Oscheius rugaoensis and *Pristionchus maupasi*, two new records of entomophilic nematodes from Iran

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Summary. A survey was carried out to determine the natural pathogens of the white grub, *Polyphylla adspersa* (Coleoptera: Scarabaeidae), in the Mashhad region, North East of Iran. Here, three species of entomophilic nematodes, namely *Oscheius rugaoensis*, *Pristionchus maupasi* and *P. pacificus*, were isolated from the white grub larvae. Measurements, illustrations, and SEM photographs of the new record are provided and their phylogenetic analyses based on 18S rDNA and ITS genes were discussed. This is the first report of *Oscheius* genus and *P. maupasi* from Iran.

Key words: 18S rDNA, insect pathology, ITS rDNA, SEM.

Entomopathogenic and insect parasitic nematodes are effective biological control agents of insects and some other pests (Godfrey *et al.*, 2005). The first nematode group, which were assigned as entomopathogenic nematodes (EPN) are species of Steinernematidae and Heterorhabditidae. In addition to EPN member, several species from other families have been identified and introduced as insect parasitic nematodes. Some species from Rhabditidae family are in this second group. Andrássy (1976) described the genus *Oscheius* and its type species, *O. insectivorus*, as a subgenus of *Rhabditis* (Dujardin, 1845). Sudhaus (1976) placed *Oscheius* in the family Rhabditidae and divided them into two groups: '*Insectivora*' group with leptoderan bursa, comprising 14 species and '*Dolichura*' group, with peloderan bursa, comprising 13 species (Sudhaus, 2011). Some representatives of this genus are obviously entomophilic, *e.g.*, *O. carolinensis* Ye, Torres-Barragan & Cardoza, 2010 was described as an entomopathogenic nematode (Ye *et al.*, 2010).

Heterorhabditidoides was described by Zhang *et al*. (2008) as a new genus of EPN. Prominent morphological similarity between *Heterorhabditidoides* and *Oscheius* was reported by Ye *et al*. (2010) and Liu *et al*. (2012), and synonimisation of these two genera was proposed with *Heterorhabditidoides chongmingensis* transferred to *Oscheius*. The pathogencity of *O. chongmingensis* to *Galleria mellonella* (Linnaeus, 1758) and *Tenebrio molitor* (Linnaeus, 1758) (Liu *et al.*, 2009) was confirmed. Later, Liu *et al*. (2012) redescribed *O. chongmingensis*. The association of *O. chongmingensis* with the bacteria in the genus *Serratia* (superfamily Enterobacteriaceae) was also demonstrated (Zhang *et al*., 2008; Liu *et al.*, 2009).

In addition to EPN, some species of nematodes are considered as insect-parasitic nematodes. Some species from this group belong to Rhabditida, the nematodes which live in soil or exist in necronemic association with insects (Poinar, 1979). *Pristionchus* (Kreis, 1932) is a genus associated with the Scarabaeoidea (Herrmann *et al*., 2006) and Lucanidae (Kanzaki *et al*., 2012). This genus currently has 36 valid species (Kanzaki *et al*., 2012). Three species of this genus, *P. pacificus* Sommer, Carta, Kim & Sternberg, 1996, *P. maupasi* (Potts, 1910) and *P. entomophagus* Steiner, 1929 are associated with *Exomala orientalis* (Bauraud, 1991), *Melolontha* sp. and *Geotrupes* sp., respectively (Hong & Sommer, 2006). Previous report showed the occurrence of this genus, with a species (*P. pacificus*) from Iran (Hassani-Kakhki *et al.*, 2013). Here we report the second species of this genus from the country. This work resulted from an ongoing project on natural enemies of the white

Table 1. Morphometric data of *Oscheius rugaoensis* (Zhang, Liu, Tan, Wang, Qiao, Yedid, Dai, Qiu, Yan, Tan, Su, Lai & Gao, 2012). Measurements in μm and in the form: mean ± standard deviation (range).

Province	Razavi Khorasan	
Locality	Mashhad	
Habitat	White grub larvae (*Polyphylla adspersa*)	
n	3♀♀	3♂♂
L	1579.2±19.1 (1562-1600)	1087.9±10.5 (1082-1100)
Aa	8.6±0.3 (8.3-8.9)	16.6±1.3 (15.7-18.08)
b	8.6±0.3 (8.3-8.9)	5.3±2.0 (2.9-6.6)
c	18.4±3.6 (15.2-22.2)	29.3±0.9 (28.2-29.9)
c'	2.7±0.4 (2.2-3.07)	1.2±0.1 (1.1-1.2)
V	47.7±0.1 (47-48)	–
Lip region diameter	13.1±1.6 (11-14)	13.6±0.3 (13-14)
Stoma	22.0±2.4 (19-24)	19.7±2.3 (17-22)
Pharyngeal corpus	105.3±1.9 (104-107)	104.1±9.9 (95-115)
Isthmus	41.5±2.4 (39-44)	36.7±0.5 (36-37)
Bulb Length	37.4±5.1 (32-41)	30.8±1.3 (29-32)
Bulb diam.	32.5±5.1 (27-36)	26.8±0.6 (26-27)
Pharynx length	184.1±8.5 (176-193)	234.1±110.4 (163-361)
Neck	206.1±10.0 (197-217)	189.5±12.6 (176-200)
Nerve ring to ant. end	164.1±3.9 (160-168)	152.7±10.3 (141-161)
Excretory pore to ant. end	209.3±17.3 (197-229)	194.7±12.7 (180-205)
Deirid to ant. end	?	?
Cuticle thickness	2.3±0.3 (2-2.5)	2.1±0.3 (1.9-2)
Body diameter: Neck base	70.7±4.2 (66-73)	48.7±1.5 (47-50)
Body diameter: Mid body	108.1±13.9 (93-119)	66.0±5.4 (60-70)
Body diameter: anus	32.2±1.8 (31-34)	31.0±2.5 (28-33)
Egg length	35.6±0.6 (35-36)	–
Egg width	23.7±1.2 (23-25)	–
G1	622.7±32.1 (600-645)	–
G2	586.4±45.0 (554-618)	–
Rectum	84.1±5.2 (80-88)	–
Tail	87.9±17.1 (71-105)	37.1±1.0 (36-38)
Vulva anterior end	753.0±9.5 (745-764)	–
Phasmid	41, 42	?
Spicules	–	61.4±4.0 (58-66)
Gubernaculum	–	28.8±1.0 (28-30)

grub, *Polyphylla adspersa* (Motschulsky, 1853) (Coleoptera: Melolonthidae) in Mashhad and urban, Razavi Khorasan province of Iran. During this work, several groups of natural enemies were collected, including two species of entomophilic nematodes that are new to the fauna of Iran.

MATERIALS AND METHODS

Sample collection. A survey was carried out during 2012-2013 to determine the natural pathogens and parasites of the white grub larvae, *P. adspersa*, in the Mashhad region, North Eastern

Iran. The white larvae were collected manually and maintained in a plastic container (10 cm diam.) filled with soil from collecting sites and fed with a piece of potato. All larvae were kept at room temperature for a few months.

