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Occurrence and distribution of nematodes in rice fields in Guilan province, Iran and the first record of *Mylonchulus polonicus* (Stefanski, 1915) Cobb, 1917 (Nematoda: Mononchina)

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Abstract: The distribution of nematodes was studied in rice fields in Guilan province, Iran, from 2014 to 2016. Nematode biodiversity of 250 soil and root samples was examined. Thirty nematode species were identified morphologically, including plant parasites, microbivores and mycetophagous and predator species. Molecular techniques were also used for further identification of three plant parasitic species. Indicators of population were also estimated. Spiral nematodes (*Helicotylenchus crenacauda* Sher, 1966 and *H. digitiformis* Ivanova, 1967) and stunt nematode (*Tylenchorhynchus agri*) were the predominant parasitic species identified. Among other species, three *mononchid* species were identified namely *Mononchus aquaticus, Mylonchulus sigmaturus* and *M. polonicus*. The species *M. polonicus* was found and reported in Iran for the first time. The two plant parasitic species *T. agri* and *Xiphinema index* were reported in association with rice in Iran for the first time. To evaluate the distribution and incidence of *Aphelenchoides besseyi* (rice white-tip nematode) in different regions of Guilan province, a total of 255 fresh seed samples were collected/inspected, of which, about 40% of them were infested with *A. besseyi*. Of the 16 studied counties, the highest percentage of infected seeds came from fields around the city of Astara (69.2%) and the second highest infection was observed near the city of Anzali (60%).

Key words: *Aphelenchoides besseyi*, free-living nematodes, plant-parasitic nematodes, *Tylenchorhynchus agri*, *Xiphinema index*

Introduction

Rice (*Oryza sativa* L.) is one of the primary staple crops internationally and is the second most important crop in Iran (Mousanejad *et al*. 2009). It is susceptible to several disease agents during its growth (Sayari *et al*. 2014). This crop is cultivated in 15 provinces in Iran but at least 69% of the rice production is found in two northern provinces, Mazandaran and Guilan. The average rice production of Guilan province, as the second largest rice producing province, is about 750,000,000 kg. This amount accounts for 40% of the country's production, and 31% of the total area of the province is under rice cultivation (Mohammadi *et al*. 2015). Rice is grown traditionally in these areas, using the most common method of rice cultivation which involves transplanting manually. Currently there are 230,000 ha of rice paddy fields in Guilan province (Ashouri 2012). Many genera and species of plant parasitic nematodes are associated with rice, but only some of these are known or suspected to cause yield loss (Bridge *et al*. 2005). *Aphelenchoides besseyi* Christie, 1942, *Hirschmanniella oryzae* (Van Breda de Haan 1902) Luc & Goodey, 1963 and *Heterodera elachista* Ohshima, 1974 are the most important species which have been found in rice-growing regions of Iran (Kheiri 1971; Minassian and Barooti 1997; Tanha Maafi *et al*. 2003). A few studies have been done on the identification of plant parasitic nematodes in Guilan province (Pedramfar *et al*. 2001; Soleymanzadeh *et al*. 2016), but identification and distribution of nematodes have not yet been studied in rice fields of Guilan province, even in some recently conducted studies (Aliramaji *et al*. 2015; Pedram and Pourjam 2014; Pedram *et al*. 2015; Roshan-Bakhsh *et al*. 2016). The aim of our study was to perform a comprehensive survey on the identification and distribution of nematodes across the rice fields of the province. Since most soil-inhabiting nematode taxa can be beneficial and may have roles in the decomposition of organic matter they could be regarded as important soil quality indicators (Yeates and Coleman 1982; Ingham *et al*. 1985; Spedding *et al*. 2004; Ravichandra 2013). Therefore the free living forms of the collected species

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of nematodes were also identified. The purposes of this study were quantifying nematode biodiversity, preparing information on population dynamics and investigating the incidence and distribution of *A. besseyi* in rice growing areas in Guilan province. Three plant parasitic taxa were also identified by using molecular techniques. The species, *M. polonicus* (Stefanski, 1915) Cobb, 1917 was reported in Iran for the first time.

Materials and Methods

During 2014–2016, a total of 250 soil samples were taken from the rhizosphere of rice at a depth of 5–25 cm, in different regions of rice fields in Guilan province, northern Iran. The samples came from near almost all the villages of the province under rice cultivation in 16 counties. The distribution map of the sampling areas is shown in Figure 1.

Each of the 250 samples was composed of 25–30 sub-samples collected from 1–2 ha. To deal with variability in the field, a systematic zigzag pattern was used for collecting sub-samples. Each sample was stored at 4°C before extracting the nematodes. Nematodes were extracted from soil using the centrifugation sugar-flotation technique (Jenkins 1964). Nematodes were also extracted from collected roots (5 g root) by maceration in a waring blender at 3,000 rpm for 2 min and extracted with a Bearmann funnel. After extraction, samples were evaluated under a dissecting microscope at 40x magnification. All nematode genera were counted with the aid of counting slides and recorded before fixing. Nematode population levels were determined and expressed as numbers per 500 cm^3 soil and 5 g root.

