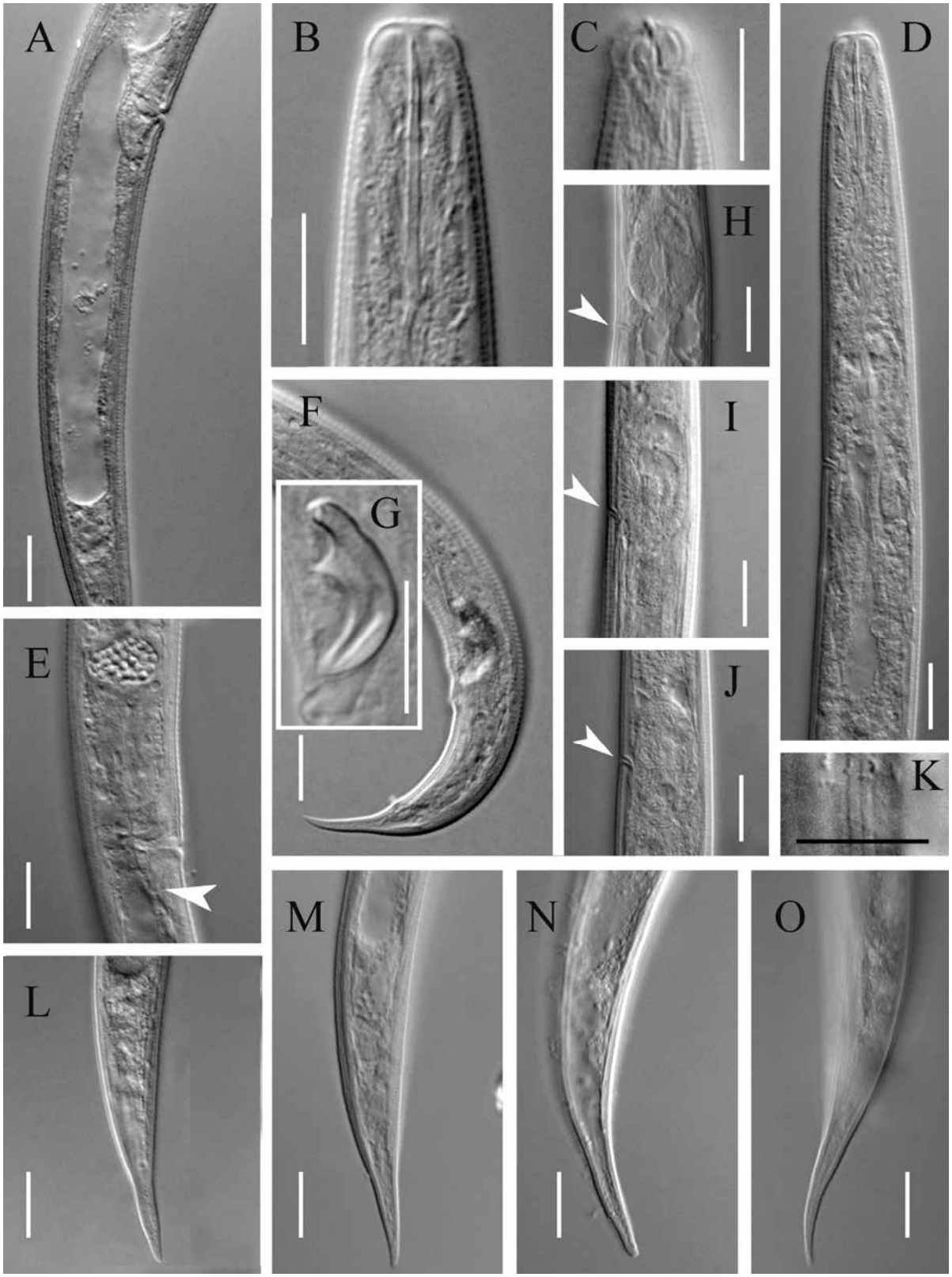

Fig. 2. *Ektaphelenchoides poinari* sp. n. A: Female, part of posterior part showing PUS and blind end of intestine; B: Anterior end in detail; C: Lip region in detail; D: Anterior region; E: Spermatheca with fine rounded sperm cells and PUS (arrow); F: Male posterior end; G: Spicule; H-J: Variation in position of E-pore (arrows); K: Three lines in lateral field; L-O: Variation in female posterior body end. All scale bars = 10 µm.

obscure, details hardly visible, uterus thick-walled, vagina not sclerotised, straight, slightly inclined anteriorly. Post-uterine sac short, less than half body width at vulva region (0.3-0.4 corresponding body width) or *ca* 8.8 % of the vulva to end of intestine distance. Intestine ends in a blind sac, vulva to end of intestine distance 64-90 µm. Anus and rectum indistinct. Distance between vulva and body end 6-7 times longer than body width at vulva. Posterior body end conical, ending to a rounded to pointed tip (Fig. 1 L-O).