Isolation of nematodes. The containers were examined daily and dead larvae were transferred to the White trap (White, 1927). Nematodes emerging from the cadavers were collected and reared for future studies using *Galleria* (Bedding & Akhurst, 1975).

Morphological characterisation. Light microscopy. For morphological study, 3-4 individual adults of both sexes were selected randomly. All nematodes were fixed with hot 4% formaldehyde and transferred to anhydrous glycerin for mounting (De Grisse, 1969). Measurements were taken using an ocular micrometer and drawings were made using a drawing tube attached to the microscope. The ratio measurements were based on slide-mounted specimens.

Scanning electron microscopy. Adult nematodes were fixed in 3% glutaraldehyde, buffered for 24 h at 4-8ºC, and then postfixed with 2% osmium tetroxide solution for 12 h. The samples were washed with sterile water three times, dehydrated in a graded ethanol series (10, 20, 50, 70 and 100%) and gold coated by mini sputter coater SC7620 (Quorum Technologies, UK). The SEM images of the species have obtained with LEO 1450VP scanning electron microscope (LEO Co. Ltd., Germany).

Molecular characterisation. DNA extraction. DNA was extracted using the 5% Chelex®100 solution. Three individual females were transferred into the 1.5 ml microtube, crushed using a micropestle in 50 µl Chelex solution and 2 µl Proteinase K then incubated at 60°C for 3 h, followed by 10 min at 95°C. After centrifugation at 13,000 rpm for 3 min, the supernatant was transferred to another 1.5 ml microtube and stored at –20°C.

ITS rDNA characterisation. For ITS region amplification, the primers 18S and 26S were used (Vrain *et al*., 1992). The PCR condition was based on Hominick *et al.* (1997). All PCR products were electrophoresed on 1% agarose gels and subsequently the gels were stained using Green viewer (SYBR). The PCR products were sequenced by Macrogen Co. (Korea). The sequences chromatograms were checked using BioEdit software (Hall, 1999). Forward and reverse sequences were assembled in DNA Baser. Twenty six sequences of the ITS region were retrieved from GenBank and aligned together with the two sequences from current study using Clustal X software (ver. 2) (Larkin *et al.*, 2007) with default parameters. The MEGA 5 program (Tamura *et al*., 2011) and K2P model (Kimura, 1980) was used to calculate nucleotide distances. Phylogenetic analyses were performed using Neighbour Joining method (Saitou & Nei, 1987) with 10000 replications of bootstrap (Felsenstein, 1985) in MEGA 5.

18S rDNA characterisation. The 18S gene was amplified using PCR primers and the condition described by Blaxter *et al.* (1998). The PCR products were electrophoresed, sequenced and assembled as described above. For phylogenetic analysis of *Oscheius* isolate, 30 sequences of 18S gene from species of family Rabditidae were retrieved from GenBank (Ye *et al.*, 2010; Zhang *et al.*, 2012) and analysed together with the sequences resulted from the current study. All sequences were aligned with the Clustal X (ver. 2). The phylogenic trees were reconstructed using Bayesian Inference (BI). Bayesian analysis was implemented on the dataset with the GTR+I+G nucleotide substitution model, using MrBayes (ver. 3.1.2) (Ronquist & Huelsenbeck, 2003). Analysis was for 1 million generations. Reconstructed tree was observed with FigTree software (ver. 1.3.1) (Rambaut, 2009). Genetic distance was calculated using K2P model implemented in the MEGA5.

For phylogenetic analysis of the *Pristionchus* isolate, 17 sequences of Diplogasteridae family were retrieved from GenBank and *Koerneria* sp. was used as an outgroup (accession numbers of used sequences are shown in Fig. 7).

DESCRIPTION

Oscheius rugaoensis (Zhang, Liu, Tan, Wang, Qiao, Yedid, Dai, Qiu, Yan, Tan, Su, Lai & Gao, 2012)

(Figs 1 & 2)

Material examined. Three amphimictic females and three males in a good state of preservation.

Measurments. See Table 1.

Female. Body almost straight, slightly curved after fixation. Cuticle annulated, annuli around 1 µm wide. Lateral field not visible. Lip region slightly offset from the neck, having six round lips, bearing small papillae. Stoma rhabditoid, 19-24 µm long, with distinct cheilo, gymno and stegostom. Cheilostom finely cuticularised. Gymnostom longer than cheilostom, having well cuticularised walls. Stegostom having glottoid apparatus, without denticle. Pharyngeal collar present, covers less than half of the stoma. Pharyngeal corpus 2.0-3.0 times

