

Morphological and molecular analysis based on sequence of the 18S rDNA of *Mononchoides adjunctus* Massey, 1966 (Nematoda: Neodiplogasteridae) from Iran

Sara Mehdizadeh^{1,2}, Ebrahim Shokoohi² and Joaquín Abolafia³

¹Young Researchers Society, Shahid Bahonar University of Kerman, Kerman, Iran;

²Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran, e-mail: eshokoohi@mail.uk.ac.ir;

³Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n. 23071-Jaén, Spain.

Accepted for publication 12 September 2013

Summary. *Mononchoides adjunctus* Massey, 1966 was isolated during a survey on free-living nematodes belonged to the family Neodiplogasteridae from a natural lawn in south-eastern Iran, near to Lout desert, in Kerman province. This population of *Mononchoides* is characterised by a dorsal claw-like tooth 2 µm long and 1 µm wide, 15 longitudinal ridges on the female body, a uterine sac associated with two dumb-bell-shaped pouches, small spicules (19-25 µm long), a short gubernaculum (11-14 µm or less than half of the spicule length), two pairs precloacal papillae, five pairs postcloacal papillae, papillae (GP⁵) comprising of three small papillae, and a long filiform tail (154-171 µm in females, 110-137 µm in males). Molecular analysis of *M. adjunctus* based on sequence of the 18S rDNA placed it together with *Tylopharynx foetidus* (EU306343) as sister group. Measurements, illustrations and phylogenetic position of *M. adjunctus* and closely related species are given.

Key words: description, *Mononchoides adjunctus*, phylogeny, taxonomy.

The genus *Mononchoides* was erected by Rahm in 1928. This genus belongs to the family Neodiplogasteridae *sensu stricto* according to Andrassy (1984, 2005), or to the family Diplogasteridae *sensu lato* according to Sudhaus & Fürst von Lieven, (2003). That first option is maintained in the present paper. The number of species and taxonomy of this genus has always been challenging according to different scientists (Calaway & Tarjan, 1973; Andrassy, 1984, 2005; Gagarin, 1998; Sudhaus & Fürst von Lieven, 2003), especially if members of genera *Diplenteron* Andrassy, 1964, *Glauxinema* Allgén, 1947, *Pareudiplogaster* Paramonov, 1952 are included or not under *Mononchoides*. In this respect, Gagarin (1998), and later Sudhaus and Fürst von Lieven (2003), considered the genus *Glauxinema* Allgén, 1947 as a junior synonym of the genus *Mononchoides*, although later Gagarin and Thanh (2006), considered this synonymisation as a mistake. On the other hand, Andrassy (2005)

maintained both genera could be separated on the basis of the morphology of the buccal cavity.

There are only a few studies about diplogasterid nematodes from Iran. The only diplogasterid reported recently has been *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996, by Hasani-Kakhki *et al.* (2013). The present paper, part of a series on nematodes belonging to the order Rhabditida from the province of Kerman (Iran), deals with one new record on diplogasterids, this time of the genus *Mononchoides* collected in natural areas. In addition, SSU rDNA analysis and phylogenetic position of a species of this genus is given.

MATERIALS AND METHODS

Nematode materials. Nematodes were extracted from soil samples by Baermann's funnel technique (1917). They were fixed with hot 4% formaldehyde solution and processed to anhydrous glycerin by the method of De Grisse (1969). Measurements were taken

