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Molecular and morphological characterization of *Pristionchus pacificus* (Nematoda: Rhabditida: Neodiplogastridae), a new record of an entomophilic nematode from Iran

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Abstract: During a survey of entomopathogenic nematodes from the Mashhad region, in northeastern Iran, an entomophilic nematode was isolated using the *Galleria* trap method. Morphological characterization showed that this isolate belongs to the family Neodiplogastridae. Detailed morphological and morphometric characterization including SEM data fit with *Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996, representing the first record of this genus and species from Iran. Iranian specimens of *P. pacificus* FUM 5 are characterized by the body length (0.69–0.83 mm), the stoma (6–10 μm) bearing a dorsal tooth and a conical elongated tail (146–220 μm). Molecular analyses using 18S and ITS rDNA genes further support this identification. Measurements, illustrations, and SEM photographs of the Iranian isolate are provided and the phylogenetic position of the species is discussed.

Key words: *Pristionchus pacificus;* SEM; ITS; 18S rDNA; Iran

Introduction

Nematodes are the most abundant metazoan in marine and terrestrial habitats and in the latter case they represent a significant component of the soil community (Caldwell 1981; Sochova et al. 2006). Ecologically, they are particularly significant in soil ecosystems where their activity can affect energy flows and nutrient cycling (Sochova et al. 2006). Due to their importance in agriculture and crop protection, a survey for isolation of native entomopathogenic and entomophilic nematodes was conducted in the Mashhad region, northeastern Iran. During this study*Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996, a Neodiplogastrid nematode, previously characterized as free-living, was recovered.

Pristionchus belongs to the family Neodiplogastridae. According to Sudhaus & Fürst von Lieven (2003) there are 28 valid genera plus other more recent described genera. Also, Andrássy (2005) reported more than 40 genera plus these new genera for this group. While most Neodiplogastrids are gonochoristic; two genera, *Diplogasteroides* de Man, 1912 and *Pristionchus* Kreis, 1932 produce self-fertilizing hermaphrodites (Mayer et al. 2007). *Pristionchus* species have an interesting necromenic association with scarab beetles. In this relationship, infective juveniles after invading a suitable host, do not feed until after host death at which time they then feed on bacteria, fungi, and other nematodes growing on the insect carcass (Rae et al. 2008; Weller et al. 2010; Morgan et al. 2011). *Pristionchus* has several species with a necromenic association including *P. pacificus* that is found on more than ten beetle species including the Oriental beetle *Exomala orientalis* (Waterhouse, 1875). The majority of *Pristionchus* species associated with beetles show high specificity to a particular beetle species. For example, two common European species, *Pristionchus maupasi* (Potts, 1910) Paramonov, 1952 and *P. entomophagus* Steiner, 1929 are found on cockchafers (*Melolontha* F., 1775) and dung beetle (*Geotrupes* Latreille, 1796), respectively (Mayer et al. 2007; Hong et al. 2008a; Rae et al. 2008; Morgan et al. 2011).

Pristionchus pacificus was firstly described by Sommer et al. (1996) and represents the most important species in the genus *Pristionchus*. This species is a satellite model nematode which used in evolutionary and developmental studies like another model species, *Caeonorhabditis elegans* (Maupas, 1900) (Rudel et al. 2005; Hong & Sommer 2006; Herrmann et al. 2007; Zauner et al. 2007; Click et al. 2009). *P. pacificus* has worldwide distribution and by now, more than 150 isolates have been recovered from soil and scarab beetles in different continents (Mayer et al. 2007; Rae et al. 2008; Kanzaki et al. 2011).

Species identification within the genus *Prision-*

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Table 1. List of primers used in this study.

Primer Name	Sequence (5′-3′)	Target Gene	Reference
G18S4	GCT TGT CTC AAA GAT TAA GCC	18S	Blaxter et al. (1998)
26R	CAT TCT TGG CAA ATG CTT TCG	18S	Blaxter et al. (1998)
18S	TTG ATT ACG TCC CTG CCC TTT	ITS	Vrain et al. (1992)
26S	TTG ATT ACG TCC CTG CCC TTT	ITS	Vrain et al. (1992)
D2F	CCT TAG TAA CGG CGA GTG AAA	28S	Stock et al. (2001)
536	CAG CTA TCC TGA GGA AAC	28S	Stock et al. (2001)

Table 2. PCR conditions for amplification of the three genes used in the present study.

Name of primer set	Cycle number	Thermal conditions
G18S4 and 26R	1	94 °C for 3 min
	35	90 °C for 60 s, 56 °C for 60 s and 72 °C for 90 s
	1	72 °C for 7 min
18S and 26S	1	94 °C for 3 min
	35	90 °C for 60 s, 55 °C for 60 s and 72 °C for 90 s
	1	72 °C for 7 min
D2F and 536	1	94 °C for 3 min
	33	94 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min
	1	72 °C for 7 min

chus, is difficult due to morphological similarity among some species (Herrmann et al. 2006; Mayer et al. 2007); DNA sequence analysis is now widely accepted in nematode identification and in assessing phylogenetic relationships (Dorris et al. 1999; Floyd et al. 2002; Blaxter 2003; Kumari & Lišková 2009; Stock 2009). Such markers including 18S ribosomal RNA (SSU) and ITS rRNA as well D2-D3 expansions of 28S rRNA (LSU) genes have been demonstrated to be helpful for species delimitation and molecular phylogenetic analysis of several groups of nematodes (Floyd et al. 2002; De Ley et al. 2005; Subbotin et al. 2007). Herein we address: 1) morphologic and morphometric characterization of the Iranian isolate (designated FUM 5) of *P. pacificus* and comparison with previous descriptions, 2) molecular characterization of the Iranian isolate using partial 18S, complete ITS rRNA and D2-D3 expansions of 28S rRNA 3) an evaluation and discussion of its phylogenetic position.

