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First report of *Meloidogyne hispanica* in Iran

Ebrahim Shokoohi[1] · **Zahra Parastar**[2] · **Hadi Panahi**[3] · **Shiva Abbaspour**[2] · **Hendrika Fourie**[1] · **Mariette Marais**[4]

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Abstract *Meloidogyne hispanica* was identified morphologically, using perineal patterns, from root samples collected from a white mulberry growing in a park in Tehran, Iran. Molecular analysis was also undertaken, based on the D2-D3 segment of the 28S rDNA region, and confirmed this population as *M. hispanica*. Phylogenetic analysis using the Bayesian inference method, places this population close to the same species from the Portugal (EU443608) and Spain (EU443606). This is the first record of *M. hispanica* in Iran.

Keywords Iran · White mulberry · *Meloidogyne hispanica* · Perineal pattern · 28S rDNA

The genus *Morus* comprises many species (Suttie 2012). The species, *Morus alba* is one of the most popular *Morus* worldwide. This plant is widely cultivated as an ornamental plant in the parks in Iran. *Meloidogyne* species were recovered from root samples collected from stunted and cholorotic trees growing in "Azalia" park in Teheran.

✉ Ebrahim Shokoohi
Ebrahim.Shokoohi@nwu.ac.za

[1] Unit for Environmental Sciences and Management, North West University, Potchefstroom, South Africa

[2] Plant Protection Clinic, District 15, Tehran Municipality, Tehran, Iran

[3] Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

[4] Nematology Unit, Biosystematic Division, Agricultural Research Council, Plant Protection Research Institute, (ARC-PPRI), Roodeplaat, Queenswood 0121, South Africa

During March 2015, root samples were collected from white mulberry in a park in Tehran (N: 35°37′51″; E: 51°28′25″). Roots were washed, cut in pieces and mature female specimens removed using a scalpel under a Nikon CH-2 stereo microscope. These specimens were fixed with a hot 4 % formaldehyde solution and transferred to anhydrous glycerine using the method of De Grisse (1969). Characteristics of the perineal patterns as described by Hirschmann (1986) were used in identifying the *Meloidogyne* species (Fig. 1).

The molecular characterisation was based on the methodology used by Rashidifard et al. (2015). The original partial 28S (D2-D3 expansion) sequence of *Meloidogyne hispanica* is deposited in GenBank under accession number KT359553. Sequencing and Bayesian inference (BI) analysis of the ribosomal DNA region D2-D3 of 28S (Fig. 2) has been obtained for this species.

The sequence lengths flanked by the forward primer D2A (5″-ACAAGTACCGTGAGGGAAAGTTG–3″) and the reverse primer D3B (5″-TCGGAAGGAACCAGCTACTA–3″) (according to Subbotin et al. 2006) of the 28S region of *M. hispanica* isolate are 694 base pairs long. The Blast test revealed that this population is seven base pairs different from the closest populations from Spain, Brazil and Portugal (EU443606, EU443607, EU443608; 98 % identity respectively). Compared with the populations from Greece (KF501128) and Spain (GQ375158), differences of eight and seven (99 % identity) base pairs, respectively, were evident.

Our phylogenetic analysis using 28S rDNA, placed the Iranian *M. hispanica* population in a clade together with other *M. hispanica* populations (Fig. 2).