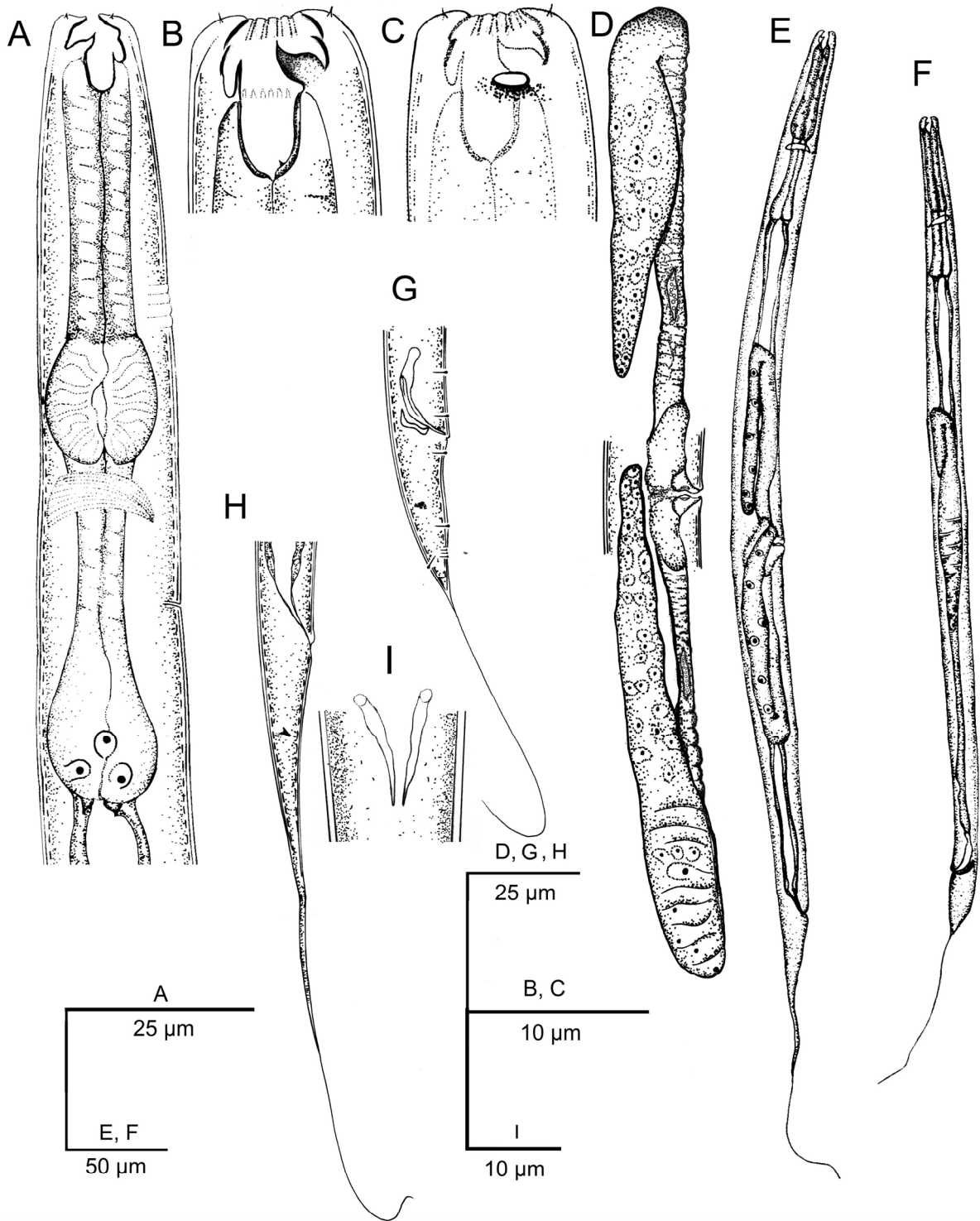


Fig. 1. *Mononchoides adjunctus* Massey, 1966. A: Neck. B: Anterior end at stoma level. C: Idem at amphid level. D: Female reproductive system. E: Entire female. F: Entire male. G: Male posterior end. H: Female posterior end. I: Spicules (ventral view).

directly using an ocular micrometer and/or a curvimeter upon drawing the corresponding organ or structure. Drawings were made using a drawing tube attached to an Olympus CH-2 microscope. LM pictures were made with a Nikon Eclipse 80i microscope equipped with a Nikon Digital Sight DS-5M camera. The terminology used to describe the morphology of the stoma and spicules follows the proposals by De Ley *et al.* (1995) and Abolafia & Peña-Santiago (2006), respectively.

Phylogenetic analysis. The sequences of several species of the superfamily Diplogasteroidea used for phylogenetic analysis were obtained from the GenBank. DNA extraction was done using an *AccuPrep* Genomic DNA extraction kit (Bioneer Corporation, Korea, <http://www.bioneer.com>) according to the manufacturer's instructions. Ten live individual specimens were picked into 1.5 ml tubes containing 5 µl double distilled water. Each tube was frozen in liquid nitrogen and was crushed using a vortex; 200 µl Tissue Lysis buffer (TL) and 20 µl proteinase K (20 mg ml⁻¹) were added. The homogenate was incubated at 60°C for 2 h. The supernatant was extracted and stored at -20°C. The forward primer SSU_F_04 (5'- GCT TGT CTC AAA GAT TAA GCC-3') and the reverse primer SSU_R_26 (5'- CAT TCT TGG CAA ATG CTT TCG-3') (Blaxter *et al.*, 1998) were used in the PCR reactions for amplification of the partial 18S region. PCR was conducted with 10 µl of the extracted DNA, 4 µl of PCR Master Mix (Kawsar Biotech company, Iran), 1 µl of each primers (10 pmol µl⁻¹) and ddH₂O to a final volume of 25 µl. The amplification was carried out using an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany), with the following parameters: 3 min at 94°C, 37 cycles of 45 s at 94°C, 45 s at 56°C and 1 min at 72°C, and finally one cycle of 6 min at 72°C followed by a holding temperature of 4°C. After DNA amplification, 5 µl of product was loaded on a 1% agarose gel (40 mM Tris, 40 mM boric acid, and 1 mM EDTA) to verify amplification. The bands were stained with 50 mM ethidium bromide and visualised and photographed on 1% agarose gel under a UV transilluminator. Product was stored at -20°C prior to sequencing. PCR product was purified for sequencing and sequenced with primers that were used for the amplification step. Sequencing was performed in both directions. The DNA sequence was edited using Chromas version 1.45 (McCarthy, 1997). Sequencing reactions were performed by the Bioneer Company (South Korea, <http://eng.bioneer.com>). Primers for the sequencing reaction were those used in the amplification step. All sequences were confirmed in both directions and repeated. The original partial 18S sequence of *Mononchoides adjunctus* Massey, 1966

was 711 bp. The sequence was deposited in GenBank under accession number KF151166. Additional sequences for the ingroups and outgroups were obtained from NCBI GenBank.