The data obtained were subjected to community analysis to determine the ecological index according to Norton (1978) as follows: the absolute frequency = number of samples containing a genus ×100/number of samples collected; relative frequency = frequency of a genus × 100/sum of frequency of all genera; absolute density = average population density (nematodes/100 cm^3 soil); relative density = average number of individual genus × 100/average number of all nematode genera; prominence value = absolute density × absolute frequency square for each certain nematode genus (absolute frequency); relative prominence = prominence value of a genus × 100/sum of prominence values of all genera. After counting, nematode specimens were killed, fixed, processed in dehydrated glycerin and mounted on glass slides according to De Grisse (1969).

For the fresh seed samples survey, 255 fresh seed samples from 16 locations were collected. The number of seed samples for each location is shown in Table 1. Nematodes were extracted from 50 g of fresh seeds (out of 500 g), randomly collected from each point, then they were extracted using the Coolen and D'Herde technique (1972). The extracted nematodes were counted and fixed. Species characterization in this survey was based on morphological and morphometric characters using available literature and their original descriptions (Hopper and Cairns 1959; Loof and Luc 1990; Nickle and Hooper 1991; Jairajpuri and Ahmad 1992; Hunt 1993; Handoo 2000; Siddiqi 2000; Andrássy 2009; Ahmad and Jairajpuri 2010).

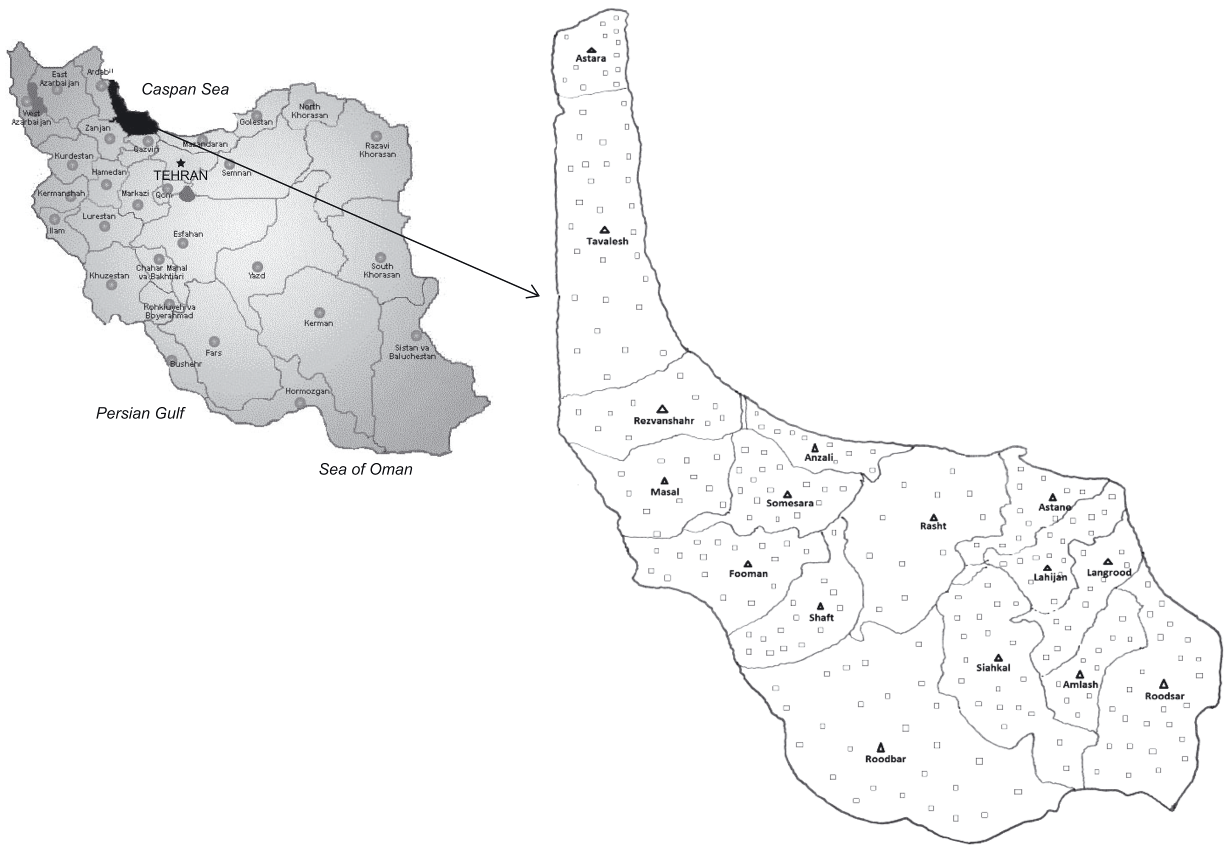

Fig. 1. Distribution map of the samples taken from rice fields in different areas of Guilan province (with the capital city, Rasht), northern Iran. Sample locations are shown with white squares; triangles indicate location of majorcounties for this study

Table 1. Incidence of *Aphelenchoides besseyi* in fresh seed samples of rice in Guilan province