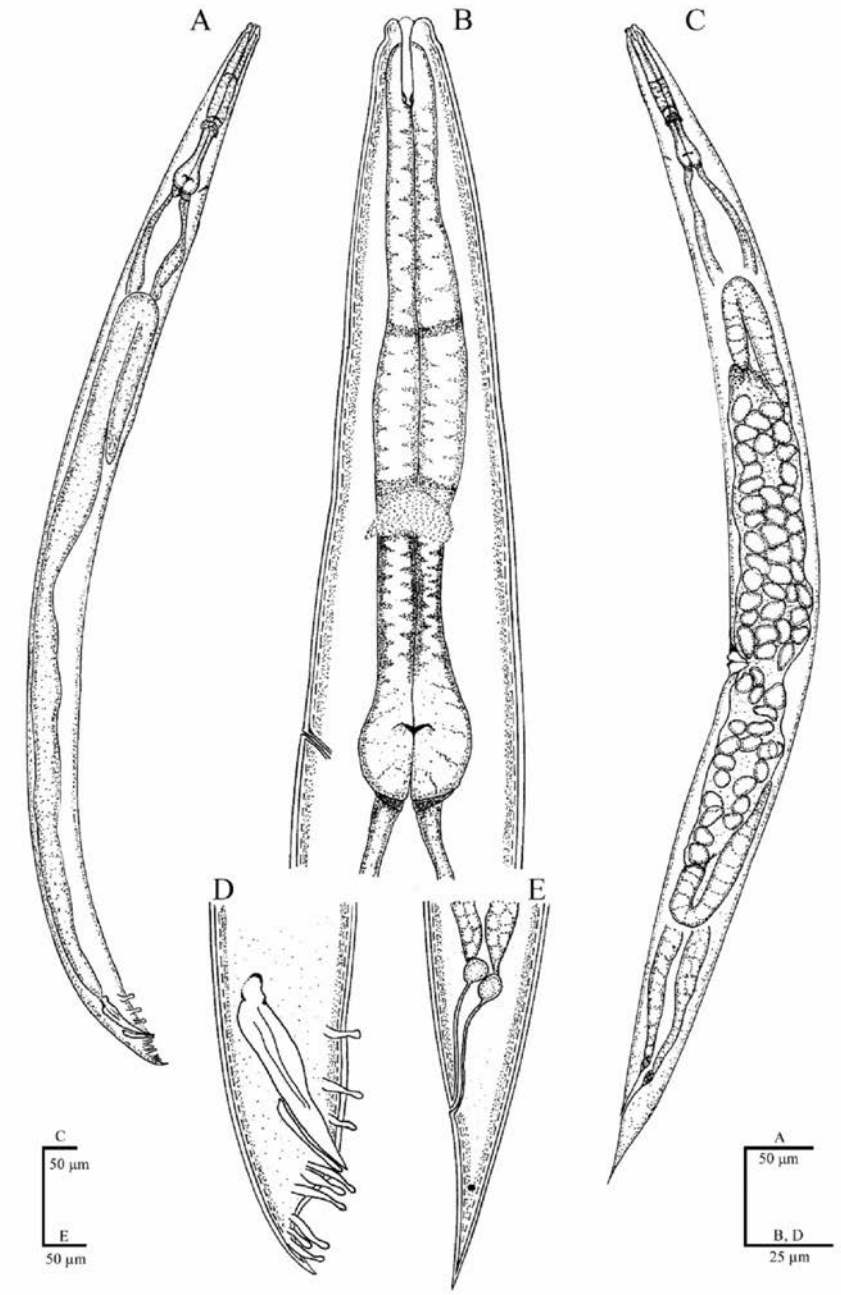

Fig. 1. *Oscheius rugaoensis* (Zhang, Liu, Tan, Wang, Qiao, Yedid, Dai, Qiu, Yan, Tan, Su, Lai & Gao, 2012). A: Entire male. B: Anterior end. C: Entire female. D: Male posterior end. E: Female posterior end.

isthmus length, with procorpus longer than metacorpus. Metacorpus distinct, swollen, 43-44 µm long. Isthmus robust and distinctly separated from metacorpus. Basal bulb spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 77-83% of neck length. Excretory pore opening at bulb level. Deirid not visible. Intestine without distinct specialisation. Reproductive system didelphic-amphidelphic. Oviduct short. Uterus tubular, filled with eggs. Vagina with fine walls, extending inward one-third of the body width. Vulva protruded, located slightly posterior to middle part of body. Rectum 2.2 times anal body diameter. Tail conical elongated, pointed distal part. Phasmid at 38-59% of tail length.

Male. General morphology similar to female. Body curved ventrally after fixation. Genital system monorchic, with testis reflexed ventrad anteriorly. Tail conical, with pointed tip and mucro. Bursa peloderan,

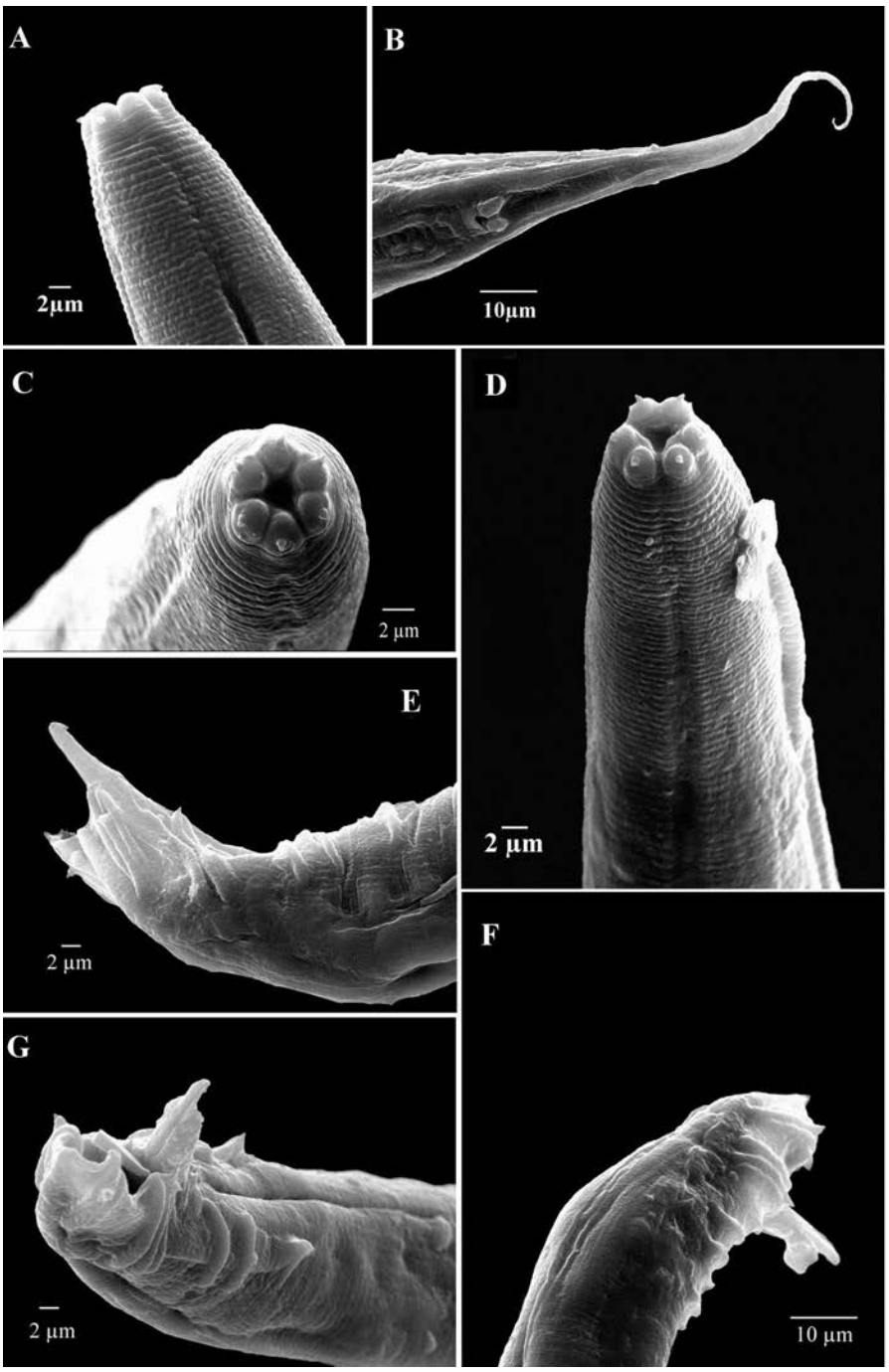

Fig. 2. *Oscheius rugaoensis* (Zhang, Liu, Tan, Wang, Qiao, Yedid, Dai, Qiu, Yan, Tan, Su, Lai & Gao, 2012) (SEM). A, D: Female anterior end (ventral view); B: Female posterior end; C: Female anterior end. (frontal view); E-G: Male posterior end.

opening anteriorly with nine pairs of papillae, three precloacal and six postcloacal, arranged in 1+1+1/3+3 pattern, the GP8th is slightly shorter. Spicules with rounded manubrium; calamus short and offset, bearing hump; and lamina curved ventrad and thinner distally with rounded tip. Gubernaculum curved ventrad.