The ribosomal SSU sequences were analysed and aligned using BioEdit (Hall, 1999). The length of alignment was 1863 bp. Phylogenetic trees were generated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The analysis under GTR model was initiated with a random starting tree and run with the Markov chain Monte Carlo (MCMC) for 10⁶ generations. The distance matrix option of Mega 5 (Tamura *et al.*, 2011) was used to calculate genetic distances according to the Maximum Composite Likelihood model of sequence evolution and bootstrap analysis was implemented with 2000 replicates. *Bunonema reticulatum* Richters, 1905 (AY593925) and *Rhabditoides inermis* (Schneider, 1866) Dougherty, 1955 (AF082996) were used as outgroups for this phylogenetic analysis. These selections were based on a study by Steel *et al.* (2011). The Bayesian tree was visualised with the TreeView program (Page, 1996).

DESCRIPTION

Mononchoides adjunctus Massey, 1966 (Figs 1 & 2)

Material examined. Four females and three males from one locality, in good state of preservation.

Measurements. See Table 1.

Female. Body 0.55–0.65 mm long. Habitus slightly curved ventrad after fixation. Cuticle annulated, lacking punctation. Annuli 1.5 µm wide. Lateral field not observed. Cuticle smoothly annulated, 1 µm at mid-body, with 15 conspicuous longitudinal ridges at midbody level. Lip region continuous with body contour, consisting of six lips, each with a small papilla, 1–2 µm long. Stoma 1.5–2.0 times longer than wide. Cheilostom wide, walls heavily cuticularised. Cheilostom subdivided into several (about 10–12) narrow, rod-like plates. Bifurcated apex of cheilorhabdia extending near labial contour. Gymnostom wide, about 5 µm. Second part of stoma consisting of gymnostom and stegostom, both anisotropic with subventral walls slightly longer than dorsal. Amphidial aperture oval shaped, located at base of dorsal tooth, about 3 µm wide. Stegostom bearing a large, claw-like dorsal tooth, 2 µm long and 1 µm wide; with a duct of dorsal gland and pointed toward anterior part of stoma. Posterior part of stegostom (= meta- and telostegostom) forming a cylindrical tube, 3–4 µm wide and 5–6 µm long. Stegostom cylindrical, 5–8 µm long and 3–5 µm wide, about 1.5 times longer than