Material and methods

Sample collection and preparation

Specimens of *P. pacificus* were obtained from soil samples using the *Galleria mellonella* (L., 1758) baiting technique (Bedding & Akhurst 1975). The collection site was a peach orchard in Masshad, Razavi Khorasan province, in northeastern Iran (36°4′ N, 59°7′ E). The infected cadavers were collected, and individually placed on a White trap to collect emerging nematode (White 1927). Harvested nematodes were stored in distilled water at 8 °C for further study.

Morphological characterization: Light and scanning electron microscopy (SEM).

Specimens were killed and fixed using hot (80 °C) 4% formaldehyde solution, and processed to anhydrous glycerin for mounting according to De Grisse (1969). Measurements were taken 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 light microscope CH-2. For SEM fixed specimens were hydrated (one day), dehydrated in a graded ethanol series (25, 30, 50, 70, 95, 100%) and finally in acetone (100%), critical point dried, coated with gold with CamScan MV2300 microscope operating at 15 mA (Shokoohi & Abolafia 2011). The terminology used to describe the morphology of the stoma follows the proposals by De Ley et al. (1995).

Molecular study

DNA extraction. Genomic DNA was extracted from an individual adult nematode by grinding in 50 µl 5% Chelex-100 (Sigma-Aldrich Chemie GmbH, Germany) and 2 µl proteinase K. The mixture was incubated at 60 °C for 2 h, followed by incubation for 10 min at 95 °C to denature the proteinase K. The extracted DNA was stored at –20 °C.

PCR conditions. A multilocus approach, including sequencing of ITS, 18S and 28S loci, was considered for molecular characterization of the new isolate of entomophilic nematode. The primers used to amplify the genes mentioned above are presented in the Table 1. The PCR mixture was carried out in a reaction volume of 25 µl, containing 2.5 µl of 10× PCR-buffer, 15.7 µl of H_2O, 1 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTPs (10 mM), 0.3 µl *Taq* polymerase (5 units/µl), 1 µl of forward primer (10 pmol/µl), 1 µl of reverse primer (10 pmol/µl), and 3 µl of template DNA. All PCR reactions were conducted in a T-Personal thermocycler (Biometra). The optimized PCR conditions are shown in the Table 2.

Sequencing. Sequencing was performed in 3730XL DNA analyzer in Macrogen Inc. (Seoul, South Korea) (http://dna.macrogen.com). All sequences were confirmed and repeated. The consensus sequence for each gene was submitted to Genbank database for further comparison using nBLAST search (http://blast.ncbi.nlm.nih.gov/Blast/).

Multiple alignments and phylogenetic analysis

For phylogenetic analysis on the basis of the 18S gene, 17 valid and verified sequences were retrieved from GenBank. The sequences were aligned using the ClustalW algorithm