Locality and habitat. The specimens were found in Mashhad (Razavi Khorasan province), North-Eastern Iran; date of sampling 2013), in association with the white grub larvae, *P. adspersa.*

Remarks. The material examined resembled *O. rugaoensis* (from Rugao, China) in having six rounded lips, conical elongated female tail,

excretory pore location (near bulb), vulval flap, peloderan bursa and arrangement of bursal papillae (1+1+1/3+3). However they differ in body length (1562-1600 µm in females and 1082-1100 µm in males *vs* 1639-2259 µm in females and 1195-1692 µm in males), tail length (71-105 µm in females and 36-38 µm in males *vs* 180-216 µm in females and 128-163 µm in males) and slightly longer gubernaculum (28-30 µm *vs* 10-27 µm). The Iranian population also is similar to *O. chongmingensis* (Zhang, Liu, Xu, Sun, Yang, An, Gao, Lin, Lai, He, Wu & Zhang, 2008); however, it differs in body length (1562-1600 µm in females and 1082-1100 µm in males *vs* 809-2220 µm in females and 822-1400 µm in males) and stoma (19-24 *vs* 8.9-12 µm in females). Liu *et al*. (2012) redescribed *O. chongmingensis*. Compared with the material examined, our population has shorter body length (*vs* 2363 µm in female and 909-1414 µm in males), longer stoma (*vs* 15 µm in female and 16 µm in males) and shorter female tail (*vs* 129 µm).

This genus and its species are reported for the first time from Iran.

Pristionchus maupasi (Potts, 1910) Paramonov, 1952

(Figs 3 & 4)

Material examined. Four amphimictic females and four males in a good state of preservation.

Measurments. See Table 2.

Female. Body almost straight, slightly curved ventrad after fixation. Cuticle smoothly annulated, lacking punctation. Annuli around 0.5 µm wide. Lateral field not observed. Lip region continuous with body contour, consisting of six slightly rounded lips, each with a small papilla. Stoma 0.8-1.3 times longer than wide. Cheilostom wide, walls heavily cuticularised. Cheilostom subdivided into four rod-like plates, cheilorhabdia. Bifurcated apex of cheilorhabdia extending out of labial contour. Gymnostome wide, about 5-6 µm. Second part of stoma consisting of gymnostom (isotopic) and stegostom (anisotopic) with subventral walls slightly longer than dorsal. Amphidial aperture oval shaped, located at base of dorsal tooth, about 2-3 µm. Stegostom bearing a large, claw-like dorsal tooth, 5 µm long and 2.5-3.4 µm wide; this with a duct of dorsal gland and pointed toward anterior part of stoma. Stegostom narrow and short, forming a cylindrical tube, 3.0-4.5 µm wide and 4.7-5.0 µm long. about 1.3 times longer than wide. Pharynx diplogasteroid, having pharyngeal corpus 1.5-2.0 times longer than postcorpus (isthmus + basal bulb). Pharyngeal procorpus cylindrical, 1.5-2.0 times metacorpus length. Metacorpus swollen, 29-46 µm long. Isthmus robust, 22-33 µm long. Basal bulb spheroid, 27-36 µm long. Cardia conoid, surrounded by intestinal tissue. Nerve ring at 75-79% of neck length, at isthmus level. Excretory pore at 85-89% of neck length, at isthmus level. Deirid not visible. Intestine without distinct specialisations. 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 1.9-2.1 times as long as the corresponding body diameter, containing an egg. 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, protruded. Rectum 0.9-1.2 times anal body diameter long. Tail firstly conical then filiform. Phasmid at 10-18% of tail length.

Male. Body 'J' shaped after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail firstly conical, then filiform, distally curved ventrad. Three pairs of precloacal genital papillae, five pairs of caudal genital papillae are present along the tail. The GP3 comprising three papillae closed each other. The GP5 is the shortest papillae. Spicules free, curved ventrally: manubrium almost straight, well cuticularised, calomus without hump, and lamina ventrally curved, pointed terminus. Gubernaculum well developed, 11-16 µm long or about 26-40% of the spicule length.

Locality and habitat. The specimens were found in Mashhad (Razavi Khorasan province), North-eastern Iran (N: 28º 36' 20.17"; E: 057º 43' 08.87"; date of sampling 2013), in association with white grub larvae, *P. adspersa*.

Remarks. The Iranian material is very similar to the original description of the *P. maupasi* provided by Potts (1910). However, it differs in body length (1562-1600 µm in females *vs* 1232-1760 µm in old hermaphrodite), neck length (197-217 µm *vs* 152-200 µm in hermaphrodite) and egg length (35-36 µm *vs* 56 µm). In the original illustration provided by Potts (1910), the second and third precloacal papillae are shown to be close to each other; however, in our specimens they are separated. In addition, the gubernaculum in our specimens is narrower (*vs* wider). These differences might be due to the host and geographical zone from where the population was isolated.

This species is reported for the first time from Iran.

Table 2. Morphometric data of *Pristionchus maupasi* (Potts, 1910) Paramonov, 1952. Measurements in μm and in the form: mean ± standard deviation (range).

Province	Razavi Khorasan	
Locality	Mashhad	
Habitat	White grub larvae (*Polyphylla adspersa*)	
n	4 ♀♀	4 ♂♂
L	1003.6±15.2 (991-1027)	881.8±30.6 (836-900)
a	15.2±1.8 (13.5-17.2)	17.8±0.6 (17.1-18.4)
b	6.5±0.21 (6.4-6.9)	6.2±0.1 (6.0-6.3)
c	4.9±0.1 (4.7-5.2)	6.4±0.4 (5.8-6.7)
V	44.6±1.0 (44-46)	–
Max. body diameter	63.9±7.7 (58-76)	49.3±1.2 (49-51)
Stoma length	14.7±3.0 (13-20)	11.6±1.1 (11-13)
Head diameter	16.1±1.3 (15-18)	13.9±2.0 (12-16)
Amphid width	2.2±0.4 (2-3)	2.5±0.4 (2-3)
Stegostom length	3.7±0.9 (3-5)	2.3±0.5 (2-3)
Stegostom width	3.0	2.0
Cheilostom width	5.2±1.5 (4-7)	5.0
Neck base	39.5±3.1 (36-44)	38.4±1.2 (36-39)
Corpus	95.1±2.4 (93-97)	87.8±1.9 (85-90)
Procorpus length	58.3±6.6 (51-65)	57.9±4.1 (54-63)
Metacorpus length	34.3±7.3 (29-46)	29.8±4.1 (24-34)
Isthmus length	25.9±4.8 (22-33)	29.2±1.9 (27-32)
Bulb length	29.7±4.3 (27-36)	24.3±1.9 (22-27)
Corpus/isthmus+bulb	33.2±5.0 (30-41)	27.4±2.1 (25-30)
Neck length	151.2±3.4 (149-156)	141.4±3.9 (136-146)
Excretory pore to ant. end	132.9±2.3 (131-136)	132.9±3.1 (129-136)
Nerve ring to ant. end	115.8±5.1 (112-124)	112.2±4.4 (107-117)
Tail length	202.6±4.7 (197-207)	137.8±12.2 (124-154)
Anal body diam. (ABD)	26.5±3.6 (22-29)	34.4±1.5 (33-36)
Anus to phasmid distance	28.8±7.9 (20-37)	28.2±0.9 (27-29)
Rectum length	26.8±6.1 (20-34)	–
Tail/rectum	7.1±1.4 (6-10)	–
Rectum /ABD	1.0±0.1 (0.9-1)	–
Vagina	18.8±2.2 (17-21)	–
Posterior gonad	309.2±6.7 (302-317)	–
Anterior gonad	315.1±16.7 (300-334)	–
Egg length	53.7±1.5 (52-55)	–
Egg width	33.7±0.6 (33-34)	–
Vulva anterior end	448.3±15.6 (436-467)	–
Vulva body diam.	63.9±7.7 (58-76)	–
Vulva to anus distance	321.8±4.9 (318-327)	–
Spicules	–	39.3±1.6 (37-41)
Spicule/ABD	–	1.1±0.0 (1-1.2)