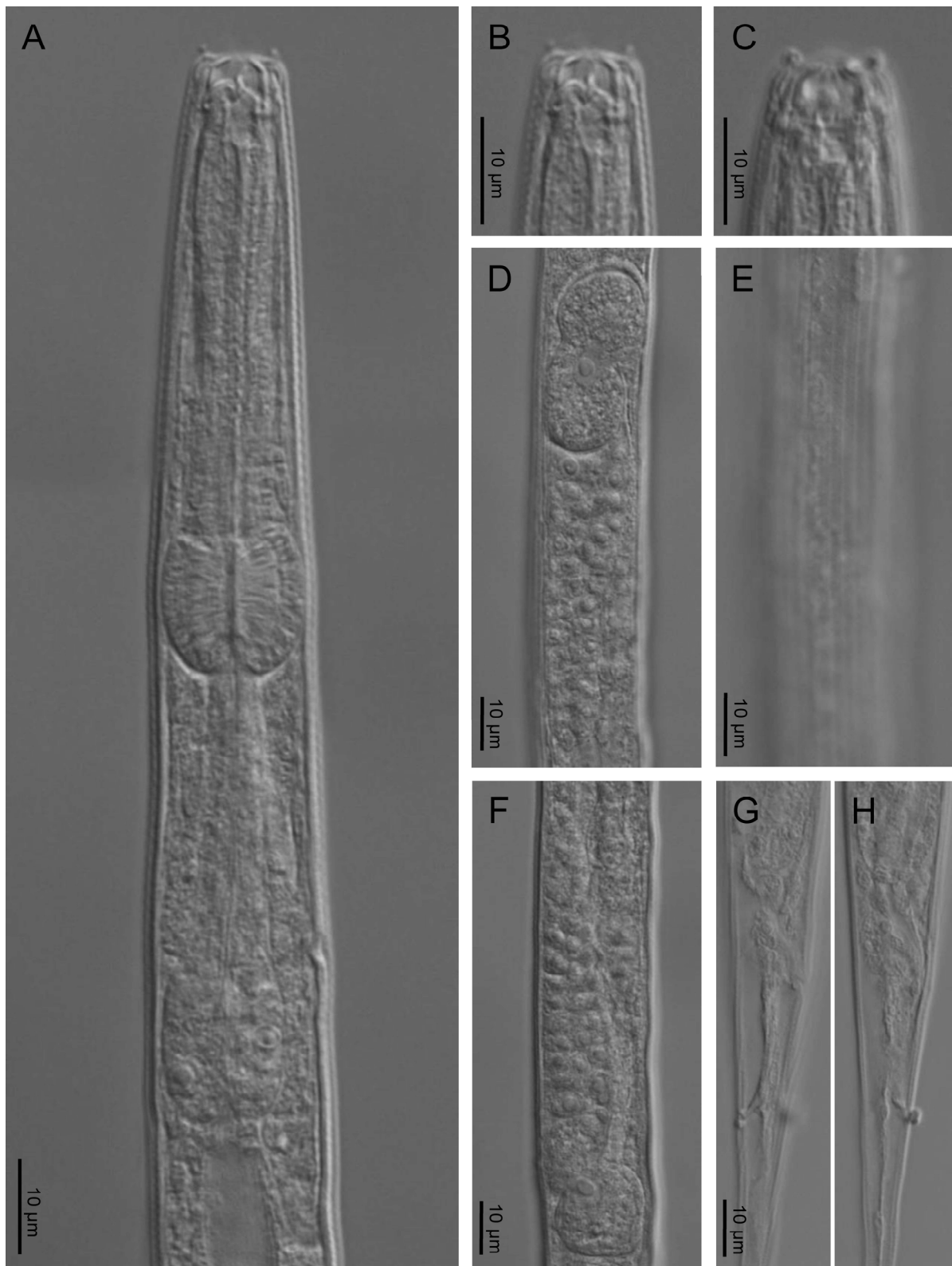


Fig. 2. *Mononchoides adjunctus* Massey, 1966. (LM, female). A: Neck. B, C: Stoma (at right and left sublateral view, respectively). D: Anterior genital branch. E: Cuticle. F: Posterior genital branch. G, H: Body posterior end at phasmids level (left and right phasmids, respectively).

wide. Pharynx diplogasteroid, having pharyngeal corpus 1.1-1.3 times longer than postcorpus (isthmus + basal bulb). Pharyngeal procorpus cylindrical, 1.5-2.0 times metacarpus length. Metacarpus swollen, 17-24 µm long. Isthmus robust, 21-27 µm long. Basal bulb ovoid, 20-24 µm long and 13-14 µm long. Cardia conoid, surrounded by intestinal tissue. Nerve ring at 67-75% of neck length, at isthmus level. Excretory pore at 71-80% of neck length, at isthmus level. Deirid not visible. Intestine without distinct specializations. Reproductive system didelphic amphidelphic with both branches equally developed. Ovaries long with oocytes arranged in one to two indistinct rows in germinal zone. Oviducts short, not well distinguished from the adjacent uterus. Uteri 4-5 times as long as the corresponding body diameter, sometimes having spermatozoa at distal part; a pair of dumb-bell-shaped pouches present at proximal part (ovijector), 17-20 µm long, connecting both uteri. Vagina with narrow lumen and extending inwards less than half of the corresponding body diameter. Vulval opening anterior to mid-body. Vulva lips weakly cuticularised, not protruding. Rectum 0.9-1.4 times anal body diameter long. Tail first conical then filiform. Phasmid at 18-20% of tail length.

Male. Body J-shaped after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail first conical, then filiform, distally curved ventrad. Two pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail. The GP⁵ (genital papillae) comprising three papillae close to each other. The GP⁶ is the shortest papillae. Spicules free, curved ventrally: manubrium almost straight and conoid, calamus with hump, and lamina ventrally curved. Gubernaculum well developed, 11-14 µm long or about 52-62% of the spicule length.

Locality and habitat. The species was collected in Andoohjerd (province of Kerman, Iran; N: 30°13'41.5"; E: 057°45'36.7"; date of sampling: 2012), associated with dominant grass species *Axonopus* sp.

Remarks. The Iranian material is similar with the original description of the *M. adjunctus* provided by Massey (1966). The Iranian material is similar to *M. adjunctus* by having small body length less than 1 mm (554-654 µm in females and 509-536 µm in males), stoma as long as lip region wide, pharyngeal corpus slightly longer than postcorpus, vulva pre-equatorial (V% = 43-45), tail with similar length (c' less than 15 in females).

However, it differs in body length (554-654 µm in females and 509-536 µm in males vs 780-870 µm in females and 700 µm in males), and c value in males (3.7-4.9 vs 3.2).

Other similar species are *M. subdentatus* (Gunhold, 1952) Andrassy, 1984 and *M. pulcherrimus* Andrassy,

Table 1. Measurements of *Mononchoides adjunctus* Massey, 1966. All measurements are in µm and in the form: mean ± s.d. (range).