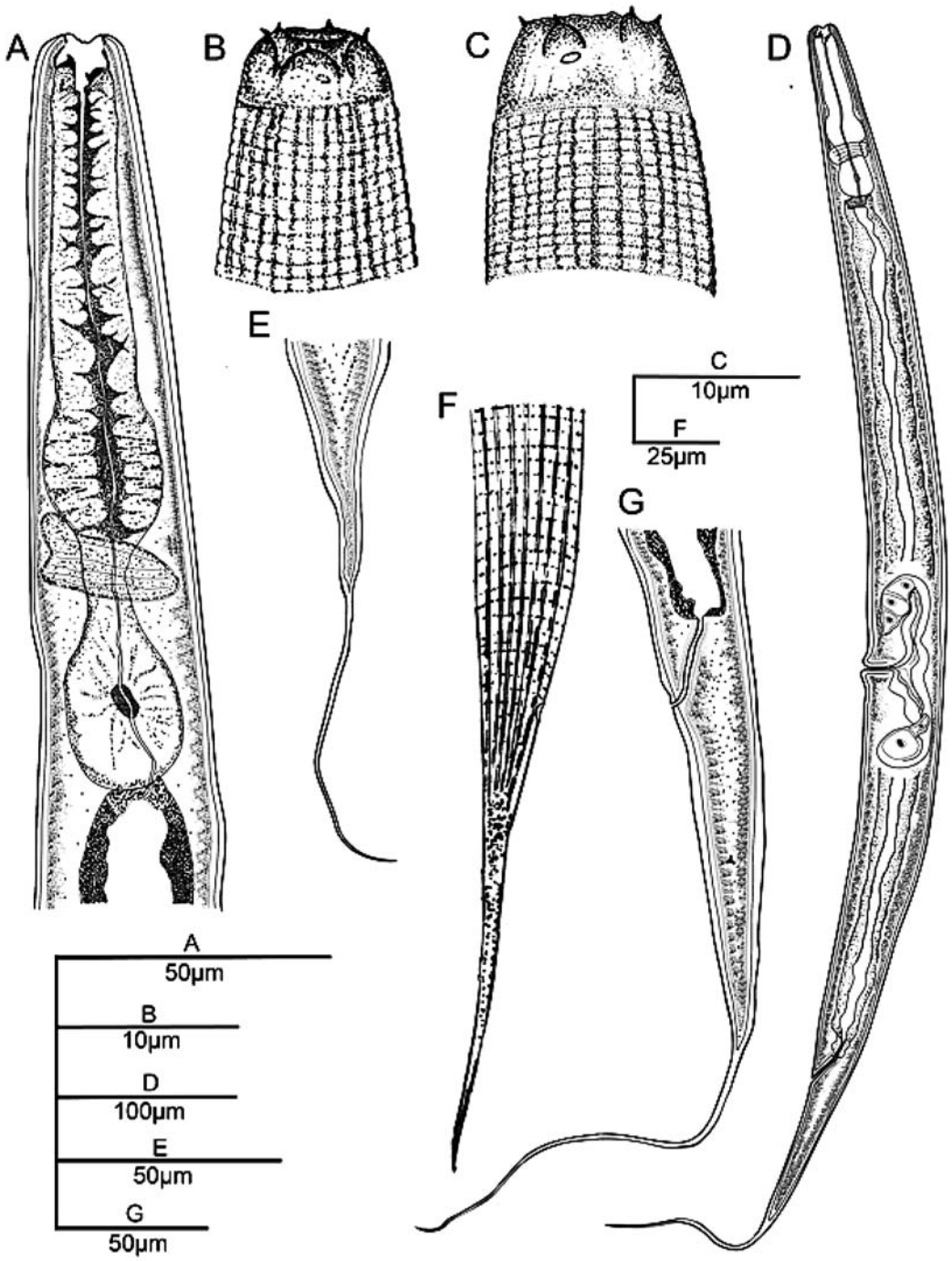

Fig. 1. *Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996. A: Anterior end; B, C: Lip region (superficial view); D: Entire female; E–G: Female tail.

(Thompson et al. 1994) and adjusted by eye. The phylogenetic analyses were performed on the Phylogeny.fr platform using maximum likelihood (ML) method (Tamura & Nei 1993) with the GTR substitution model (Dereeper et al. 2008). For calculation of Neighbor-Joining (NJ) tree (Saitou & Nei 1987) under the Kimura 2 parameter substitution model (Kimura, 1980) MEGA 5 software (Tamura et al. 2011) was used (Fig. 4). An 18S sequence of the closely related genus *Koerneria* (*K. lucani*; AB597233) was used as an outgroup for both analyses (Herrmann et al. 2006; Mayer et al. 2007). The NJ tree was bootstrapped 10,000 replicates (Felsenstein 1985).

***Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996** (Figs 1, 2)

Population from Mashhad, province of Razavi Khorasan, Iran (11 ♀♀) (measurements, see Table 3).

Female. Body 0.69–0.83 mm long. Habitus mostly straight after fixation. Cuticle "single". Without punctuation, 2 µm wide at midbody, bearing longitudinal lines which each one comprising two closely lines (visible under LM). Annuli 1.5 µm wide, with conspicuous longitudinal ridges (*ca.* 12 at head level). Lat-