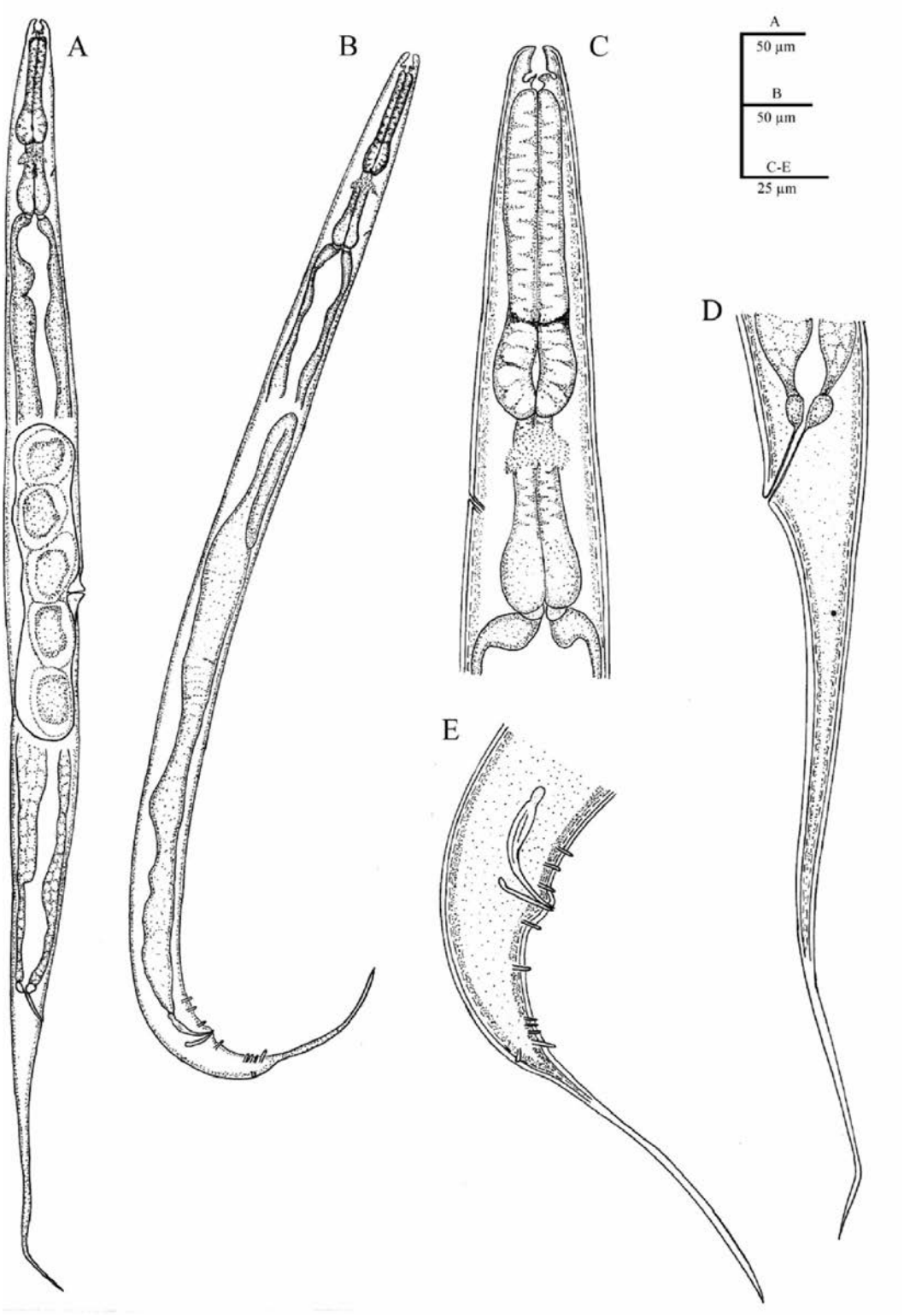

Fig. 3. *Pristionchus maupasi* (Potts, 1910) Paramonov, 1952. A: Entire female. B: Entire male. C: Anterior end. D: Female posterior end. E: Male posterior end.

Molecular characterisation. The ITS regions including ITS1, 5.8S and ITS2 and 18S rDNA were used for identification and phylogenetic analysis of isolated species. All obtained DNA sequences were submitted in GenBank with accession numbers KF500233 to KF500240.

Molecular analysis of *O. rugaoensis*. The length of 18S rDNA for FUMN101 and FUMN102 isolates were 760 bp. NBLAST analysis based on 18S gene for the Iranian population attributed 100 and 99% similarities with *O. rugaoensis* (JQ002566) and *O. chongmingensis* (EF503692), respectively.

The multiple alignment of a 704 bp segment of 18S gene for 31 taxa indicated that 378 sites were conserved, 321 sites were variable and 272 sites were parsimony informative. The phylogenetic trees reconstructed based on 18S sequence, using the Bayesian analysis, showed *O. rugaoensis* forms a monophyletic group with *O. chongmingensis*; *Rhabditis* sp., and the '*Insectivora*' group of *Oscheius* were placed in a single clade (Fig. 5).

Mean interspecific distance of 18S sequences was 0.158% (range 0.00-0.329%), which was calculated by the K2P model. There was no intraspecific difference between the Iranian isolates and *O. rugaoensis* (JQ002566) but there was a 0.002% difference with *O. chongmingensis* (EF503692).

NBLAST analysis based on ITS gene for FUMN101 and FUMN102 gave 99% and 98% similarities with *O. chongmingensis* (EF503690) and *O. rugaoensis* (JQ002565), respectively. The length of ITS rDNA region of *O. rugaoensis* was 814 bp (ITS1 = 421 bp, 5.8S rDNA = 154 bp, and ITS2 = 239 bp). There were six nucleotide insertions in ITS1 and ITS2 genes.