Province; Locality; Habitat	Kerman; Andoohjerd; grass	
n	4♀♀	3♂♂
L	611.4±42.2 (554-654)	521.2±13.9 (509-536)
a	26.4±0.6 (25.7-27.0)	31.3±1.6 (29.5-32.3)
b	5.4±0.1 (5.3-5.5)	5.6±0.2 (5.4-5.8)
c	3.7±0.1 (3.6-3.8)	4.4±0.6 (3.7-4.9)
c'	12.7±0.8 (12-14)	7.5±0.8 (7-8)
V	44.2±0.6 (43-45)	–
Lip region width	10.8±0.3 (10.5-11.0)	8.3
Seta length	1.5±0.6 (1.0-2.0)	1.0
Amphid width	4.7±0.5 (4-5)	4.0
Stoma length	11.3±1.0 (10-12)	9.0±0.5 (8-9)
Cheilostom width	2.9	2.8
Stegostom length	6.1±1.5 (5-8)	5.6±0.9 (5-6)
Stegostom width	4.4±1.3 (3-6)	4.0±0.5 (4-5)
Procorpus length	33.8±0.6 (33-34)	29.3±1.4 (28-31)
Metacarpus length	20.8±2.7 (18-23)	17.3±1.1 (17-18)
Isthmus length	23.8±2.1 (21-26)	23.5±1.4 (22-25)
Bulb length	21.8±1.5 (21-23)	15.7±1.6 (15-18)
Neck length*	113.2±6.2 (105-119)	93.8±1.1 (93-94)
Excretory pore position	84.6±10.5 (75-96)	74.5±0.7 (74-75)
Nerve ring position	70.6±3.9 (67-74)	66.2±2.0 (65-68)
Body diameter at neck base level	20.0±1.6 (18-22)	16.0±1.4 (15-18)
Midbody diameter	23.2±1.5 (22-25)	16.7±0.9 (16-18)
Body diameter at anus/ cloaca level	12.9±0.7 (12-14)	14.8±1.3 (14-16)
Anterior genital branch	105.5±16.1 (83-117)	–
Posterior genital branch	111.6±12.2 (100-124)	–
Vagina	8.3±0.6 (8-9)	–
Vulva to anus distance	201.8±15.1 (185-222)	–
Rectum length	14.2±2.3 (13-18)	–
Tail length	163.4±7.2 (154-171)	119.9±15.0 (110-137)
Spicule length	–	21.8±2.6 (19-24)
Gubernaculum length	–	12.3±1.3 (11-14)

* Stoma + pharynx length

Table 2. Estimates of genetic distance of 18S rDNA region among *Mononchoides* and closely related species studied using Maximum Composite Likelihood method.

	Species	1	2	3	4	5	6
1	<i>Mononchoides striatus</i> JF769024						
2	<i>Tylopharynx foetidus</i> EU306343	0.124					
3	<i>Mononchoides composticola</i> GU943511	0.116	0.064				
4	<i>Mononchoides composticola</i> GU943512	0.116	0.064	0.000			
5	<i>Neodiplogaster</i> sp. AB478640	0.120	0.074	0.064	0.064		
6	<i>Mononchoides adjunctus</i> KF151166	0.205	0.147	0.147	0.147	0.143	