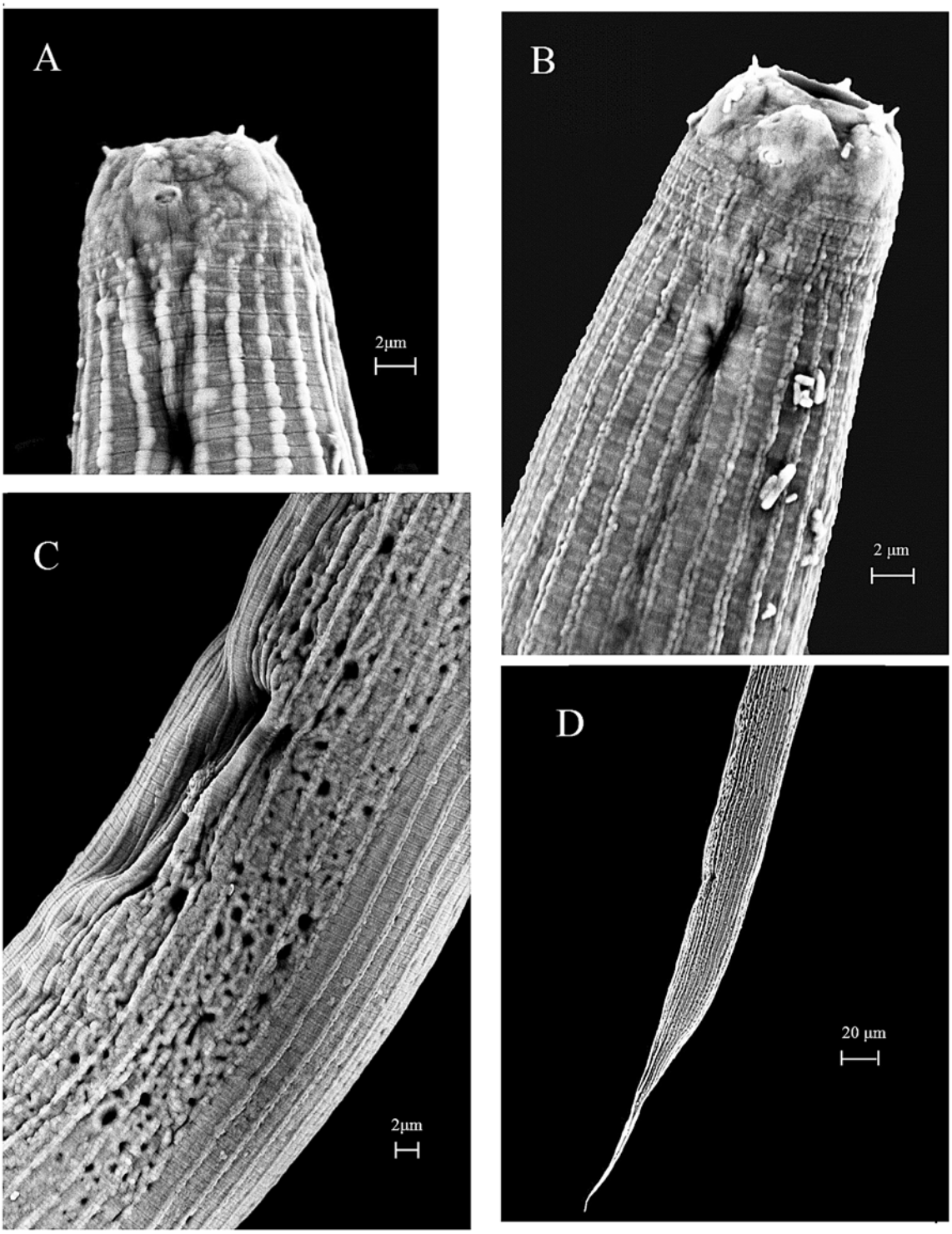

Fig. 2. *Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996 (SEM): Lip region of eurostomatous female: A (lateral view); B (ventrolateral view). C: Cuticle. D: Female tail.

eral field not observed. Lip region continuous with the neck, bearing six separated lips and four cephalic papillae. Stoma as long as wide. Cheilostom wide, with well-developed and cuticularised rhabdia. Gymnostom slightly wider than cheilostom and stegostom. Gymnostome and stegostome anisotopic with subventral walls slightly longer than dorsal. Pro-mesostegostome 9 µm wide. Stegostome bearing claw like tooth, 2–3 × 2 µm. Posterior part of stegostom including meta and telostegostom, telostegostome short, 2 µm diam. and 0.8 µm long. Amphid openings oval, 1.1 µm wide. Pharyngeal corpus cylindrical, 2 times isthmus length. Metacorpus swollen, 20 × 22 µm. Basal bulb spheroid, 21 µm wide and 32 µm long. Cardia conoid. Nerve ring at isthmus level, at 53% of neck length. Excretory pore not visible. Deirid not observed. Intestine without distinct specialization. Reproductive system amphidelphic, both branch equally developed. Uterus tubular,

Table 3. Measurements of *Pristionchus pacificus* Sommer, Carta, Kim et Sternberg, 1996 from Iran and USA [all measurements in μm and the format: mean ± standard deviation (range)].

Locality	Iran, Khorasane Razavi province	Unites States, Pasadena*
Habitat	Soil (Peach orchard)	Soil (Flower garden)
N	11 ♀♀	121 ♀♀
Body Length	763 ± 53 (690–834)	750–1410
a	20.2 ± 3.2 (16.4–23.1)	12.1–17.4
b	7.2 ± 1.6 (5.3–9.1)	5.6–9.1
c	4.4 ± 0.4 (3.6–5.0)	3.8–6.7
c'	9.4 ± 2.6 (5.8–14.5)	5.0–9.6
V	44.6 ± 4.1 (35–50)	42–59
Stoma length	8.7 ± 1.8 (6–10)	8–15
Stoma width	8.3 ± 1 (6–10)	7–13
Procorpus + metacorpus	72.7 ± 14.2 (57–91)	80–105
Isthmus + bulb	36.6 ± 9.4 (20–49)	45–70
Pharynx length	109.3 ± 21.8 (77–137)	120–170
Vulval anterior end.	339.8 ± 25 (276–366)	410–670
Midbody diameter	38.3 ± 4.1 (31–43)	–
Anal body diameter	19.8 ± 6.8 (11–37)	22–43
Egg length	45 ± 7.1 (37–54)	?
Tail length	175 ± 26.9 (146–220)	170–302

Explanations: * according Sommer et al. (1996).

1.1 times as long as the corresponding body diameter. Uterine egg measuring 28 × 39 μm. Oviduct short. Vagina extending inwards 0.3–0.4 times the corresponding body diameter with narrow lumen and protruded lips. Rectum as long as anal body diameter. Tail conical elongated. Phasmid at 22% of tail length.

Male. Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad. Three pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail. This information is based on SEM photographs. Measurements of males are not available, because they appear to have been distorted by suboptimal fixation.

Analysis of rDNA sequences

To confirm the morphological identification of the nematode isolate of *P. pacificus*, a selected sample was analyzed by molecular approaches. Complete sequence of the ITS as well as partial sequence of 18S were determined and deposited in GenBank under the accession numbers JQ699288, JN039364 (ITS sequences) and JQ699287 (18S sequence). The D2-D3 expansion segment of 28S rRNA was not amplified in this study. NBLAST search through GenBank data showed that the Iranian isolate has high similarity with those sequences available for *P. pacificus*.