Male. Rare. Body slender, tapering gradually to both ends, J-shaped after heat relaxation. General morphology similar to that in female, except sexual characters and stronger ventrally bent posterior body end. Testis single, expanded anteriorly. Spicules arcuate, separate, *ca* 2.2 times longer than capitulum width, having well developed condylus with rounded tip, pointed rostrum, lamina/calomus complex smoothly rounded and no cucullus. The single midventral precloacal papilla (P1) and the distal subventral pair of papillae (P4) not observed. One pair (P2) just before cloacal opening and another pair (P3) at *ca* 34% of tail length posterior to cloacal aperture (Fig. 3 A & B). Tail conoid with rounded terminus.

Type habit and locality. The original population was recovered from the bark of a dead *Pinus sylvestris* L. and soil around its trunk in a small park, Poonak square, Tehran, Iran in February 2011.

Other locality. The second population (few individuals) was recovered from decaying dung samples collected from the city of Shahr-e-Rey.

Type material. Holotype female, five paratype females and one paratype male deposited at Nematode Collection of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Three female paratypes deposited at each of the following collections: CABI Europe-UK, Egham, Surrey, UK; USDA Nematode Collection, Beltsville, MD, USA and Department of Nematology, Agricultural University, Wageningen, the Netherlands.

DIAGNOSIS AND RELATIONSHIPS

Ektaphelenchoides poinari sp. n. is characterised by females with 477-565 µm long body, the offset lip region 7.5-9.5 µm wide, separated from the rest of the body by a shallow constriction, 18-23 µm long stylet lacking knobs at the base, the position of an excretory pore at the level to slightly posterior of the metacorpus base, three incisures in the lateral field, the short PUS (*ca* 0.4 corresponding body width), the posterior end of the body conical, rare males with spicules having the rounded condylus, the pointed rostrum and the blunt tip. By having a short PUS, the new species comes close to six known species of the genus, namely *E. attenuata*, *E. kelardashtensis*, *E. musae*, *E. pini*, *E. sylvestris* and *E. winteri*. Compared to *E. attenuata*, the new species has a shorter body (477-565 *vs* 870-950 µm), a posteriorly located vulva (V = 77.8-80.4 *vs* 60-63), an anteriorly located excretory pore (65-72 *vs* 97-100 µm from anterior), the shorter PUS (5-8 *vs* 23-28 µm) or 0.3-0.4 *vs* 1/2 vulval body diameter and a conical tail with the rounded tip *vs* filiform. Compared to *E. kelardashtensis*, the new species has a longer stylet (18-23 *vs* 13-16 µm), a posteriorly located vulva (V = 77.8-80.4 *vs* 61.5-68.0) and a conical tail with the rounded tip *vs* filiform. Compared to *E. musae*, the new species has a posteriorly located vulva (V = 77.8-80.4 *vs* 64-69), the shorter PUS (5-8 *vs* 9-19 µm long) and a conical tail with the rounded tip *vs* filiform. Compared to *E. pini*, the new species has an anteriorly located excretory pore (65-72 *vs* 94-123 µm from anterior end), a hemizonid posterior to

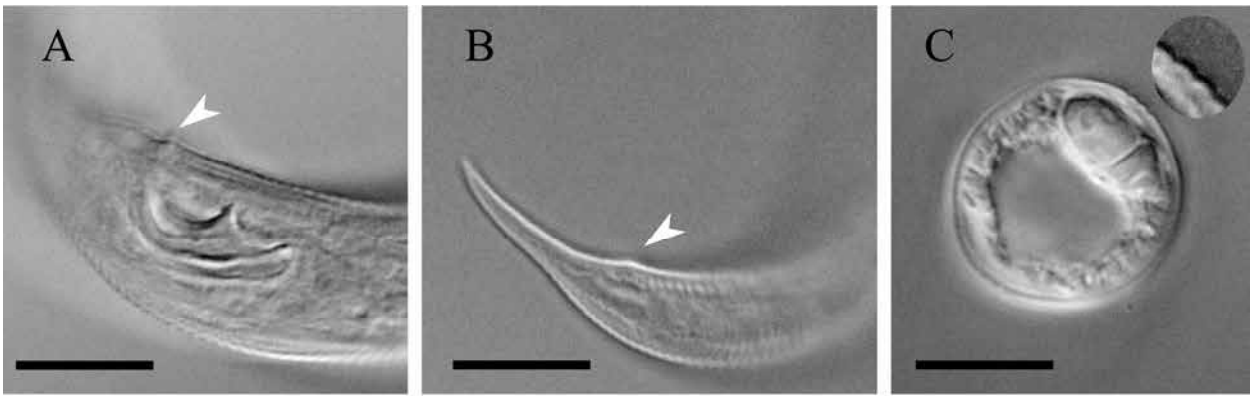

Fig. 3. *Ektaphelenchoides poinari* sp. n. A: The subventral papillae pair (P2) just before cloacal opening (arrow); B: The other pair of subventral papillae (P3) (arrow); C: Cross section showing lateral field with three incisures. All scale bars = 10 µm.