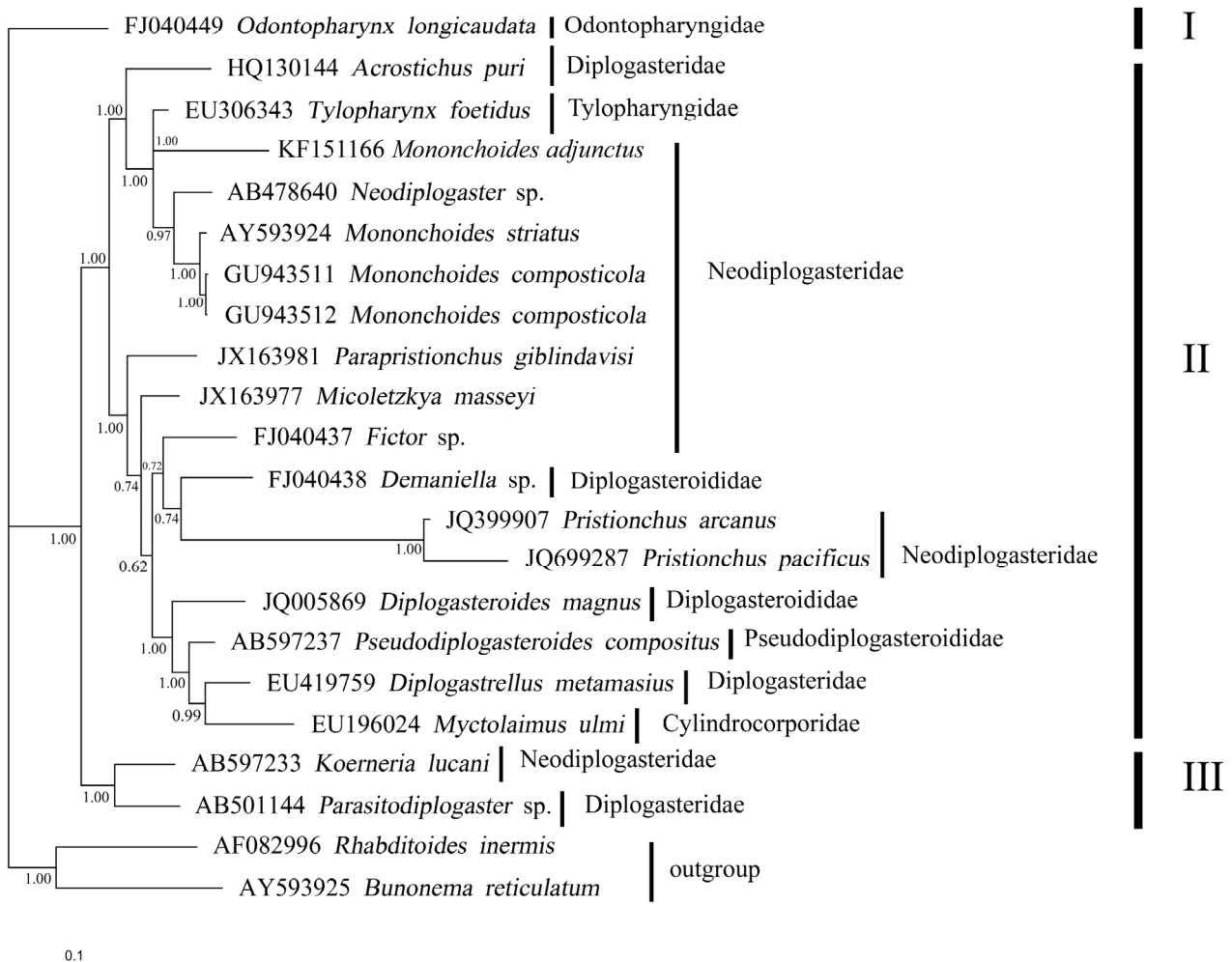


Fig. 3. The Bayesian Inference tree of known and newly sequenced *Mononchoides* species from Iran based on sequence of the 18S rDNA region.

1987. Our specimens compared with *M. subdentatus* has stoma slightly longer than wide (*vs* wider than long), longer female tail (154-171 *vs* 146-158 μm ; the measurement taken from the original measurement). In comparison with the latter species it has longer female (554-654 *vs* 880-1020 μm), shorter female tail (*vs* 200-215 μm), and spicules (19-24 *vs* 36-38 μm). Furthermore, Iranian specimens are similar to *M. striatulus* (Fuchs, 1933) Goodey in Goodey (1963); however, in our specimens spicules are free (*vs* spicules fused at their posterior half).

This species is reported for the first time from Iran.

DISCUSSION

The tree contains three main clades: I) *Odontopharynx longicaudata* de Man, 1912; II) *Acrosticus puri* Kanzaki, Giblin-Davis, Zeng, Ye, Center & Thomas, 2010; *Tylopharynx foetidus* (Bütschli, 1874) Goodey, 1928; *Neodiplogaster* sp.; *Mononchoides* spp.; *Mikoletzkyia masseyi* Susoy, Kanzaki & Herrmann, 2013; *Fictor* sp.; *Demaniella* sp.; *Pristionchus* spp.; *Parapristionchus giblindavisi* Kanzaki, Ragsdale, Herrmann, Mayer, Tanaka & Sommer, 2012; *Diplogasteroides magnus* (Völk, 1950) Weingärtner, 1953; *Pseudodiplogasteroides compositus* Körner, 1954; *Diplogastrellus metamasius* Kanzaki, Giblin-Davis, Zeng, Ye & Center, 2008 and *Myctolaimus ulmi* (Goodey, 1930) Sudhaus & Fürst von Lieven, 2003; and III) *Koerneria lucani* (Körner, 1954) Meyl, 1960 and *Parasitodiplogaster* sp.

Figure 3 shows the evolutionary relationships of the material examined as derived from molecular analysis. The sequence of Iranian *M. adjunctus* is included, together with other two *M. composticola* and *M. striatus* sequences, in a clade dominated by long-tailed Diplogasteroidea taxa, confirming other recent studies, *e.g.*, those by Steel *et al.* (2011) and Kanzaki *et al.* (2011). Genetic distance according Maximum Composite Likelihood among 18S rDNA region of *Mononchoides* species showed that *M. adjunctus* has the maximum genetic distance (0.205) with *M. striatus* (AY593924) from The Netherlands (Table 2). Furthermore, in comparison with *M. composticola* (GU943511; GU943512) reported from Belgium, its genetic distance is 0.116.

The phylogenetic analysis places *Mononchoides* close to *Tylopharynx foetidus*, in agreement with Steel *et al.* (2011). However, our analysis agrees with the previously published 18S rDNA trees of Diplogasteromorpha (van Megen *et al.*, 2009; Steel *et al.*, 2011; Kanzaki *et al.* 2011), in the fact that the 18S rDNA gene does provide resolution within the diplogastrids for the phylogenetic analysis within this group of nematodes.