Sampling locations	Number of samples	Number of seed samples infested with *A. besseyi*	Percent of seed samples infested with *A. besseyi*	Average numbers of *A. besseyi* per 1,000 seeds
Amlash	10	1	10.00	0–38*
Anzali	10	6	60.00	0–760**
Astane	15	18	53.33	0–605**
Astara	13	9	69.20	0–770**
Foman	16	9	56.30	0–515**
Lahijan	15	5	33.33	0–167*
Langrood	12	3	25.00	0–60*
Tavalesh	28	13	46.42	0–560**
Masal	11	2	18.18	0–90*
Rasht	11	6	54.54	0–598**
Rezvanshahr	10	1	10.00	0–98*
Roodbar	28	2	7.00	0–54*
Roodsar	26	9	34.61	0–22*
Shaft	14	5	35.71	0–258*
Siahkal	20	8	40.00	0–560**
Somesara	16	6	37.50	0–480*

*low infestations (0–500 nematodes per 1,000 seeds); **moderate infestations (500–1,000 nematodes per 1,000 seeds)

For molecular identification purposes, three selected individuals of plant parasitic taxa (*A. besseyi*, *H. oryzae* and *H. elachista*) were further identified by using molecular markers. Two genomic fragments, 28S rDNA D2/D3 and internal transcribed spacer (ITS) (ITS1-5.8S--ITS2) regions, were sequenced. Each individual was transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, QIAGEN Inc., Valencia CA, USA) on a clean slide and crushed using a cover slip. The suspension was collected by adding 20 μl AE buffer. DNA samples were stored at –20°C until used for polymerase chain reaction (PCR) amplification. Primers used for D2-D3 amplification were forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse primer D3B (5′-TGCGAAGGAACCAGCTACTA-3′) (Nunn 1992). The ITS1 region was amplified using forward primer TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and reverse primer AB28 (5′-ATATGCTTAAGTTCAGC GGGT-3′) as described in Vovlas *et al.* (2008). The information of PCR is according to Panahandeh *et al.* (2015). The PCR products were sequenced in both directions using PCR primers with an ABI 3730XL sequencer (Bioneer Corporation, South Korea). Newly obtained sequences for these species were deposited in GenBank database (accession numbers KX622689, KX622690 and KX622691 for 28S rDNA D2/D3 fragments of *A. besseyi*, *Tylenchorhynchus agri* and *H. elachista* respectively, and KX622692 for ITS of *H. elachista*).

Results

In this study, thirty species of soil inhabiting nematodes were identified. Free-living nematodes were more diverse in the number of species (about 70%) compared with plant parasitic nematodes (about 30%). *Tripylla* sp. and *Mononchus* sp. (with 4.8% and 4.4% frequency) had the highest populations among free-living nematodes. In comparison to the populations of other groups, the order Mononchida Jairajpuri, 1969 (Jairajpuri 1969) and other beneficial predatory nematodes showed the lowest populations in the studied areas (Table 2).

The results of this survey indicated that the plant-parasitic nematodes, *Helicotylenchus* spp. (33%) and *T. agri* (31.4%), were found to be the predominant genera compared with others in the studied fields. *Heterodera elachista* (1.79%) was the next most populous and *Criconemella paragoodeyi* Choi & Geraert, 1975 was the least populous (0.1%). The rice white-tip nematode, *A. besseyi*, was observed at low frequency in soil but had a moderate population in fresh seed samples compared with soil in the studied areas. Among the 255 fresh seed samples, 113 seed samples (40.4%) were infested with *A. besseyi* in the studied area. Of these samples, 33.3% had low infestations (0–500 nematodes per 1,000 seeds) and 67.98% had moderate infestations (500–1,000 nematodes per 1,000 seeds). Among the studied locations, Astara and Anzali had the largest percent of infected fresh seed samples and also the highest level of infection. Roodsar had the lowest number of infestation (0–22 nematodes per 1,000 seeds). Fukano (1962) determined the economic damage threshold density for *A. besseyi* to be 3,000 live nematodes per 1,000 seeds, which provides a useful basis for damage prediction (Bridge *et al.* 2005). Based on this threshold, about 67% of the infected fresh seed samples had more than 500 nematodes per 1,000 seeds, a threshold creating a need for management programs in order to prevent greater crop losses.

In the present study *M. polonicus* was found for the first time in Iran's nematode fauna. Morphometric data of the species are given in Table 3. It is characterized by having a medium sized body, smooth cuticle (under light microscopy), about 2.5–3.0 μm wide at midbody,

Table 2. List of nematodes identified in rice fields in Guilan province of Iran and some of their ecological features calculated according to Norton (1978). Root (5 g)/Soil (500 cm^3)