The multiple alignment of an 802 bp segment of ITS regions for 32 taxa indicated that 47 sites were conserved, 698 sites were variable and 557 sites were parsimony informative. The obtained results of ITS regions were similar to those that resulted from the 18S gene. In the phylogenetic analysis based on the ITS gene, FUMN101 and FUMN102 isolates of *O. rugaoensis* (JQ002565), *O. chongmingensis* (EF503690) and *O. carolinensis* (FJ547240) ('*Insectivora*' group) were placed in the first clade. ITS sequence analysis showed similarity of FUMN101 and FUMN102 with *Oscheius* with 100% bootstrap support (Fig. 6).

The mean interspecific distance of ITS sequences was 0.559% (range 0.00-1.70%) which was calculated by the K2P model. The nucleotide distance between the Iranian population of *O. rugaoensis* and *O. chongmingensis* and *O. rugaoensis* were 0.011 and 0.018%, respectively.

Molecular analysis of *P. maupasi*. NBLAST analysis based on 18S gene sequence for FUMN103 and FUMN104 attributed 100% similarity with *P. maupasi* (FJ040443) and *P. pacificus* (AF083010), respectively. The multiple alignment of an 822 bp segment of ITS regions for 32 taxa indicated that 617 sites were conserved, 186 sites were variable and 55 sites were parsimony informative.

In the current study, two species of *Pristionchus* were identified. Also, in the phylogenetic tree the FUMN103 isolate of *P. maupasi* with *P. maupasi* (FJ040444) were placed in a subclade with 100% bootstrap support (Fig. 7).

The mean interspecific distance of 18S sequences was 0.050% (range 0.00-0.227%), which was calculated by the K2P model. There were no nucleotide differences between the FUMN103 isolate and *P. maupasi* (FJ040443), and the FUMN104 isolate and *P. pacificus* (AF083010).

There were only two records in GenBank for sequences of ITS regions from *Pristinochus* genus. Thus, we could not perform any analysis.

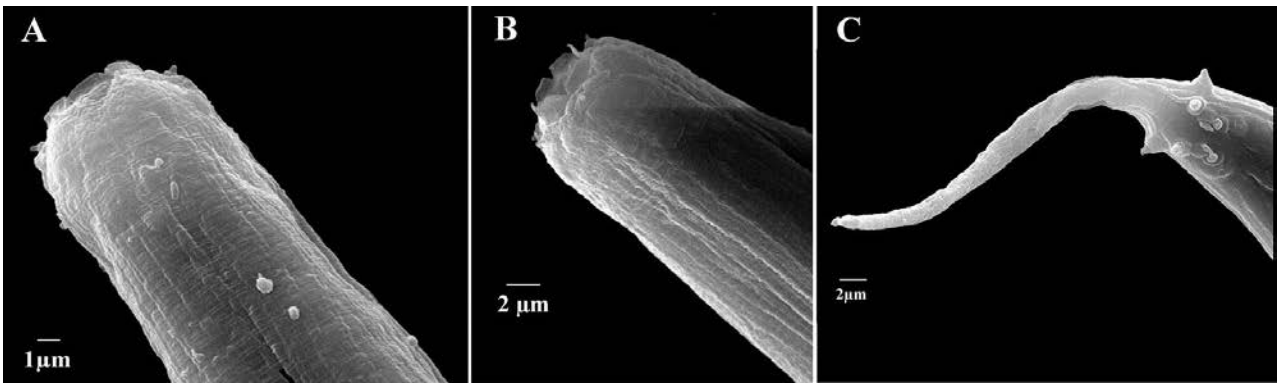

Fig. 4. *Pristionchus maupasi* (Potts, 1910) Paramonov, 1952 (SEM of male). A, B: Anterior end (Ventral and lateral view, respectively); C: Posterior end.

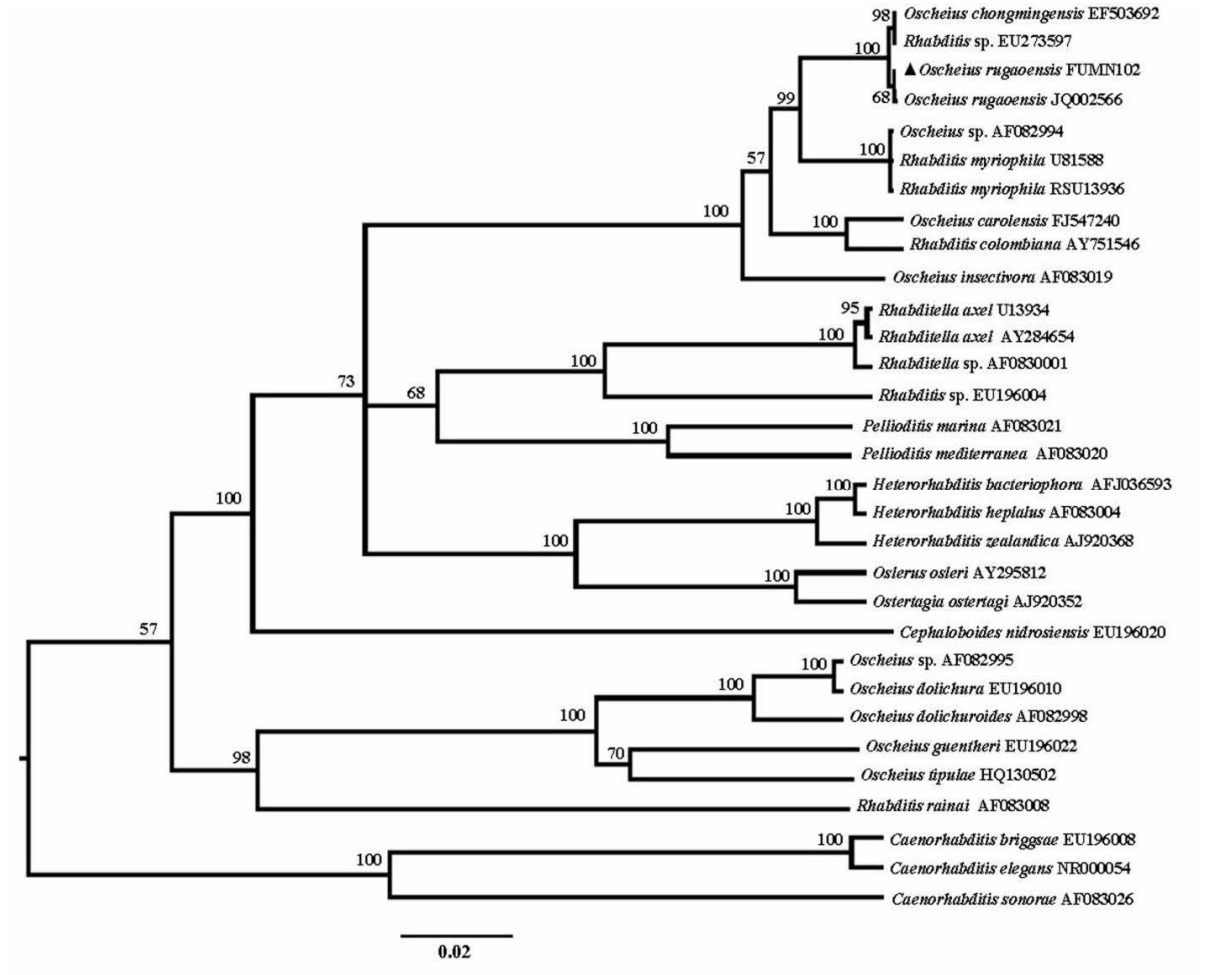

Fig. 5. Phylogenetic relationships of *Oscheius rugaoensis* and other closely related species reconstructed by Bayesian analysis based on 18S rDNA sequences data.