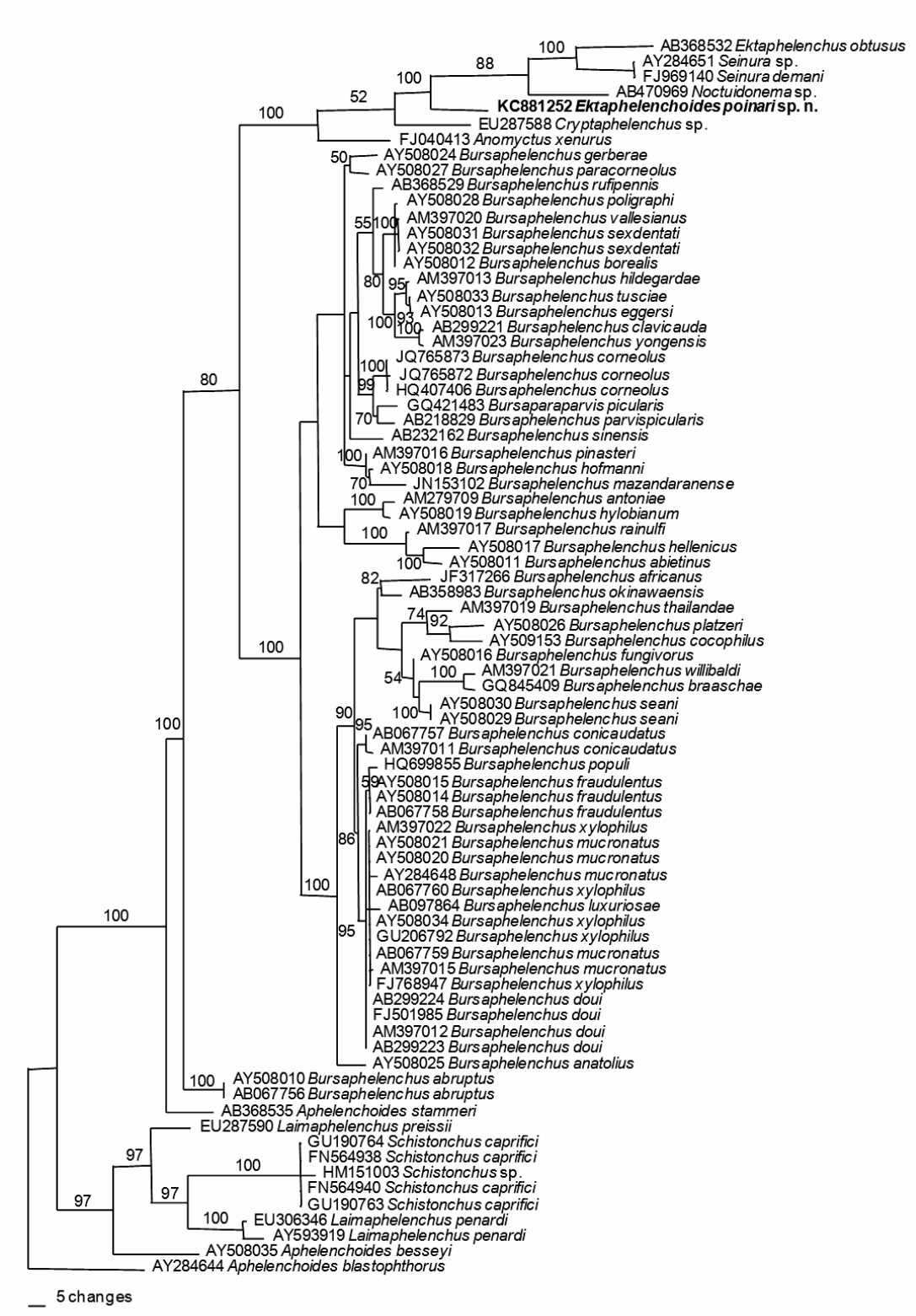

Fig. 4. The 10001st Bayesian tree inferred from SSU under GTR+I+G model (lnL = 4913.3193; freqA = 0.2563; freqC = 0.1919; freqG = 0.2626; freqT = 0.2892; R(a) = 1.3177; R(b) = 3.8058; R(c) = 1.1678; R(d) = 0.2231; R(e) = 8.3565; R(f) = 1; Pinva = 0.4171; Shape = 0.4241). Posterior probability values exceeding 50% are given on appropriate clades.