Nematode species/character	Absolute frequency [%]	Relative frequency [%]	Absolute density	Prominence value [%]
Aphelenchoides spp. (*Aphelenchoides besseyi, A. bicaudatus*)	0.29	0.29	1.60	5.30
Aporcelaimellus obtusicaudatus	0.95	0.95	5.30	5.35
Aquatides aquaticus	1.01	1.02	2.80	10.71
Chronogaster sp.	2.98	3.07	4.50	19.64
Criconemella paragoodeyi	0.11	0.11	2.00	1.78
Dorylaimoides elegans	0.47	0.47	8.00	1.78
Helicotylenchus crenacauda	24.22	31.00	11.94	60.71
Helicotylenchus digitiformis	8.90	9.70	11.50	23.21
Heterodera elachista	1.79	1.82	6.00	8.90
Ischiodorylaimus cognatus	1.19	1.20	3.30	10.71
Labronemella labiata	0.29	0.29	2.50	3.57
Laimydorus pseudostegnalis	1.49	1.51	3.10	14.20
Leptonchus sp.	0.23	0.23	4.00	1.78
Lindseyus costatus	1.20	1.21	2.60	14.20
Mesodorylaimus litoralis	1.55	1.57	2.60	17.85
Mononchus aquaticus	4.47	4.67	7.50	17.85
Mylonchulus spp. (*M. polonicus* & *M. sigmaturus*)	0.169	0.16	3.00	3.00
Oxydirus oxycephalus	0.40	0.47	4.00	3.57
Paractinolaimus decraemerae	0.35	0.35	3.00	5.35
Plectus spp. (*P. aquatilis* & *P. parientinus*)	3.20	3.31	11.00	8.90
Rhyssocolpus vinciguerrae	1.40	1.40	12.00	3.57
Tylenchidae (*Basiria graminophila, Neopsilen chusmagnidens, Irantylenchus clavidorus, Filenchus facultativus & F. polyhypnus*)	1.61	1.64	2.45	19.64
Tylenchorhynchus agri	31.40	45.00	11.19	83.90
Thornenema baldum	1.19	1.20	1.25	28.57
Tobrilidae (*Tobrilus, Eutobrilus*)	3.80	3.95	9.20	12.50
Tripyla sp.	4.80	5.04	8.10	17.85
Xiphinema index	0.29	0.29	5.00	1.78

Table 3. Morphometric characters for female *Mylonchulus polonicus* (Stefanski, 1915) Cobb, 1917 (measurements are in µm except "L" in mm)

Population characters	Present study	Khan *et al*. 2002	Andrássy, 2009	Mulvey, 1961
n	4	7	–	–
L	1.91±0.4(1.87–1.95)	1.8±0.1(1.6–2.0)	1.6–2.5	1.82
a	28.1±1.4(27–29.2)	36.7±2.1(33–41)	28–38	33.10
b	3.6±0.34(3.4–3.8)	3.8±0.2(3.6–4.3)	3.2–3.7	3.50
c	19±1.4(18–20)	17.01±0.5(16–18)	16–25	20.10
c′	2.1±0.2(2.0–2.3)	2.8±0.1(2.6–3.0)	2.8–4.0	–
V	62.5±2.4(61–64)	63.5±1.3(61–66)	56–66	62.50
Buccal cavity diameter	20.5±1.8(19–22)	19–23	18–22	–
Buccal cavity length	36.7±1.5(35.5–38)	32–36	33–36	–
Pharynx length	505±22.1(490–520)	453–536	480–510	–
Nerve ring from ant. end	194±7.5(188–200)	133–145	–	–
G1	7.4±0.1(7.4–7.6)	–	–	–
G2	9.6±0.1(9.5–9.7)	–	–	–
Anterior genital gonad length	255±4.8(250–260)	–	–	–
Posterior genital gonad length	197.5±2.7(195–200)	–	–	–
Tail length	94±2.1(93–96)	107.5±6.3(95–117)	80–120	–

n – number of nematodes counted; L – nematode total length; a – body length/greatest body diameter; b – body length/distance from anterior to esophagointestinal valve; c – body length/tail length; c′ – tail length/tail diameter at anus or cloaca; V – %distance of vulva from anterior; G1 – overall length of the anterior ovary from vulva × 100/body length; G2 – overall length of the posterior ovary from vulva × 100/body length