DISCUSSION

The current work provided the first data about insect-parasitic nematodes associated with *Polyphylla* genus. Previous research showed the natural occurrence of some insect pathogenic nematodes in the white grub larvae, *e.g.*, *Steinernema glaseri* Steiner, 1929, *S. carpocapsae* Weiser, 1955 and *Heterorhabditis bacteriophora* Poinar, 1976 (Karimi 2007; Karimi *et al*., 2009a, b) and the entomophilic nematode *P. pacificus* (Hasani-Kakhki *et al.*, 2013). In this study, three entomophilic nematodes, *O. rugaoensis*, *P. maupasi* and *P. pacificus*, were isolated from the larvae of *P. adspersa*. This report includes a new genus (*Oscheius*) and two new species (*O. rugaoensis* and *P. maupasi*) for Iranian fauna of the entomophilic nematodes.

Different insect hosts were reported for the species belong to *Oscheius* and *Pristionchus*. Previously, species in the *Insectivora* group of *Oscheius* were considered as parasites of insect and other arthropods. For example, *O. colombianus* Stock, Caicedo & Calatayud, 2005 was found in the subterranean burrower bug, *Cyrtomenus bergi* Froeschner, 1960, *O. insectivora* (Körner, 1954) in lucanid beetles (Körner, 1954), *O. myriophila* Poinar, 1986 in the millipede, *Oxidus gracilis* (Poinar, 1986), and *O. necromenus* Sudhaus & Schulte, 1989 in diplopods (Liu *et al.*, 2011). Our unpublished data about virulence potential of *O. rugaoensis*, implied the high entomopathogenic potential of this species. Recent research on *Oscheius* confirmed the entomopathogenic potential of *Oscheius* species, such as *O. chongmingensis*, *O. carolinensis* and *O. rugaoensis* (Zhang *et al*., 2008; Ye *et al*., 2010; Zhang *et al*., 2012). The high virulence

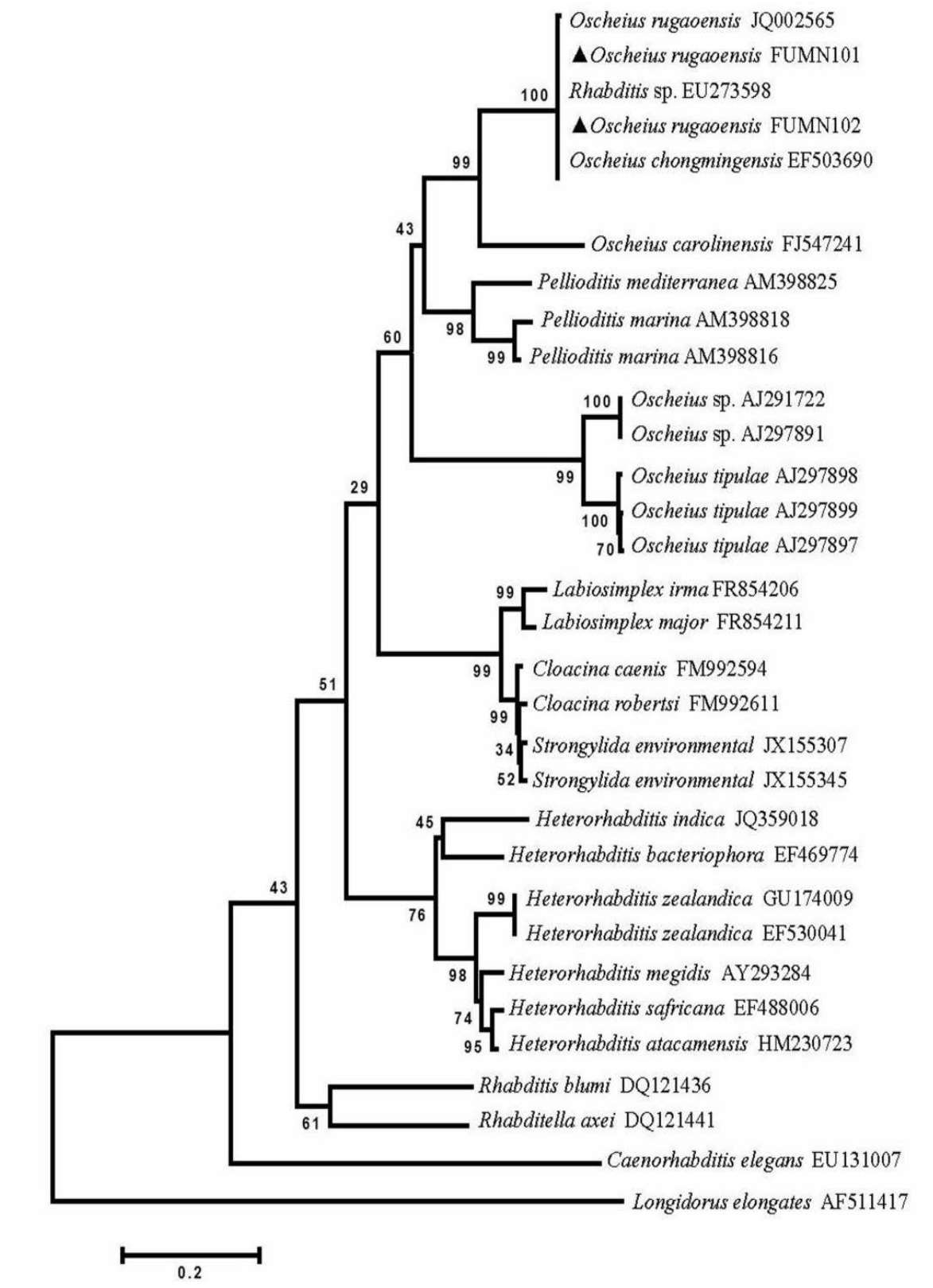

Fig. 6. Phylogenetic relationships of *Oscheius rugaoensis* and other closely related species reconstructed by neighbor joining based on ITS rDNA sequences data.

Fig. 7. Phylogenetic relationships of *Pristionchus* genus reconstructed by Bayesian analysis based on 18S rDNA sequences data. *Koerneria* (JX163979) was included as an outgroup.

of *Oscheius* may be attributed to associated bacteria. Different bacteria are reported to have association with Rhabditida. Among those, *Serratia* sp. was isolated from *O. rugaoensis* and *S. nematodiphida* was isolated from *O. chongmingensis* (Zhang *et al*., 2008; 2012). To our knowledge, there is no data about the potential of associated bacterium with *O. rugaoensis* as well the role of this bacterium in pathogenicity and the nematode life cycle. Future research could provide new insight into characterisation of those bacteria and their possible role in the biology of the nematode.