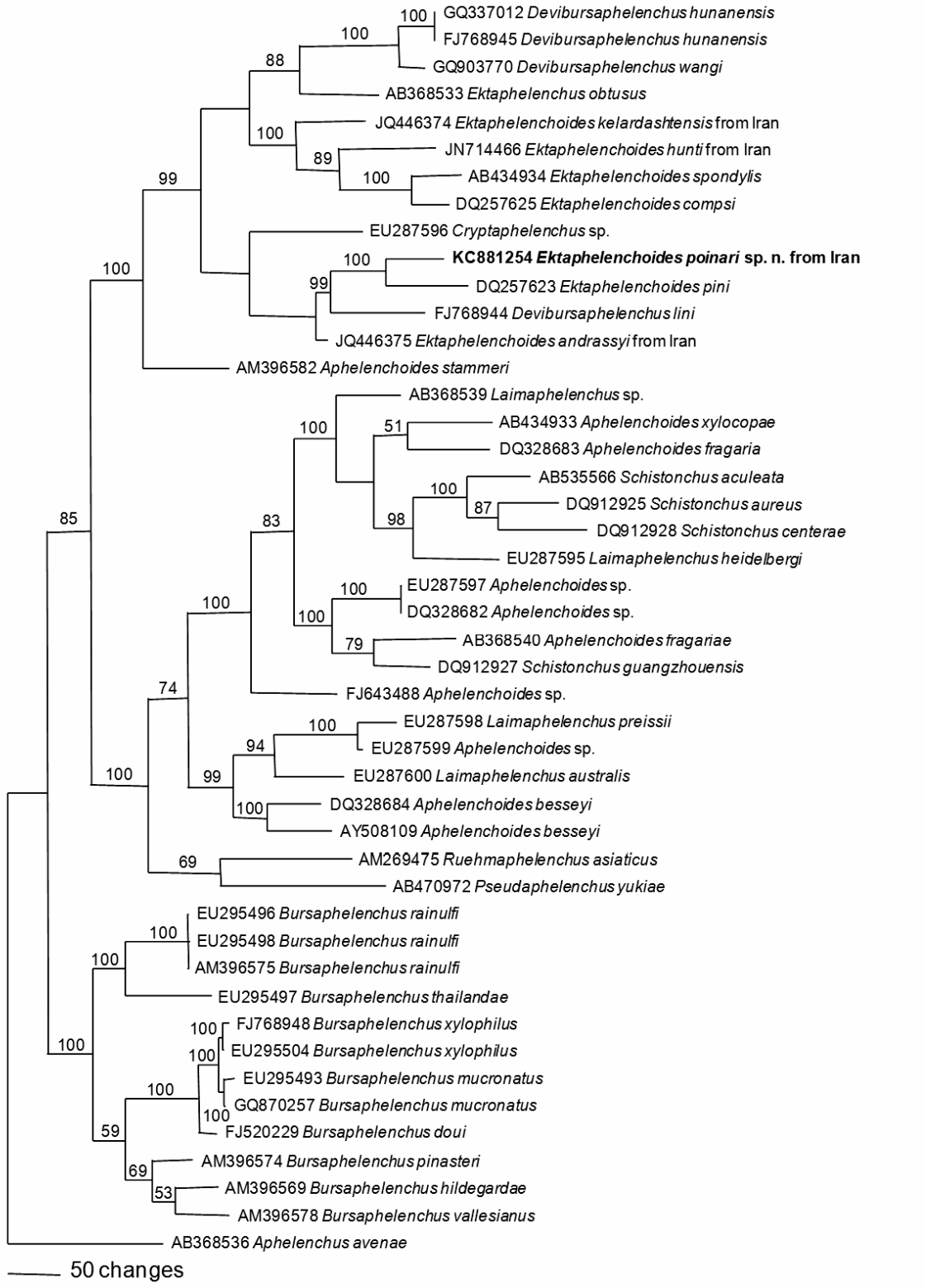

Fig. 5. The 10001st Bayesian tree inferred from LSU D2D3 under TVM+I+G model (-lnL = 16729.0039; freqA = 0.1695; freqC = 0.19; freqG = 0.3218; freqT = 0.3186; R(a) = 1.1046; R(b) = 4.0379; R(c) = 1.4553; R(d) = 0.58; R(e) = 4.0379; R(f) = 1; Pinva = 0.1688; Shape = 0.7095). Posterior probability values exceeding 50% are given on appropriate clades.

excretory pore *vs* immediately posterior and a conical tail with the rounded tip *vs* filiform. Compared to *E. sylvestris*, the new species has a shorter body (477-565 *vs* 644-843 μm), a posteriorly located vulva (V = 77.8-80.4 *vs* 75.0-76.5) and a different position of the excretory pore (at the level of the metacorpus base to slightly posterior to the metacorpus base *vs* posterior to metacorpus). Compared to *E. winteri*, the new species has a shorter body (477-565 *vs* 993-1460 μm), an anteriorly located vulva (V = 77.8-80.4 *vs* 78-85) and the shorter PUS (0.3-0.4 *vs* 0.4-0.8 corresponding body width).