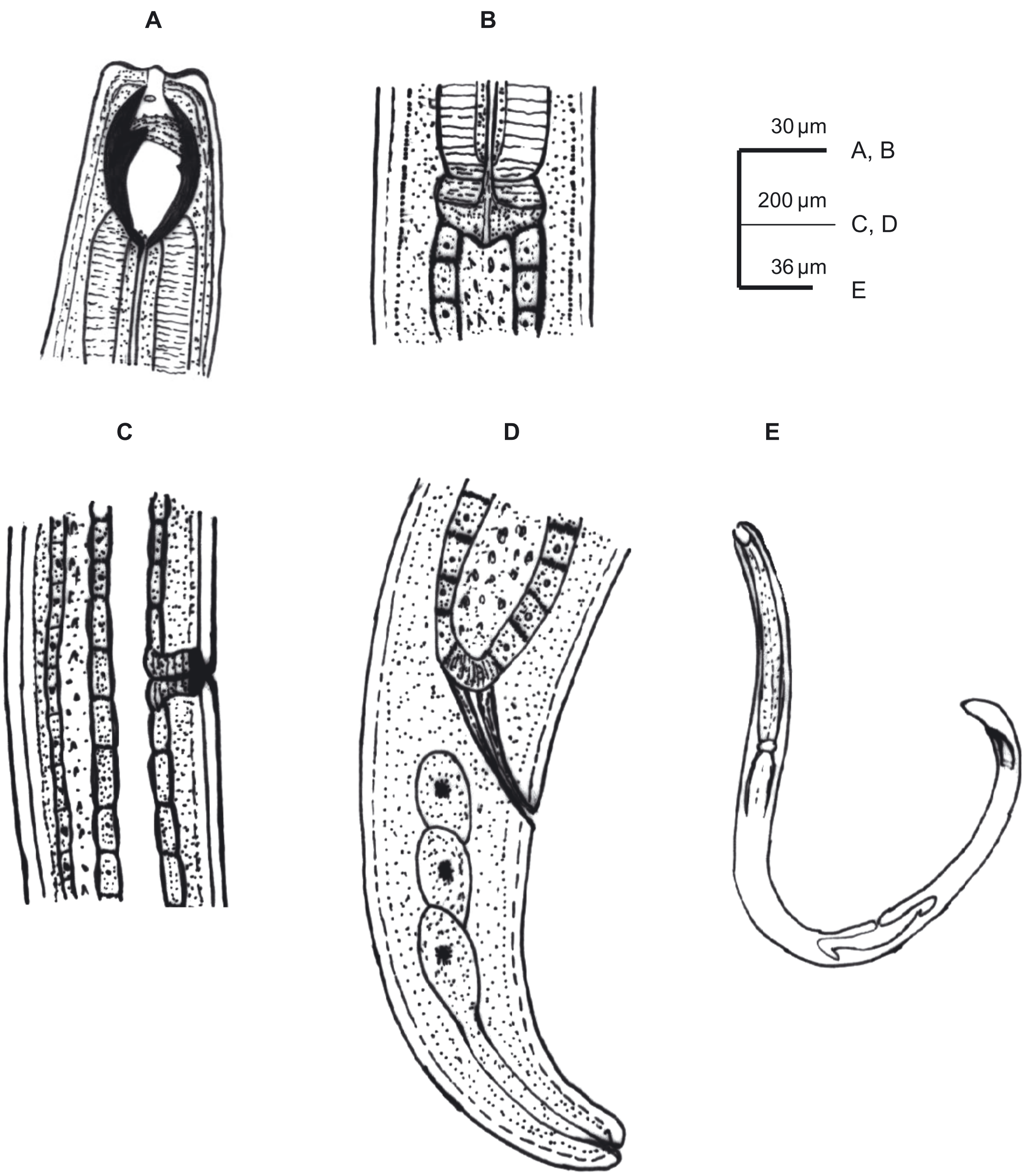

Fig. 2. Female *Mylonchulus polonicus*: A – head; B – pharyngo-intestine junction; C – vulva region; D – tail; E – whole body

C-shaped open body upon fixation, amphids cup-like with oval aperture, papillae slightly protruding, wide buccal cavity with thick walls, a small dorsal claw like tooth, directed forward and situated in the anterior half of the buccal cavity, its apex at 71–74% of length of buccal cavity length from its base, two smaller sub median teeth, the large dorsal tooth apex facing forward, sub ventral small teeth and irregular rasp-like denticles in 6–7 rows, vertical walls of prominent buccal cavity and barrel-shaped buccal capsule with a tapering base (Fig. 2). A nerve ring encircles the pharynx at the anterior end of the body, transverse vulva, pars refringens vaginae appearing as two triangular-like sclerotized pieces, advulval papillae absent, the female reproductive system didelphic-amphidelphic, small ovaries and reflexed with a single row of oocytes, except at the tip of the ovary, rectum about equal to the diameter of the anal body, sphincter present, female tail rather long, ventrally arcuate, bluntly-conoid and three caudal glands distinct in tandem (Fig. 2D). Currently, eight species of the genus *Mylonchulus* have been reported in Iran [*M. brachyuris* (Bütschli, 1873) Cobb, 1917; *M. cf. hawaiiensis* (Cassidy, 1931) Goodey, 1951; *M. kermaniensis* Shokoohi, Mehrabi-Nasab and Mirzaei, 2013; *M. lacustris* (Cobb in Cobb 1915) Andrássy, 1958; *M. minor* (Cobb, 1893) Cobb, 1916; *M. nainitalensis* Jairajpuri, 1970; *M. paitensis* Yeates, 1992; *M. sigmaturus* Cobb, 1917] (Farahmand *et al*. 2009; Ghaderi *et al*. 2012; Koohkan *et al*. 2014).