Heterorhabditidoides genus was described by some morphological characters such as six separate lips with minute sensilla, pore-like amphidial apertures and median female vulva. Zhang *et al*. (2008) compared their data only with EPN species and assigned a new genus without comparing their samples with *Oscheius* species. Subsequent research on this genus showed their similarity with '*Insectivora*' group of *Oscheius* (Zhang *et al*., 2012). These finding were confirmed using DNA sequences and phylogenetic analysis of 18S and 28S rDNA genes and demonstrated that *O. rugaoensis* belongs to the '*Insectivora*' group (Zhang *et al*., 2012). *Oscheius rugaoensis* has high similarity with *O. chongmingensis* but can be distinguished from it based on some morphological characteristics (*e.g*., body length, male ridge pattern of the lateral field, stoma size and vulval flap in the female) as well as molecular data (Zhang *et al*., 2012). The 18S rDNA and ITS sequences of both *O. chongmingensis* and *O. rugaoensis* differ in 2 and 12 nucleotides, respectively. The analyses of DNA sequences between ITS and 18S genes sequences of *O. chongmingensis* and *O. rugaoensis* showed 98% and 99% similarity, respectively.

Another critical criteria for species separation between *O. chongmingensis* and *O. rugaoensis* is a cross-breeding test. Zhang *et al*. (2012) showed reproductive isolation between those species, when their cross hybridisation test failed. This status was conflicted when Zhang *et al*. (2012) used the nucleotide distance to distinguish *Heterorhabditidoides* from *Oscheius*. They stated the nucleotide distance between 18S and ITS genes of *Heterorhabditidoides vs* the '*Insectivora*' group of *Oscheius* were less than those value between the '*Insectivora*' group and the '*Dolichura*' group. Thus they suggested changing the genus of '*Insectivora*' group (*O. carolinensis*, *O. colombianus*, *O. myriophila* and *O. insectivorus*) to *Heterorhabditidoides* and the '*Dolichura*' group was introduced as *Oscheius* (Zhang *et al*., 2012).

We followed the hypothesis of Ye *et al.* (2010) and Liu *et al*. (2012), who interpreted the results of phylogenetic analysis on DNA sequences of 18S rDNA and D2D3 region of 28S rDNA genes and suggested *Heterorhabditidoides* as junior synonym of *Oscheius*, so they transferred *H. chongmingensis*

to *O. chongmingensis*. We consider that the comments by Ye *et al.* (2010) about the species status of *Oscheius* looks more realistic, but still more evidence is needed to explain why phylogenetic analyses categorise different species of *Oscheius* genus into different clades. Briefly, the major concern about molecular analysis on *Oscheius* species is their paraphyletic status. This genus comprises different species in various groups. Phylogenetic analysis on the basis of 18S gene indicated *Oscheius* (*Heterorhabditidoides*) and the '*Insectivora*' group are sister groups and monophyletic, whereas the '*Dolichura*' group and the '*Insectivora*' group was paraphyletic (Zhang *et al*., 2012). When we used the dataset used by Ye *et al*. (2010) together with our sequences to reconstruct a phylogenetic tree, the paraphyletic status of *Oscheius* was confirmed and our new isolate grouped in a clade near the only another isolate of *Oscheius*. While Zhang *et al.* (2008) used ITS sequences of *Oscheius* (*Heterorhabditidoides*) to infer their phylogeny, we declined to show this result as a phylogenetic tree. We agree with the comment of Ye *et al*. (2010) that this issue could be due to high divergent of ITS gene. It is not easy to prepare a robust homologous multiple alignment for this reason. The reconstructed phylogenetic tree using ITS sequences revealed that two populations of *O. chongmingensis* (China) and two populations of *O. rugaoensis* (China and Iran) were placed in one clade. But in the phylogenetic tree based on the 18S rDNA sequences, *O. rugaoensis* and *O. chongmingensis* were placed in different subclades with 68 and 98% value of bootstrap support, respectively. Paraphyletic relationships among *Oscheius* species is a major problem that requires a comprehensive phylogenetic study on all available species of this genus from different geographical origins. Further work is needed to be done to resolve the question of monophyletic or paraphyletic relationships between species of *Oscheius*.

Pristionchus maupasi is the second species of this genus belonging to the family Neodiplogasteridae found in Iran. Our finding about association of *Pristionchus* with the white grub species in the Scarabaeidae family is in agreement with previous research. Several field studies indicated that *Pristionchus* species have close associations with scarab beetles (Herrmann *et al*., 2006). Necromenic behaviour of *Pristionchus* was a clear trait in our observations. *Pristionchus maupasi* was described by Potts (1910) and isolated primarily from *Melolontha* in the Europe. Here, we introduced the *P. adspersa* as a novel host for *Pristionchus* genus as well *P. maupasi*. This species has been isolated from different life stages of the scarabs including larvae, pupae and adults (Hong *et al*., 2008), while our sample emerged from the larvae. The present study provides new information using combined data of morphology, morphometric and DNA sequences analysis to characterise *P. maupasi*.

Pristionchus maupasi is a cryptic species and is close to *P. aerivorus* and *P. pseudaerivorus*. Mayer *et al*. (2007) surveyed the phylogeny of *Pristionchus* based on SSU gene (27 ribosomal protein genes) and noted that SSU is a useful dataset for species identification within the genus. However, this gene is not a suitable marker for differentiation between cryptic species (*e.g.*, *P. maupasi* and *P. aerivorus*) (Mayer *et al*., 2007). In our study, analysis of the 18S rDNA gene sequence distinguished *P. maupasi* from *P. aerivorus* and *P. pseudaerivorus* with a single nucleotide difference. Also, phylogenetic analysis based on 18S rDNA gene indicated that *P. pacificus* and *P. maupasi* are paraphyletic. This conflict needs to be investigated by molecular analysis using more species from a wide geographical and ecological origin. In addition, the rich and diverse ecosystems of Iran need to be surveyed by extensive sampling.

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R. Darsouei, J. Karimi and E. Shokoohi. *Oscheius rugaoensis* и *Pristionchus maupasi* – два новых для Ирана вида энтомофильных нематод.

Резюме. В рамках исследовательского проекта, направленного на изучение природных врагов белых хрущей *Polyphylla adspersa* (Coleoptera: Scarabaeidae), в окрестностях Мешхеда на северо-востоке Ирана у личиночных стадий жуков были обнаружены три вида энтомофильных нематод: *Oscheius rugaoensis*, *Pristionchus maupasi* и *P. pacificus*. Для первых двух видов приводятся измерения, рисунки и фотографии, полученные в СЭМ, а также результаты филогенетического анализа по последовательностям 18S и ITS рДНК. Это первое сообщение о находке *P. maupasi* и вида рода *Oscheius* в Иране.