Remark. Description of the new species is based on the original population. The data of the second population is given in Table 1.

Molecular characterisation and phylogeny of *Ektaphelenchoides poinari* sp. n. For molecular analyses, the 690-bp ribosomal DNA small subunit (SSU) (KC881252), the 744-bp ribosomal DNA large subunit (LSU) D2D3 (KC881254) and the 889-bp ribosomal DNA internal transcribed spacer 1 (ITS1) (KC881253) were sequenced. A Blastn search of these sequences revealed the strongest match to aphelenchids, but nothing was identical. The species with close identity were included in the phylogenetic analyses.

Figure 4 presents a phylogenetic tree based on the SSU of many aphelenchids. Using *Aphelenchoides blastophtorus* Franklin, 1952 as the outgroup, *Ektaphelenchoides poinari* sp. n. is in the 100%-supported clade which includes *Ektaphelenchus obtusus* Thorne & Malek, 1968, *Seinura demani* (T. Goodey, 1928) J.B. Goodey, 1960, *Seinura* sp., *Noctuidonema* sp., *Cryptaphelenchus* sp. and *Anomyctus xenurus* Allen, 1940. Based on currently valid sequences of SSU for the mentioned genera, it would be difficult to resolve the phylogenetic relations between them. Further sequences of morphologically similar genera like that group of aphelenchids that lack a functional rectum are needed to enable assessment of the usefulness of 18S rDNA sequences to infer the relationships between them.

Figure 5 presents the phylogenetic tree based on the LSU of many aphelenchids. Using *Aphelenchus avenae* Bastian, 1865 as the outgroup, *Ektaphelenchoides poinari* sp. n. is in a 100%-supported clade which includes many *Ektaphelenchoides* species, namely *E. pini*, *E. andrassyi*, *E. compsi*, *E. spondylis*, *E. hunti*, *E. kelardashtensis* and *Ektaphelenchus obtusus*. *Ektaphelenchoides poinari* sp. n. is unique and different from three other described species from Iran. This clade also contains five species of the genera *Devibursaphelenchus* Kakulia, 1967, *Aphelenchoides* Fischer, 1894 and *Cryptaphelenchus* Fuchs, 1937, and *Aphelenchoides stammeri* Körner, 1954 is in the basal position. All genera in this fully supported clade except *A. stamerri*, are members of Ektaphelenchinae Paramonov, 1964 (Braasch, 2009). All of them lack a functional rectum and anus.

Figure 6 presents a phylogenetic tree based on the ITS region of some aphelenchids. Using *Aphelenchoides* sp. as the outgroup, *Ektaphelenchoides poinari* sp. n. is closest to *E. pini*, the only sequenced *Ektaphelenchoides* species for this region and some species in *Devibursaphelenchus*.

Etymology. The new species named in honour of Prof. George O. Poinar Jr, the well-known pioneer scientist in systematics of insect associated nematodes.

List of the species of *Ektaphelenchoides* Baujard, 1984

Type species. *Ektaphelenchoides pini* (Massey, 1966) Baujard, 1984

= *Seinura pini* Massey, 1966

E. pini was described as *Seinura pini* by Massey in association with *Dendroctonus adjunctus* in a ponderosa pine.

Other valid species:

E. andrassyi Atighi, Pourjam, Pedram, Ye & Aliramaji, 2013;

E. andrassyi was isolated from bark samples of a beech tree (*Fagus orientalis* Lipsky) with bark beetle galleries from Kelardasht forests, Mazandaran province, northern Iran.

E. attenuata (Massey, 1974) Baujard, 1984;

= *Seinura attenuate* Massey, 1974

E. attenuata was described as *Seinura attenuata* by Massey (1974) in association with *Dendroctonus terebrans* in a loblolly pine.

E. compsi Baujard, 1984;

E. compsi was isolated from xylem of *Pinus laricio* Poir, Loiret, France.

E. hunti Atighi, Pourjam, Pedram, Ye & Robbins, 2012;

E. hunti was isolated from bark samples of a beech tree (*Fagus orientalis* Lipsky) with bark beetle galleries from Kelardasht forests, Mazandaran Province, northern Iran.