Analysis of the 18S rDNA region, based on NJ method (Fig. 4) showed three main clades (I, II and III). NJ analysis placed the Iranian isolate of *P. pacificus* with three other isolates of this species in clade I. However, NJ analysis showed that *P. pacificus* FUM 5 differs from all other known isolates. The 18S sequences comparing *P. pacificus* FUM5 with other isolates of the species (i.e., with accession numbers PPU81584, DQ270018 and AF083010) were 0.35, 0.41 and 0.39%, respectively. Bootstrap support for the association between the new isolate of *P. pacificus* and the other three known isolates was 88%, with 10000 replicates in the NJ tree. Two isolates of *P.* cf. *pacificus* (AB597235 and AB597236) were shown to be sister to a clade of four other isolates including FUM5 isolate. The nearest clade (II) to *P. pacificus* includes species *P. pauli* Herrmann, Mayer et Sommer, 2006, *P. marianneae* Herrmann, Mayer et Sommer, 2006 and *P. maupasi*. The clade III included some other species, *P. lheritieri* Maupas, 1919, *P. entomophagus* and *P. uniformis* Fedorko et Stanuszek, 1971. The monophyletic clade containing the new isolate of *P. pacificus* was strongly supported the tree obtained from NJ analysis. Analyses by NJ and ML method showed similar results for new isolate of *P. pacificus*. The topology of ML tree (Fig. 3) is consistent with the position of the species and new isolate resulted by NJ (Fig. 4).

Discussion

Previously, *P. pacificus* was isolated as a new species from Pasadena, California, USA (Sommer et al. 1996). In this study, a single population of *P. pacificus* from a peach orchard of Mashhad, Razavi Khorasan province, Iran was shown to agree with the original description by Sommer et al. (1996). This species resembles to *P. maupasi*, although it is distinctive by the longitudinal lines comprised of closely apposed pairs on its cuticle (*vs.* not lacking such lines). Also, *P. pacificus* is similar to *P. lheritieri*, however *P. pacificus* differs in the morphology of lip region (slightly indented *vs* not indented) and by teeth that are more robust and anteriorly directed (*vs* less robust and less anteriorly directed). The papillae arrangement of the male tail of the Iranian population of *P. pacificus* fits well with the original description. Other minor differences among isolates may be expected due to geographical distribution and habitat.

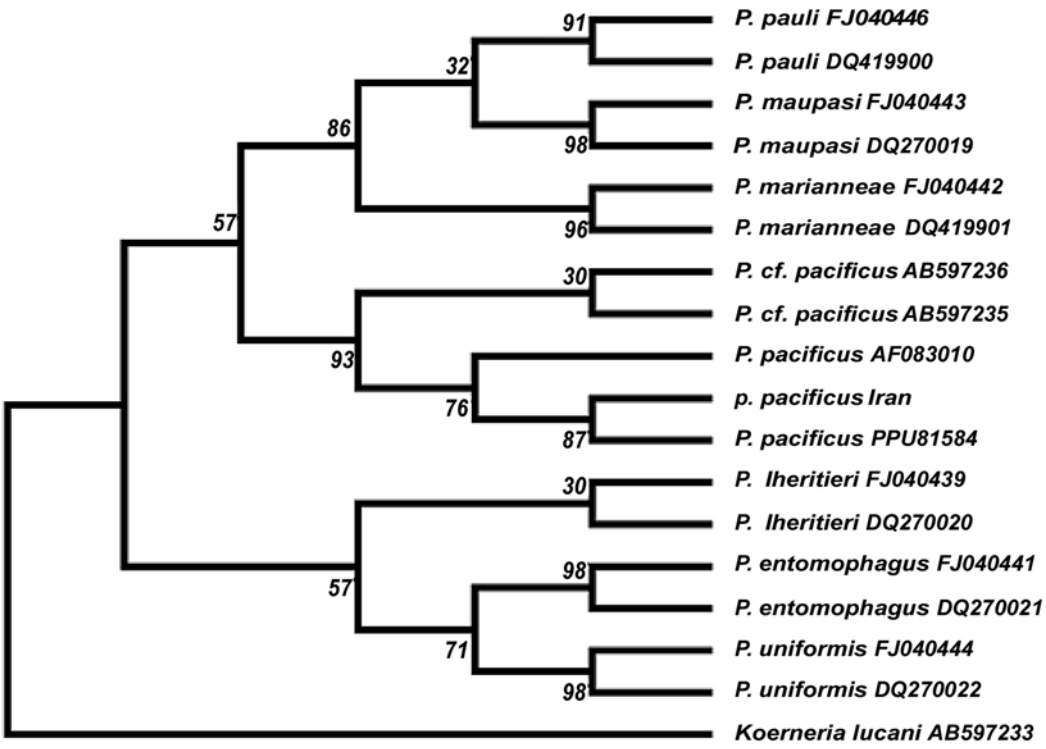

Fig. 3. Maximum likelihood cladogram of *Pristionchus* species based on analysis of 18S rRNA gene sequences.