Mylonchulus polonicus closely resembles *M. lacustris* but there are some significant differences e.g. it has a larger body (vs. 1.1–1.5 mm) and longer tail (vs. c' = 1.5–2.0). Andrássy (1992) synonymized *M. polonicus* with *M. montanus* described by Mulvey (1961). Our material is in agreement with the description given by Mulvey (1961) in both the measurements and morphological characters. Also a slight difference in value (27–29.2 vs. 33.1) was observed. In comparison with the specimens studied by Andrássy (2009), the Iranian population has no remarkable

Table 4. Distribution of nematodes identified in rice fields in Guilan province, Iran (with location of sampling). Root (5 g)/Soil (500 cm^3)

Nematode species	Astara	Tavalesh	Rezvanshahr	Masal	Anzali	Somesara	Foman	Rasht	Shaft	Astane	Lahijan	Siahkal	Langrood	Roodbar	Amlash	Roodsar
Aphelenchoides (*A. besseyi* & *A. bicaudatus*)	+	+	+	0	+	+	+	+	+	0	0	+	+	0	+	0
Aporcelaimellus obtusicaudatus	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aquatides aquaticus	+	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
Chronogaster sp.	+	+	0	0	0	0	+	0	0	++	0	0	0	+	+	+
Criconemella paragoodeyi	+	0	0	0	+	0	+	0	0	+	0	+	0	0	0	0
Dorylaimoides elegans	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Helicotylenchus spp.	+++	+++	++	+	++	+++	+++	+++	++	+	++	++	+	+	+	+
Heterodera elachista	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0
Ischiodorylaimus cognatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Labronemella labiata	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0
Laimydorus pseudostegnalis	+	++	+	+	+	+	+	++	+	+	+	+	+	+	+	+
Leptonchus sp.	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lindseyus costatus	+	+	0	0	0	0	+	0	0	+	0	0	0	0	0	+
Mesodorylaimus litoralis	+	+	+	0	0	+	+	+	+	++	+	+	0	0	0	0
Mononchus aquaticus	++	++	+	+	+	++	++	++	+	+	+	+	+	+	+	+
Mylonchulus spp. (*M. polonicus* & *M. sigmaturus*)	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxydirus oxycephalus	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
Paractinolaimus decramerae	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0
Plectus spp. (*P. aquatilis* & *P. parientinus*)	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	0
Rhyssocolpus vinciguerrae	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thornenema baldum	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tobrilidae (*Tobrilus*, *Eutobrilus*)	++	+	+	+	+	++	+	0	0	+	+	0	+	0	+	0
Tripyla sp.	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	0
Tylenchorhynchus agri	+++	+++	++	+	++	+++	+++	+++	++	+	++	++	++	+	++	+
Tylenchidae (*Basiria*, *Neopsilenchus*, *Irantylenchus*, *Filenchus*)	0	+	0	0	0	+	+	+	0	0	0	0	0	0	0	0
Xiphinema index	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = not recorded; + = present in survey; ++ = common; +++ = widespread. Relative density > 30% = widespread, relative density > 10–30% = common and relative density < 10% = present in survey

differences in morphology and morphometric data. Compared with the data given by Khan and Araki (2002), our population has slightly smaller ranges for the index "a" (27–29.2 vs. 33–41) and tail length (93–96 vs. 95–117 μm). The species, only found in one soil sample (Table 4), was collected from a rice field next to the Caspian Sea in Tavalesh county (southwest of the Caspian Sea, 140 km northwest of Rasht. GPS coordinates: 37°21'54" North, 50°5'34" East).

The current study presents new data on the diversity and distribution of nematode fauna in rice paddies in Guilan province, Iran. Most of the common species of plant parasitic nematodes observed in this study were similar to those reported in a previous survey in rice fields in Guilan by Pedramfar *et al*. (2001). However, the plant parasitic nematodes mentioned below which have previously been identified in rice include: *A. besseyi*, *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941; *Basiria graminophila* Siddiq, 1959; *Criconemella paragoodeyi*, *Filenchus facultative* (Szczygiel, 1970) Brzeski, 1982; *F. polyhypnus* (Steiner & Albin, 1946) Meyl, 1961; *Helichotylenchus crenacauda*, *H. digitiformis*, *Heterodera oryzae* Luc & Berdon, 1961 and *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971. The two species *Heterodera elachista* and *T. agri* were reported for the first time as associates with rice in Guilan province. The 28S rDNA D2/D3 sequence of the Iranian population of *T. agri*, found and sequenced in the present study (morphometric data and comparison with other populations are given in Table 5, with accession number KX622690) was identical to the sequence of the same genomic fragment of the species with accession number KJ461559 sequenced by Handoo *et al*. (2014), and only one nucleotide difference was observed in the overlapping part of two sequences, which is a common intra-species variation. *T. agri* has already been reported in Iran from the rhizospheres of pomegranate, date and sour lemon (Mojtahedi *et al*. 1983; Nowruzi and Barooti 2001).