E. kelardashtensis Atighi, Pourjam, Pedram, Ye, Robbins & Namjou, 2012;

E. kelardashtensis was isolated from bark samples of an unidentified tree with bark beetle galleries from Kelardasht forests, Mazandaran province, northern Iran.

Table 2. Diagnostic morphometric data and morphological characters of species of *Ektaphelenchoides*.

Species	L	a	b	V	Stylet	Lateral lines	Position of excretory pore	End of body shape	Spicule	Reference
E. andrassyi	480-621	34.9-43.0	6.5-8.7	71.9-75.0	12.5-16.0	3	Base of metacorpus to posterior	Conical	13-17	Atighi *et al.*, 2013a
E. attenuata	870-880	34-42	12-13	61-63	17-19	Obscure	Posterior to base of metacorpus	Filiform	-	Massey, 1974, Baujard,1984
E. compsi	700-900	38-49	8-10	73.5-77.0	18-24	Obscure	Posterior to base of metacorpus	Conical	19-24	Baujard, 1984
E. hunti	711-929	35.9-44.5	7.7-10.0	70.4-72.6	16-23	3	At the middle of metacorpus to slightly posterior and at 2-8 µm distances from the base of metacorpus.	Conical	12.5-17.5	Atighi *et al.*, 2012
E. kelarda-shtensis	433-577	34.7-44.4	8.0-11.2	61.5-68.0	13-16	Obscure	Posterior to base of metacorpus	Filiform	8-10	Atighi *et al.*, 2013b
E. musae	500-710	28-33	7.4-9.8	64-69	18.5-22.0	Obscure	Posterior to base of metacorpus	Filiform	-	Baujard, 1984
E. pini	720	33	9	74	23	3	Posterior to base of metacorpus	Filiform	22-28	Massey, 1966, Baujard, 1984
E. ruehmi	355-487	31.3-38.0	5.3-7.0	67.3-73.3	9.0-12.5	3	Posterior to base of metacorpus	Filiform	13	Yaghoubi et al., 2014
***E. poinari* sp. n.**	477-565	25.1-31.1	6.3-8.4	77.8-80.4	18-26	Obscure	Level of the metacorpus base to slightly posterior	Conical	15	Present study
E. spondylis	595-710	28.6-47.3	7.0-8.7	71.2-72.3	19-23	Obscure	Posterior to base of metacorpus	Conical	23-26	Kanzaki *et al.*, 2009
E. sylvestris	644-843	25-32	8-11	75.0-76.5	18-23	3	Posterior to base of metacorpus	Conical	-	Pedram *et al.*, 2012
E. winteri	993-1460	25-35	9.0-16.6	78-85	19-26	Obscure	Posterior to base of metacorpus	Conical	23-27	Hooper, 1995