REFERENCES

- ABOLAFIA, J. & PEÑA-SANTIAGO, R. 2006. Nematodes of order Rhabditida from Andalucia Oriental, Spain. The family Panagrolaimidae, with a compendium of species *Panagrolaimus* and a key to their identification. *Journal of Nematode Morphology and Systematics* 8: 133-160.
- ALLGÉN, C.A. 1947. Weitere Untersuchungen über schwedische Nematoden. *Kunliga Fysiografiska Sällskapet I Lund Förhandlingar* 7: 1-10.
- ANDRÁSSY, I. 1964. Neue Nematoden-Arten aus Ungarn, III. Fünf neue Arten. *Opuscula Zoologica Budapest* 5: 9-23.
- ANDRÁSSY, I. 1984. *Klasse Nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida)*. Deutschland, Berlin, Akademie Verlag. 509 pp.
- ANDRÁSSY, I. 1987. The free-living nematode fauna of the Kiskuns g National Park. In: *The Fauna of the Kiskuns g National Park* (S. Mahunka Ed.). pp. 15-46. Hungary, Budapest, Academy Publisher.
- ANDRÁSSY, I. 2005. *Free-living nematodes of Hungary (Nematoda errantia)*. Vol. 1. *Pedozoologica Hungarica* 3. 518 pp.
- BAERMANN, G. 1917. Eine einfache Methode zur Auffindung von *Ankylostomum* (Nematoden) Larven in Erdproben. *Geneeskunding Tijdschrift voor Nederlandsch-Indië* 57: 131-137.
- BLAXTER, M.L., DE LEY, P., GAREY, J.R., LIU, L.X., SCHELDAMAN, P., VIERSTRAETE, A., VANFLETEREN, J.R., MACKAY, L.Y., DORRIS, M., FRISSE, L.M., VIDA, J.T. & THOMAS, W.K. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* 392: 71-75.
- BÜTCHLI, O. 1874. Zur Kenntniss der freilebenden Nematoden insbesondere der des Kieler Hafens. *Abhandlungen der Senckenbergischen naturforschenden Gesellschaft* 9: 1-56.
- CALAWAY, T.W. & TARJAN, C.A. 1973. A compendium of the genus *Mononchoides* Rahm, 1928 (Diplogasterinae: Nematoda). *Journal of Nematology* 5: 107-116.
- DE GRISSE, A. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen van de Rijksfaculteit Landbouwetenschappen Gent* 34: 351-369.
- DE LEY, P., VAN DE VELDE, M.C., MOUNPORT, D., BAUJARD, P. & COOMANS, A. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica* 41: 153-182.

- DE MAN, J.G. 1912. *Odontopharynx longicaudata* n.g. n.sp. eine neue Form von Anguilluliden. *Zoologische Jahrbücher (Systematik)* 33: 637-642.
- DOUGHERTY, E.C. 1955. The genera and species of the subfamily Rhabditinae Micoletzky, 1922 (Nematoda). A nomenclatorial analysis including an addendum on the composition of the family Rhabditidae Örley, 1880. *Journal of Helminthology* 29: 105-152.
- FUCHS, G. 1933. Einige Nematoden bei *Scolytus scolytus* F. *Capita Zoologica (Os-Gravenhage)* 4: 1-45.
- GAGARIN, V.G. & NGUYEN VU THANH. 2006. Re-classification of Neodiplogasteridae with notes on the genus *Glauxinema* Allgén, 1947 and description of *G. aquaticum* sp. n. from Vietnam (Nematoda). *Zoosystematica Rossica* 15: 1-6.
- GAGARIN, V.G. 1998. A review of the genus *Mononchoides* Rahm, 1928, with description of two new species from European Russia (Nematoda: Diplogasterida). *Zoosystematica Rossica* 7: 229-238.
- GOODEY, T. 1928. The species of the genus *Aphelenchus*. *Journal of Helminthology* 6: 121-160.
- GOODEY, T. 1930. A new species of the nematode genus *Cylindrogaster*. *Journal of Helminthology* 8: 89-92.
- GOODEY, T. 1963. *Soil and freshwater nematodes*. 2. ed. (J.B. Goodey Ed.). London, Methuen & Co.; New York, Wiley & Sons. 544 pp.
- GUNHOLD, P.P. 1952. Über die in Kompost lebenden Nematoden. *Bonner Zoologische Beiträge* 3: 151-166.
- HALL, T.A. 1999. BioEdit: A user-friendly biological sequence alignment and analysis program for Windows 95/98/NT. *Nucleic Acid Symposium Series* 41: 95-98.
- HASANI-KAKHKI, M. KARIMI, J. & SHOKOOHI, E. 2013. Molecular and morphological characterization of *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996 (Nematoda, Rhabditida, Neodiplogasteridae), a new record of an entomophilic nematode from Iran. *Biologia* 68: 910-917.
- KANZAKI, M., RAGSDALE, E.J. HERRMANN, M., MAYER, W.E., TANAKA, R. & SOMMER, R.J. 2012. *Parapristionchus giblindavisi* n. gen., n. sp. (Rhabditida: Diplogasteridae) isolated from stag beetles (Coleoptera: Lucanidae) in Japan. *Nematology* 14: 933-947.
- KANZAKI, N., GIBLIN-DAVIS, R.M., ZEN, Y., YE, W. & CENTER, B.J. 2008. *Diplogastrellus metamasius* n. sp. (Rhabditida: Diplogasteridae) isolated from *Metamasius hemipterus* (Olivier) (Coleoptera: Dryophthoridae) in southern Florida and Costa Rica. *Nematology* 10: 853-868.
- KANZAKI, N., GIBLIN-DAVIS, R.M., ZENG, Y.S., YE, W.M., CENTER, B.J. & THOMAS, W.K. 2010. *Acrostichus puri* n. sp. (Nematoda: Diplogasteridae), a phoretic associate of *Augochlora pura mosieri* Cockerell (Hymenoptera: Halictidae). *Nematology* 12: 49-64.
- KANZAKI, N., TAKI, H., MASUYA, H., OKABE, K., TANAKA, R. & ABE, F. 2011. Diversity of stag beetle-associated nematodes in Japan. *Environmental Entomology* 40: 281-288.
- KÖRNER, H. 1954. Die Nematodenfauna des vergehenden Holzes und ihre Beziehungen zu den Insekten. *Zoologische Jahrb. cher (Systematik)* 82: 245-353.
- 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.
- MC CARTHY, C. 1997. Chromas, Version 1.41, Griffith University, Brisbane.
- MEYL, A.H. 1960. *Die freilebenden Erd- und Süßwassernematoden (Fadenwürmer)*. In: Die Tierwelt Mitteleuropas, vol. 1 (5a) (P. Brohmer, P. Ehrmann & G. Ulmer Eds.). pp. 164. Deutschland, Leipzig, Quelle & Meyer.
- PAGE, R.D.M. 1996. TreeView: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12: 357-358.
- PARAMONOV, A.A. 1952. [An attempt of ecological classification of the nematodes associated with plants]. *Trudy Gelmintologicheskoi Laboratorii Akademiya Nauk SSSR* 6: 338-369. (In Russian).
- RAHM, G.F. 1928. Alguns nematodes parasitas e semi-parasitas das plantas culturaes do Brasil., *Archivos do Instituto de Biologia e Defesa Agricola e Animal* 1: 239-251.
- RICHTERS, F. 1905. *Bunonema reticulatum* n. sp. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 15: 46-47.
- RONQUIST, F. & HUELSENBECK, J. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- SCHNEIDER, A.F. 1866. *Monographie der Nematoden*. Deutschland, Berlin. 357 pp.
- SOMMER, R.J., CARTA, L.K., KIM, S.Y. & STERNBERG, P.W. 1996. Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda: Neodiplogasteridae). *Fundamental and applied Nematology* 19: 511-521.
- STEEL, H., MOENS, T., SCHOLAERT, A., BOSHOF, M., HOUTHOOFD, W. & BERT, W. 2011. *Mononchoides composticola* n. sp. (Nematoda: Diplogasteridae) associated with composting processes: morphological, molecular and autecological characterisation. *Nematology* 13: 347-363.