The species *H. elachista* has previously been reported from rice fields in Mazandaran province by Tanha Maafi

Table 5. Morphometric characters of *Tylenchorhynchus agri* Ferris, 1963 population (measurements are in μm except "L" in mm)

Population characters	Present study		Ferris, 1963		Nickle, 1991	
	female	male	female	male	female	male
n	12	4	10	–	10	10
L	737±100(637–810)	695±5(682–700)	700(660–770)	660(540–720)	700(660–770)	660(540–720)
a	29.54±2.5(26–32.3)	31.5±1.5(29.4–33)	30(28–33)	33(29–36)	30(28–33)	33(29–36)
b	5.4±1.5(4.8–6.8)	5.1±1.5(4.0–6.5)	5.1(4.7–5.5)	5.1(4.3–5.6)	5.1(4.7–5.5)	5.1(4.3–5.6)
c	14.3±2.2(13.6–16.1)	14.0±1.0(13.2–14.5)	18(15–21)	16(15–18)	18(15–21)	16(15–18)
c′	2.8±0.5(2.1–3.4)	3.0±0.6(2.7–3.7)	2.6	–	–	–
V/T	55.2±3.5(52.8–58)	53±2.5(49–55.3)	56(55–58)	52(48–56)	56(55–56)	52(48–56)
Stylet length	20.8±2.5(19–23)	19.0±1.2(18.6–20.0)	21(20–23)	20(19.5–21)	21(20–23)	20(19.5–21.0)
DGO	24±3(21–26)	–	23–24	–	–	–
MB	48.3±4.5(44–52.3)	46.9±3.2(46–51.6)	–	–	–	–
Excretory pore	96±4.5(92–101)	98.5±6.5(93.2–106)	–	–	92(88–98)	88–105
Oesophagus length	122±10(114–132)	119±10(110–129)	–	–	116(108–128)	111–128
Tail	48.5±5.5(42–54)	45.3±4.2(41.8–49.8)	–	–	35(32–39)	–
Head annuli	4	–	4	–	–	–
Tail annuli	23±2.5(21–25)	–	22(18–26)	–	–	–
Spicules	–	23±3.5(21–26)	–	24(22–25)	–	24(22–25)
Gubernaculum	–	11.5±1.5(10.9–12.4)	–	13.4(12.5–14.4)	–	13.4(12.5–14.4)

n – number of nematodes counted; L – nematode total length; a – body length/greatest body diameter; b – body length/distance from anterior to esophagointestinal valve; c – body length/tail length; c′ – tail length/tail diameter at anus or cloaca; V/T – distance of vulva from anterior/length of male gonad relative to body length; DGO – dorsal espohageal gland orific; MB – distance from anterior to median bulb relative to length of oesophagus

Table 6. Morphometric data for female *Xiphinema index* Thorne and Allen, 1950 (measurements are in μm except "L" in mm)

Population characters	Present study	Thorne and Allen, 1950	Heyns, 1971	Andrassy, 2009	Hunt, 1993
n	9	–	10	–	–
L	2.9±0.3(2.5–3.2)	3.4	3.25(2.9–3.6)	2.9–3.4	3.1(2.91–3.28)
a	56±3.5(52.3–60.4)	58	57(54–61)	54–66	62(58–66)
b	6.6±0.8(5.7–7.5)	7.6	6.6(6.2–8)	6.0–7.6	6.8(6.0–7.7)
c	73.4±10.8(65–84)	76	88(72–98)	75–95	84(75–93)
c′	1.1±0.1(1–1.2)	–	0.9(0.7–1.1)	0.8–1.3	1.12(1.0–1.3)
V	38.4±2.5(37–41)	38	41(40–42)	38–42	39.4(38–40)
Odontostyle	128±7.5(121–134)	–	129(123–134)	120–130	126(119–129)
Odontophore	73.5±5.5(69–78.2)	–	78(74–81)	70–80	70(63–78)
Stylet total length	195±11.5(185–209)	–	206(197–215)	–	196(190–206)
Guiding ring from anterior end	107.8±7.1(102–115)	–	–	–	–
Diam. at mid-body	50.4±4.0(46–54)	–	–	–	–
Anterior genital tract length	318.5±63(252.5–380)	–	–	–	–
Posterior genital tract length	400±45(355–460)	–	–	–	–
Tail	37.3±2.5(36–39)	–	–	–	–
Hyaline portion of tail	18.5±2.5(17–21)	–	–	–	–

n – number of nematodes counted; L – nematode total length; a – body length/greatest body diameter; b – body length/distance from anterior to esophagointestinal valve; c – body length/tail length; c′ – tail length/tail diameter at anus or cloaca; V – %distance of vulva from anterior *et al*. (2003). In this study the collected population of this species was sequenced for its 28S rDAN D2/D3 and ITS fragments (accession numbers KX622691 and KX622692, respectively). The ITS sequence of our population was identical to the corresponding sequences of the species deposited into the GenBank (accession numbers HM560778 and AF498391) (identity = 100%). A 98–99% identity was observed while comparing with several other populations of the species accessible in the database. The 28S rDNAD2/D3 sequence of the presently sequenced population of *A. besseyi* with accession number KX622689 was also identical to the same genomic fragment of several populations of the species available in the GenBank (99–100% identity was observed).

Based on morphological and morphometric characters, *Xiphinema index* Thorne and Allen, 1950 was identified for the first time in association with rice in Iran. Morphometric data and a comparison with other populations of the species are presented in Table 6.