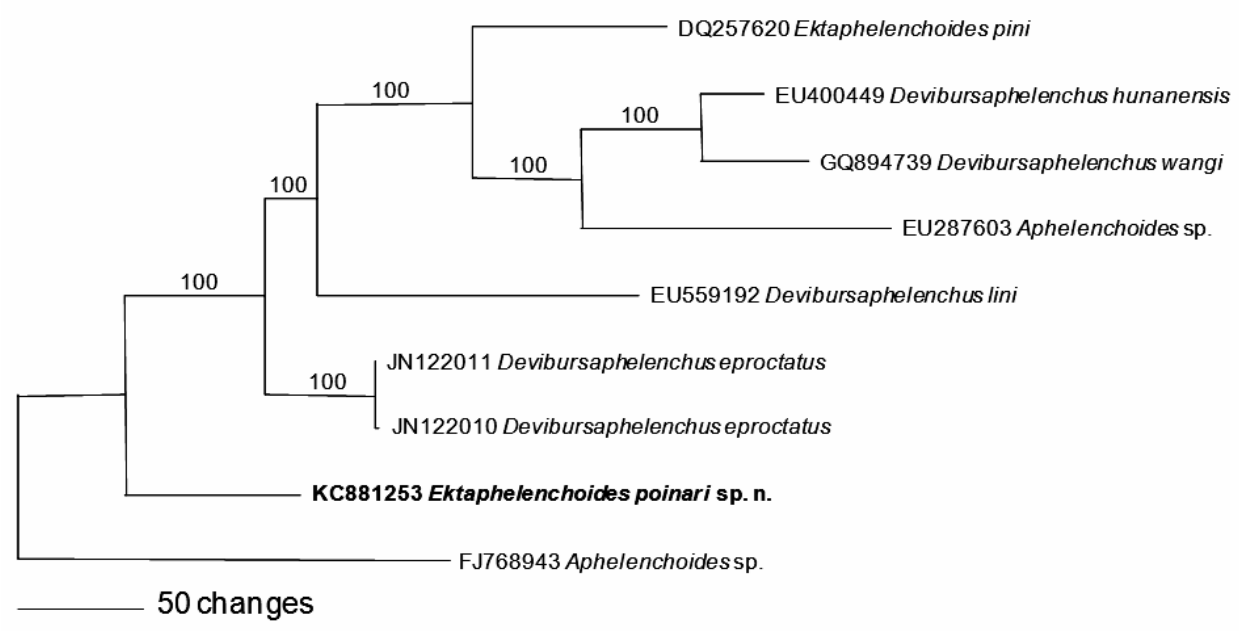

Fig. 6. The 10001st Bayesian tree inferred from ITS under Chi-square = 76.809094 (df = 24), P = 0.00000019 model (lnL = 5384.542; freqA = 0.00284; freqC = 0.001759; freqG = 0.002223; freqT = 0.003177; R(a) = 1.295; R(b) = 9.6763; R(c) = 2.0628; R(d) = 0.9699; R(e) = 12.2474; R(f) = 1; Pinva = 0; Shape = 0.7401). Posterior probability values exceeding 50% are given on appropriate clades.

E. musae Baujard, 1984;

E. musae was isolated from banana corn tissue from the Ivory Coast.

E. poinari sp. n. – present study.

E. spondylis Kanzaki, Giblin-Davis & Center, 2009;

E. spondylis was isolated from body cavity of *Spondylis buprestoides* from Japan.

E. sylvestris Pedram, Pourjam, Atighi, Ye & Houshmand, 2012;

E. sylvestris was isolated from the bark of a dead *Pinus sylvestris* L. in a small park, Poonak square, Tehran, Iran.

E. winteri Hooper, 1995;

E. winteri was found attached to larvae of *Xylodiplosis* sp. (Diptera: Cecidomyidae) emerging from logs of oak, *Quercus robur* L., cut from trees at Crickley Hill, Gloucestershire, England.

Identification of *Ektaphelenchoides* species. The diagnostic morphometric data and morphological characters of ten valid species and one new species of the genus are given in Table 2. The morphometric data are given according to the original descriptions and any subsequent re-descriptions of the species. The characters most useful for separating species delimitation include length of body, PUS and stylet, the position of an excretory pore and vulva (V), the number of lines in a lateral field and shape of body end in females, and length and shape of spicules in males.

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REFERENCES

ATIGHI, M.R., POURJAM, E., PEDRAM, M., YE, W. & ROBBINS, R.T. 2012. Molecular and morphological characterization of *Ektaphelenchoides hunti* sp. n. (Nematoda: Ektaphelenchinae) from north of Iran. *Russian Journal of Nematology* 20: 37-44.

ATIGHI, M.R., POURJAM, F. ALIRAMAJI, M.,YE, W. & PEDRAM, M. 2013. Molecular and morphological characterization of *Ektaphelenchoides andrassyi* sp. n. (Nematoda: Ektaphelenchinae) from northern Iran. *Journal of Nematode Morphology and Systematics* 16: 17-23.

ATIGHI, M.R., POURJAM, E., PEDRAM, M., YE, W., ROBBINS, R.T. & NAMJOU, S. 2013. Molecular and morphological characterization of *Ektaphelenchoides kelardashtensis* sp. n. (Nematoda: Ektaphelenchinae) from northern Iran. *Russian Journal of Nematology* 21: 23-30.

BAUJARD, P. 1984. Remarques sur la sous-famille des Ektaphelenchinae Paramonov, 1964 et proposition d'*Ektaphelenchoides* n. gen. (Nematoda: Aphelenchoididae). *Revue de Nématologie* 7: 147-171.

BRAASCH, H. 2009. Re-establishment of *Devibursaphelenchus* Kakuliya, 1967 (Nematoda, Aphelenchoididae) and proposal for a new combination of several *Bursaphelenchus* species. *Journal of Nematode Morphology and Systematics* 12: 1-5.

CHERRY, T., SZALANSKI, A.L., TODD, T.C. & POWERS, T.O. 1997. The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology* 29: 23-29.

DE GRISSE, A.T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nematodes phytoparasitaires. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 34: 351-369.

FLOYD, R., ABEB, E., PAPERT, A. & BLAXTER, M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* 11: 839-850.

HOOPER, D.J. 1995. *Ektaphelenchoides winteri* n. sp. (Nematoda: Ektaphelenchidae) from wood fly larvae *Xylodiplosis* sp. (Diptera: Cecidomyidae). *Fundamental and Applied Nematology* 18: 465-470.