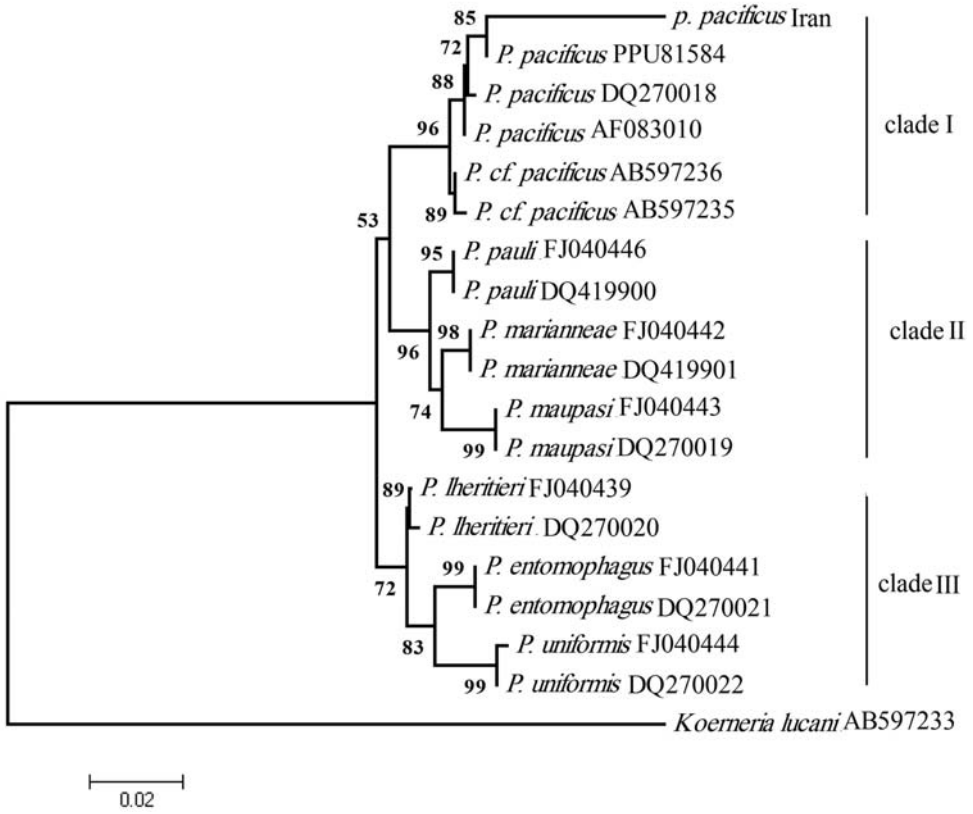

Fig. 4. Phylogenetic relationships among *Pristionchus* spp. based on partial 18S rRNA gene sequences inferred by neighbor-joining analysis, using Kimura two-parameter. *Koerneria lucani* was used as an outgroup. Numbers represent bootstrap frequencies (10,000 replicates).

Morphological identification of nematodes is often difficult and time consuming due to variable and overlapping characters as well as problems of interpreting plesiomorphic states and convergent (homoplasious)

features (Dorris et al. 1999; Stock 2002; Blaxter 2003; Stock & Reid 2004; Kumari & Lišková 2009). Furthermore, most of the characters used for species identification are only good for diagnostic purposes without phylogenetic value (Dorris et al. 1999; Stock 2002; Stock & Reid 2004). For these reasons, methods such as molecular analysis have particular value for identifying nematodes (Dorris et al. 1999; Blaxter 2003; Kumari & Lišková 2009). Partial 18S rRNA sequences are used extensively as molecular markers for taxonomic identification as well as phylogenetic inference in most groups of nematodes (Floyd et al. 2002; Abebe et al. 2011). Herrmann et al. (2006) and Mayer et al. (2007) used 18S sequence for identification and phylogenetic inference of *Pristionchus* species. Specifically they demonstrated that this gene serves as a fast and accurate barcoding marker for species identification with sufficient resolution within the genus *Pristionchus*. The present study has fairly good agreement with the phylogeny of *Pristionchus* species as proposed by Herrmann et al. (2006) and Mayer et al. (2009) based on the molecular phylogeny of partial 18S.

Based on the SSU-sequence dataset, *P. pacificus,* within Neodiplogastridae, belongs to clade V, proposed by Blaxter et al. (1998); clade V also includes Rhabditida such as *C. elegans*, the vertebrate-parasitic order Strongylida, and the entomopathogenic genus *Heterorhabditis* Poinar, 1976 (Blaxter et al. 1998; Mayer et al. 2007). Herrmann et al. (2006) and Brown et al. (2011) demonstrated that *P. pacificus* species have nictation behaviour and association with insects. Consequently, it may not be surprising that this species is placed with free living and parasitic nematodes in one clade. Brown et al. (2011) suggested that nictation behaviour in *P. pacificus* serves in host-finding behaviour. Speculation is that nictation or nictation-like host finding behaviors are crucial during the initial steps of parasitism evolution (Brown et al. 2011). Association with dead insects as in the *Pristionchus* genus could be interpreted as representing a pre-adaptation for the evolution of true parasitism, including an environment furthering adaptation to low oxygen levels, high temperatures and toxic host enzymes (Rae et al. 2008).