Discussion

Xiphinema spp. are large nematodes and economically important pests of several crops. These nematodes not only directly damage their hosts by direct feeding on root cells, but also can transmit plant pathogenic viruses. Several species of *Xiphinema* Cobb, 1913 have so far been recorded from the rhizosphere of rice namely *X. cavenessi* Luc, 1973 in Côte d'Ivoire; *X. insigne* Loos, 1949 in India; *X. nigeriense* Luc, 1961; and *X. orbum* Siddiqi, 1963 in India, and *X. oryzae* Bos and Loof, 1985 in Nigeria, *X. seredouense* Luc, 1975 in Guinea but none of these species are known to be harmful (Bridge *et al*. 2005). Furthermore, *Xiphinema ifacolum* Luc, 1961 can cause significant yield loss in rice when it interacts synergistically with other root pathogens. Even alone at low population densities, it can reduce rice yield (Lamberti *et al*. 1987). Because of the presence of *X. index* in a number of the surveyed rice fields in the present study, its tentative effects on rice crop loss should be further investigated.

Helicotylenchus spp. were the most prevalent plant-parasitic nematodes in the studied area. The economic importance of these nematodes on rice crop loss has not been well studied. *Helicotylenchus* spp. are often considered as mild pathogens (Norton 1974) and can increase susceptibility to other plant pathogenic fungi in order to gain access to the host root cells.

Tylenchorhynchus spp. are common nematodes in rice fields throughout the world, and their damage is accentuated by an aggregation phenomenon known as 'swarming' (Joshi and Hollis 1976). *Tylenchorhynchus annulatus* has the widest distribution and is the main species found in irrigated rice fields. Other less commonly reported species of the genus in rice fields are *T. annulatus*; *T. brassicae* Siddiqi, 1961; *T. clarus* Allen, 1955; *T. clavicaudatus* Seinhorst, 1963; *T. claytoni* Cobb, 1913; *T. crassicaudatus* Siddiqi, Mukherjee & Dasgupta, 1982; *T. elegans* Siddiqi, 1961; *T. karnalensis* Saha, Singh, Lal & Kaushal, 2002 *T. mashoodi* Siddiqi & Basir, 1959 and *T. nudus* Allen, 1955 (Khan *et al*. 1990; Haidar *et al*. 1996; Khan and Shaukat 2000). However, none of these species have been shown to cause remarkable damages to rice in the field (Bridge *et al*. 2005). The high population density of *T. agri* recovered in this study emphasizes the need for further studies on its potential pathogenicity and crop losses on rice.

Heterodera elachista has an important role in yield loss in rice and despite low levels of infection with this species, management programs against it should be applied. *A. besseyi* causes white=tip disease in rice and it has worldwide distribution in rice fields. It causes up to 60% crop losses in various infested regions (Bridge *et al*. 2005). The nematode was first reported in Iran by Kheiri (1971) and is widely distributed in the rice growing areas in northern parts of the country including Guilan province (Talachian and Akhiani 1976; Elahinia and Mahdavian 1998; Jamali *et al*. 2006).

Based on our results, about 40.4% of collected fresh seed samples of rice were infested with *A. besseyi* and about 27.1% of fresh seed samples had more than 500 nematodes per 1,000 seeds. Due to the economic importance of rice in Guilan province and the devastating effects of this nematode species on susceptible cultivars, management methods should be considered to reduce nematode population levels in these areas.

Free-living nematodes have very important and beneficial roles in the decomposition of organic material and the recycling of nutrients in soil. They serve as important environmental indicators, and the predator forms could potentially be bio-control agents against parasitic forms. In the present survey, based on morphological and morphometric characteristics, a number of free living nematodes were identified and their distribution and population density were studied. The identified species were as follows: *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968; *Aquatides aquaticus* (Thorne, 1930) Heyns, 1968; *Chronogaster* sp.; *Dorylaimoides elegans* Thorne & Swanger, 1936; *Eutobrilus* sp.; *Ischiodorylaimus cognatus* Andrássy, 1983; *Labronemella labiate* Andrássy, 1985; *Laimydorus pseudostagnalis* (Micoletzky, 1927) Siddiqi, 1969; *Leptonchus* sp.; *Lindseyus costatus* Ferris & Ferris, 1973; *Mesodorylaimus litoralis* Loof, 1969; *Mononchus aquaticus* Coetzee, 1968; *Mylonchulus polonicus*; *Mylonchulus sigmaturus*; *Oxydirus oxycephalus* (De Man, 1885) Thorne, 1939; *Paractinolaimus decraemerae* Pedram, Niknam, Vinciguerra, Ye & Robbins, 2010; *Plectus aquatilis* Andrassy, 1985; *Plectus parientinus* Bastian, 1865; *Rhyssocolpus vinciguerrae* Pedram, Porurjam, Robbins, Ye & Pena-Santigo, 2011; *Thornenema baldum* (Thorne, 1939) Andrássy, 1959; *Tobrilus* sp. and *Tripyla* sp.

All of the abovementioned free living nematodes, except *L. costacus*, were first found in the rhizosphere of rice in Iran by Coomans and Kheiri in 1986. Earlier, they had been reported in the rhizosphere of other plants from the country (Ghaderi *et al*. 2012).

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