HUNT, D.J. 1993. *Aphelenchida, Longidoridae and Trichodoridae. Their Systematics and Bionomics.* UK, CABI Publishing. 352 pp.

HUNT, D.J. 2008. A checklist of the Aphelenchoidea (Nematoda: Tylenchina). *Journal of Nematode Morphology and Systematics* 10: 99-135.

KANZAKI, N. 2014. *Ektaphelenchoides spondylis* is a predatory nematode. *Nematology* 16: 245-247.

KANZAKI, N. & FUTAI, K. 2002. A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the xylophilus group. *Nematology* 4: 35-41.

KANZAKI, N., GIBLIN-DAVIS, R.M. & CENTER, B.J. 2009. Description of *Ektaphelenchoides spondylis* sp. n. (Nematoda: Ektaphelenchinae) isolated from *Spondylis buprestoides* (Coleoptera: Cerambycidae) in Japan. *Nematology* 11: 181-188.

LARGET, B. & SIMON, D.L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750-759.

MASSEY, C.L. 1966. The nematode parasites and associates of *Dendroctonus adjunctus* (Coleoptera: Scolytidae) in New Mexico. *Annals of the Entomological Society of America* 59: 424-440.

MASSEY, C.L. 1974. *Biology and Taxonomy of Nematode Parasites and Associates of Bark Beetles in the*

United States. Agriculture Handbook (№ 446). USA, USDA, Forest Service, 233 pp.

MULLIN, P.G., HARRYIS, T.S. & POWERS, T.O. 2005. Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology* 7: 59-79.

NUNN, G.B. 1992. *Nematode molecular evolution*. Ph.D. dissertation: University of Nottingham, UK, 192 pp.

PEDRAM, M., POURJAM, E., ATIGHI, M.R., YE, W. & HOUSHMAND, A. 2012. *Ektaphelenchoides sylvestris* sp. nov. (Nematoda: Ektaphelenchinae) from Iran. *Annales zoologici* 62: 325-329.

POSADA, D. & CRANDALL, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

RONQUIST, F. & HUELSENBECK, J. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

VRAIN, T.C., WAKARCHUK, D.A., LEVESQUE, A.C. & HAMILTON, R.I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Journal of Nematology* 29: 250-254.

WHITEHEAD, A.G. & HEMMING J.R. 1965. A comparison of some quantitative methods for extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55: 25-38.

YAGHOUBI, A., POURJAME, E., ATIGHI, M.R. & PEDRAM, M. 2014. Molecular and morphological characterization of *Ektaphelenchoides ruehmi* sp. n. (Nematoda: Ektaphelenchinae) from southwestern Iran. *Russian Journal of Nematology*: 22: 31-38.

YE, W.M., GIBLIN-DAVIS, R.M., BRAASCH, H., MORRIS, K. & THOMAS, W.K. 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43: 1185-1197.

Aliramaji, F., E. Pourjam, M.R. Atighi, W. Ye, A. Roshan-Bakhsh, M. Pedram. Описание *Ektaphelenchoides poinari* sp. n. (Nematoda: Ektaphelenchinae) из Ирана и сводка по валидным видам рода *Ektaphelenchoides* Baujard, 1984.

Резюме. Приведено описание и иллюстрации для нового вида *Ektaphelenchoides poinari* sp. n., сопровождающееся данными по морфометрии и молекулярно-таксономическим особенностям. Новый вид характеризуется длиной тела у самок 477-565 мкм; обособленной губной областью шириной 7.5-9.5 мкм, отделенной от остального тела неглубоким сужением; стилетом длиной 18-23 мкм, не имеющим утолщений при основании; положением экскреторной поры на уровне или несколько сзади от основания метакорпуса; тремя инцизурами в латеральном поле; коротким рудиментом задней матки – задним маточным мешком (PUS), составляющим 0.3-0.4 ширины тела в этом месте; коническим задним концом тела; редко встречающимися самцами; спикулами с округлым кондилюсом, заостренным рострумом и тупой дистальной оконечностью. Новый вид близок к *E. attenuata*, *E. kelardashtensis*, *E. musae*, *E. pini*, *E. sylvestris* и *E. winteri*, но отличается от них более коротким PUS, формой задней оконечности тела у самок и самцов, положением вульвы, а также молекулярно-таксономическими особенностями. *Ektaphelenchoides poinari* sp. n. отличается от других видов, изученных молекулярными методами, по последовательностям малой субъединицы рибосомы, D2D3-сегмента последовательности большой субъединицы и ITS 1-участка. Филогенетический анализ всех этих последовательностей показывает, что *E. poinari* sp. n. близок к другим видам родов *Ektaphelenchoides* и *Deviburs aphelenchus*. Дана сводка по всем валидным видам рода, основанная на морфологических и морфометрических критериях.