- SUDHAUS, W. & FÜRST VON LIEVEN, A. 2003. A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda). *Journal of Nematode Morphology and Systematics* 6: 43-90.
- SUSOY, V., KANZAKI, M. & HERRMANN, M. 2013. Description of the bark beetle associated nematodes *Micoletzkyia masseyi* n. sp. and *M. japonica* n. sp. (Nematoda: Diplogastridae). *Nematology* 15: 213-231.
- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739.
- VAN MEGEN, H., VAN DEN ELSSEN, S., HOLTERMAN, M., KARSSSEN, G., MOOYMAN, P., BONGERS, T., HOLOVACHOV, A., BAKKER, J. & HELDER, J. 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* 11: 927-950.
- VÖLK, J. 1950. Die Nematoden der Regenwürmer und aasbesuchenden Käfer. *Zoologische Jahrbücher (Systematik)* 79: 1-70.
- WEINGÄRTNER, I. 1953. Die Nematoden des Kompostes. *Sitzungsberichte der Physikalisch-medizinischen Sozietät zu Erlangen* 76: 86-107.

Mehdizadeh, S., Shokoohi, E. and Abolafia, J. Анализ *Mononchoides adjunctus* Massey, 1966 (Nematoda: Neodiplogasteridae) из Ирана по результатам изучения морфологии вида и последовательности 18S rDNA.

Резюме. Нематоды *Mononchoides adjunctus* Massey, 1966 обнаружены во время сбора нематод семейства Neodiplogasteridae в луговой почве близ пустыни Лут в провинции Керман (юго-восточная часть Ирана). Особи этой популяции *Mononchoides* характеризуются наличием в стоме дорсального когтевидного зуба длиной 2 мкм и диаметром 1 мкм; 15-ю продольными ребрами на теле самок; матками, связанными с двумя карманами гантелевидной формы; небольшими спикулами (19-25 мкм в длину); коротким рульком (11-14 мкм или менее половины длины спикулы); двумя парами преклоакальных папилл; пятью парами постклоакальных папилл, (GP⁵) из трех мелких папилл; длинным нитевидным хвостовым филаментом (154-171 мкм у самок, 110-137 мкм у самцов). Молекулярный анализ *M. adjunctus* по последовательностям 18S rDNA показал близость их к *Tylopharynx foetidus* (EU306343). Приводятся результаты измерений, рисунки, фотографии, а также данные по филогенетическим связям *M. adjunctus* и близких видов.