Some differences between *P. pacificus* and *C. elegans* with respect to the development of the vulva, the gonad and the buccal cavity, have contributed to this species being an ideal satellite organism in comparative studies with *C. elegans* (Rudel et al. 2005; Hong & Sommer 2006; Herrmann et al. 2007; Zauner et al. 2007; Click et al. 2009). Morgan et al. (2012) mentioned that the complex effects of environmental, ecological and geological factors can influence local adaptation and genotypic evolution in *P. pacificus*. They showed that differential colonization and host associations have achieved a richly diverse pattern in *P. pacificus* (Morgan et al. 2012). While *P. pacificus* has developed as a model system in evolutionary developmental biology in the last decade, finding a new isolate of this model may provide new insight into how redundant developmental mechanisms evolve (Hong et al. 2008b).

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References

Abebe E., Mekete T. & Thomas W.K. 2011. A critique of current methods in nematode taxonomy. Afr. J. Biotechnol. **10:** 312–323. DOI: 10.5897/AJB10.1473

Bedding R.A. & Akhurst R.J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica **21:** 109–110. DOI: 10.1163/187529275X00419

Blaxter M. 2003. Counting angels with DNA. Nature **421:** 122–124. DOI: 10.1038/421122a

Blaxter M.L., De Ley P., Garey, J.R., Liu L.X., Scheldeman P., Vierstraete A., Vanfleteren J.R., Mackey L.Y., Dorris M., Frisse L.M., Vida J.T. & Thomas K. 1998. A molecular evolutionary framework for the phylum Nematoda. Nature **392:** 71–75. DOI: 10.1038/32160

Brown F.D., D'anna I. & Sommer R.J. 2011. Host-finding behaviour in the nematode *Pristionchus pacificus*. Proc. R. Soc. B. **278:** 3260–3269 DOI: 10.1098/rspb.2011.0129

Caldwell J.R. 1981. Biomass and respiration of nematode populations in two moss communities at Signy Island, Maritime Antarctic. Oikos **37:** 160–166.

Click A., Savaliya C.H., Kienle S., Hermann M. & Pires-Dasilva A. 2009. Natural variation of outcrossing in the hermaphroditic nematode *Pristionchus pacificus*. BMC Evol. Biol. **9:** 75. DOI: 10.1186/1471-2148-9-75

De Grisse A.T. 1969. Redscription ou modification de quelques techniques utilises dans l'etude des nematodes phytoparasitaries. Meded. Rijks Faculteit der Landbouwe Tenschappen Gent **34:** 351–369.

De Ley P., De Ley I., Morris K., Abebe E., Mundo M., Yoder M., Heras J., Waumann D., Rocha-olivares A., Burr J., Baldwin J. & Thomas W. 2005. An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. Phil. Trans. R. Soc. B. **360:** 1945–1958. DOI: 10.1098/rstb.2005.1726

De Ley P., De Velde M.C., Mounport D., Baujard P. & Coomans A. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhaditidae, with a proposal for a revised stomaterminology in Rhabditida (Nematoda). Nematologica **41 (4-5):** 153–182. DOI: 10.1163/003925995X00143

De Man J.G. 1912. *Odontopharynx longicaudata* n. g. n. sp. Eine neue from von Anguilluliden. Zool. Jahrb. Jena Abt. Syst. **33:** 637–642.

Dereeper A., Guignon V., Blanc G., Audic S., Bufet S., Chevenet F., Dufayard J.-F., Guindon S., Lefort V., Lescot M., Claverie J.M. & Gascuel O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. **36:** W465–W469. DOI: 10.1093/nar/gkn180

Dorris M., De Ley P. & Blaxter M.L. 1999. Molecular analysis of nematode diversity and the evolution of parasitism. Parasitol. Today **15:** 188–193. DOI: 10.1016/S0169-4758(99)01439-8

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39:** 783–791. DOI: 10.2307/2408678

Floyd R., Eyualem A., Papert A. & Blaxter M. 2002. Molecular barcodes for soil nematode identification. Mol. Ecol. **11:** 839–850. DOI: 10.1046/j.1365-294X.2002.01485.x

Herrmann M., Mayer W.E., Hong R.L., Kienle S., Minasaki R. & Sommer R.J. 2007. The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. Zool. Sci. **24:** 883–889. DOI: 0289-0003-24-9-883

Hong R.L. & Sommer R.J. 2006. *Pristionchus pacificus*: a well-rounded nematode. Bioessays **28:** 651–659. DOI: 10.1002/bies.20404

Hong R.L., Svatos A., Herrmann M. & Sommer R.J. 2008a. The species-specific recognition of beetle cues by the nematode *Pristionchus maupasi*. Evol. Dev. **10 (3):** 273–279.

Hong R.L., Witte H. & Sommer R.J. 2008b. Natural variation in *Pristionchus pacificus* insect pheromone attraction involves the protein kinase EGL-4. Proc. Natl. Acad. Sci. U.S.A. **105:** 7779–7784. DOI: 10.1073/pnas.0708406105

Kanzaki N., Taki H. Masuya H., Okabe K. Tanaka R. & Abe F. 2011. Diversity of stag beetle-associated nematodes in Japan. Environ. Entomol. **40:** 281–288. DOI: 10.1603/EN10182

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:** 111–120. DOI: 10.1007/BF01731581

Kreis H.A. 1932. Beiträge zur Kenntnis pflanzenparasitischer Nematoden. Zeitschr. Parasitenkd. **5:** 184–194. DOI: 10.1007/BF02120641

Kumari S. & Liskova M. 2009. Molecular confirmation of *Xiphinema italiae* Meyl, 1953 (Nematoda: Longidoridae) from the Slovak Republic. Helminthologia **46:** 131–134. DOI: 10.2478/s11687-009-0025-8

Mayer W.E., Herrmann M. & Sommer R.J. 2007. Phylogeny of the nematode genus Pristionchus and implications for biodiversity, biogeography and the evolution of hermaphroditism. BMC Evol. Biol. **7:** 104. DOI: 10.1186/1471-2148-7-104

Mayer W.E., Herrmann M. & Sommer R.J. 2009. Molecular phylogeny of beetle associated diplogastrid nematodes suggests host switching rather than nematode-beetle coevolution. BMC Evol. Biol.**9:** 212. DOI: 10.1186/1471-2148-9-212

Morgank K., Mcgaughran A., Villate L., Herrmann M., Witte H., Bartelmes G., Rochat J. & Sommer R.J. 2012. Multi locus analysis of *Pristionchus pacificus* on La Re'union Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare out-crossing. Mol. Ecol. **21:** 250–266. DOI: 10.1111/j.1365-294X.2011.05382.x

Rae R., Riebesell M., Dinkelacker I., Wang O., Herrmann M., Weller A.M., Dieterich C. & Sommer R.J. 2008. Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. J. Exp. Biol. **211:** 1927–1936. DOI: 10.1242/jeb.014944

Rudel D., Riebesell M. & Sommer R.J. 2005. Gonadogenesis in *Pristionchus pacificus* and organ evolution: development, adult morphology and cell–cell interactions in the hermaphrodite gonad. Dev. Biol. **277:** 200–221. DOI: 10.1016/j.ydbio.2004.09.021

Saitou N. & Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406–425.

Sudhaus W. & Furst V.L.A. 2003. A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda). J. Nematode Morp. Syst. **6:** 43–90.

Shokoohi E. & Abolafia J. 2011. New data and SEM observations of six known species of the superfamily Rhabditoidea Örley, 1880 (Rhabditida) from Tehran province (Iran). J. Nematode Morph. Syst. **14 (1):** 39–54.

Sochova I., Hofman J. & Holoubek I. 2006. Using nematodes in soil ecotoxicology. Environ. Int. **32:** 374–383. DOI: 10.1016/j.envint.2005.08.031

Sommer R.J. 2006. *Pristionchus pacificus*. In: The *C. elegans* Research Community (eds), Worm Book. http://www.wormbook.org, doi/10.1895/wormbook.1.102.1. (accessed 05.03.2011)

Sommer R.J., Carta L.K., Kim S.Y. & Stenberg P.W. 1996. Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda: Neodiplogasteridae). Fund. Appl. Nematol. **19:** 511–521.

Stock S.P. 2009. Molecular approaches and taxonomy of insect parasitic and pathogenic nematodes, pp. 70–98. In: Stock S.P., Vandeberg J., Boemare N. & Glazer I. (eds), Insect Pathogens: Molecular Approaches and Techniques. Wallingford, UK: CABI Publishing, 417 pp. ISBN: 9781845934781 1845934784

Stock S.P., Campbell J. F. & Nadler S.A. 2001. Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. J. Parasitol. **87:** 877–889. DOI: 10.2307/3285148

Stock S.P. & Reid A.P. 2004. Biosystematics of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae): current status and future directions, pp. 435–446. In: Cook R. & Hunt D.J. (eds), Proceedings of the Fourth International Congress of Nematology, June 8–13, 2002, Tenerife, Spain, Nematology Monographs and Perspectives 2, Brill Academic Publ., 866 pp. ISBN13: 9789004139213

Subbotin S., Sturhan D., Volvas N., Castillo P., Tanyi T., Moens M. & Baldwin J. 2007. Application of the secondary structure model of rRNA for phylogeny: D2-D3 expansion segments of the LSU gene of plant parasitic nematodes from the family Hoplolaimidae Filipjev, 1934. Mol. Phylogenet. Evol. **43:** 881–890. DOI: 10.1016/j.ympev.2006.09.019

Tamura K. & Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10:** 512–526. PMID: 8336541

Tamura K., Dudley J., Nei M. & Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:** 1596–1599. DOI: 10.1093/molbev/msm092

Thompson J.D., Higgins D.G. & Gibsson T.J. 1994. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680. DOI: 10.1093/nar/22.22.4673

Vrain T.C., Wakarchuk D.A., Levesque A.C. & Hamilton R.I. 1992. Intraspecific rDNA restriction fragment length polymorphisms in the *Xiphinema americanum* group. Fund. Appl. Nematol. **15:** 563–574.

Weller A.M., Mayer W.E., Rae R. & Sommer, R.J. 2010. Quantitative assessment of the nematode fauna present on Geotrupes dung beetles reveals species-rich communities with a heterogeneous distribution. J. Parasitol. **96:** 525–531. DOI: http://dx.doi.org/10.1645/GE-2319.1

White G.F. 1927. A method for obtaining infective nematode larvae from cultures. Science **66:** 302–303. DOI: 10.1126/science.66.1709.302-a

Zauner H., Mayer W., Herrmann M., Weller A., Erwig M. & Sommer R.J. 2007. Distinct patterns of genetic variation in *Pristionchus pacificus* and *Caenorhabditis elegans*, two partially selfing nematodes with cosmopolitan distribution. Mol. Ecol. **16:** 1267–1280. DOI: 10.1111/j.1365-294x.2006.03222.x

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