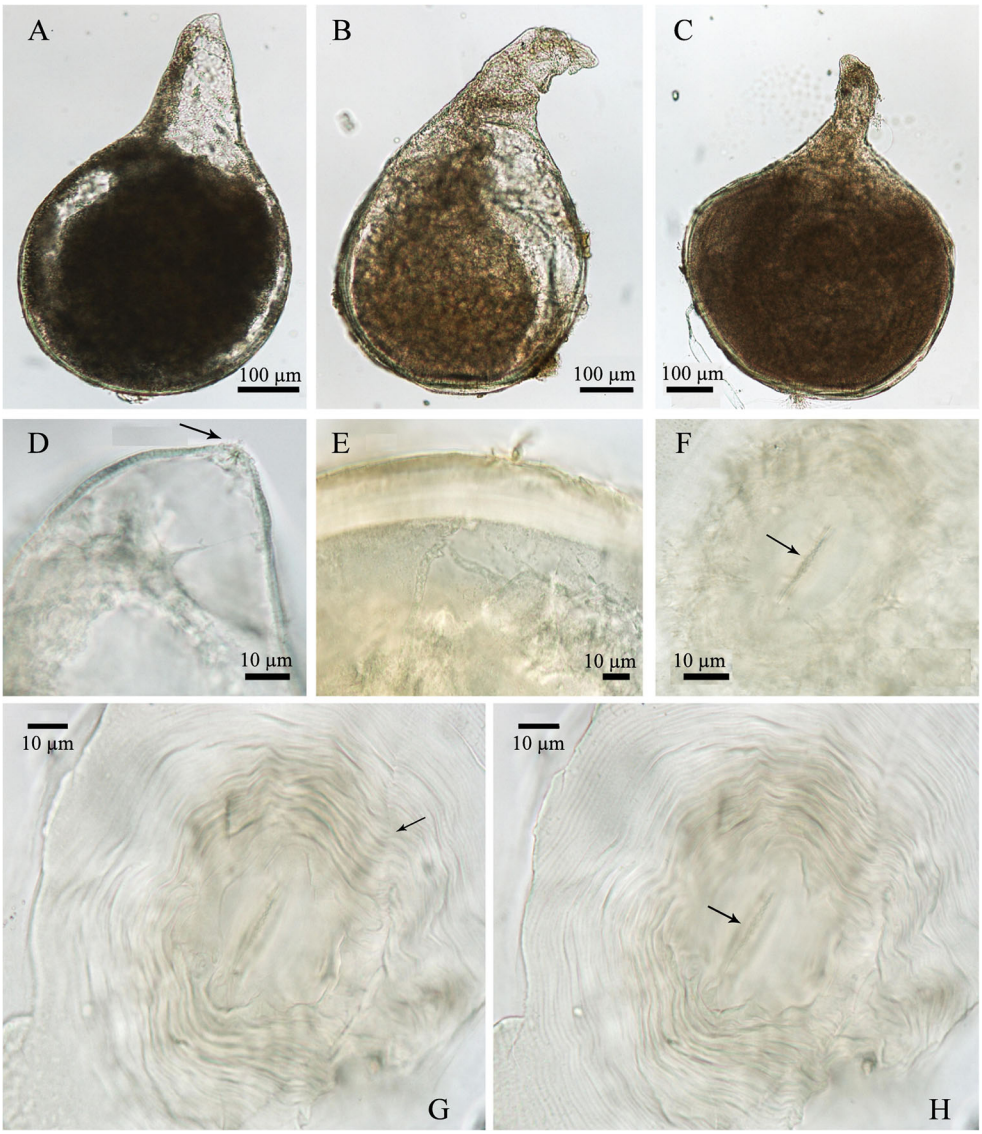

Fig. 1 *Meloidogyne hispanica* Hirschmann 1986. **a-c**: Entire female. **d**: Anterior end of a typical female (*arrow indicates lip region*). **e**: Cuticle of a typical female. **f**: Vulva of a typical female. **g**, **h**: Perineal patterns of two typical females

Molecularly characterised species of *M. hispanica* thus form a monophyletic group. The phylogeny of *Meloidogyne* spp. has been studied (De Ley et al. 2002; Tigano et al. 2005) using 18S rDNA and 18S rDNA and mtDNA, respectively. However, *M. hispanica* was not included in these studies. *Meloidogyne hispanica* and *M. ethiopica* are place together in a group, however, they differ in terms of the stylet of the female (small *vs* large), phasmids (not prominent *vs* prominent) and lateral line (conspicuous *vs* not conspicuous). Other genes e.g. mtDNA may separate these two species clearly. Two permanent microscope slides, containing the perennial patterns and females of *M. hispanica*, respectively, were deposited in the National Collection of Nematodes (NCN) at the Nematology Unit, Biosystematics Division, Agricultural Research Council (ARC) - Plant Protection Research Institute (PPRI) (Pretoria, South Africa) with slide numbers 50192 and 50193. According to the literature, this is the first record of *M. hispanica* in Iran. *Morus alba* seems to be affected by *M. hispanica* and management practices need to be put in place to control the nematode and to prevent its dispersal to other parks in Tehran.

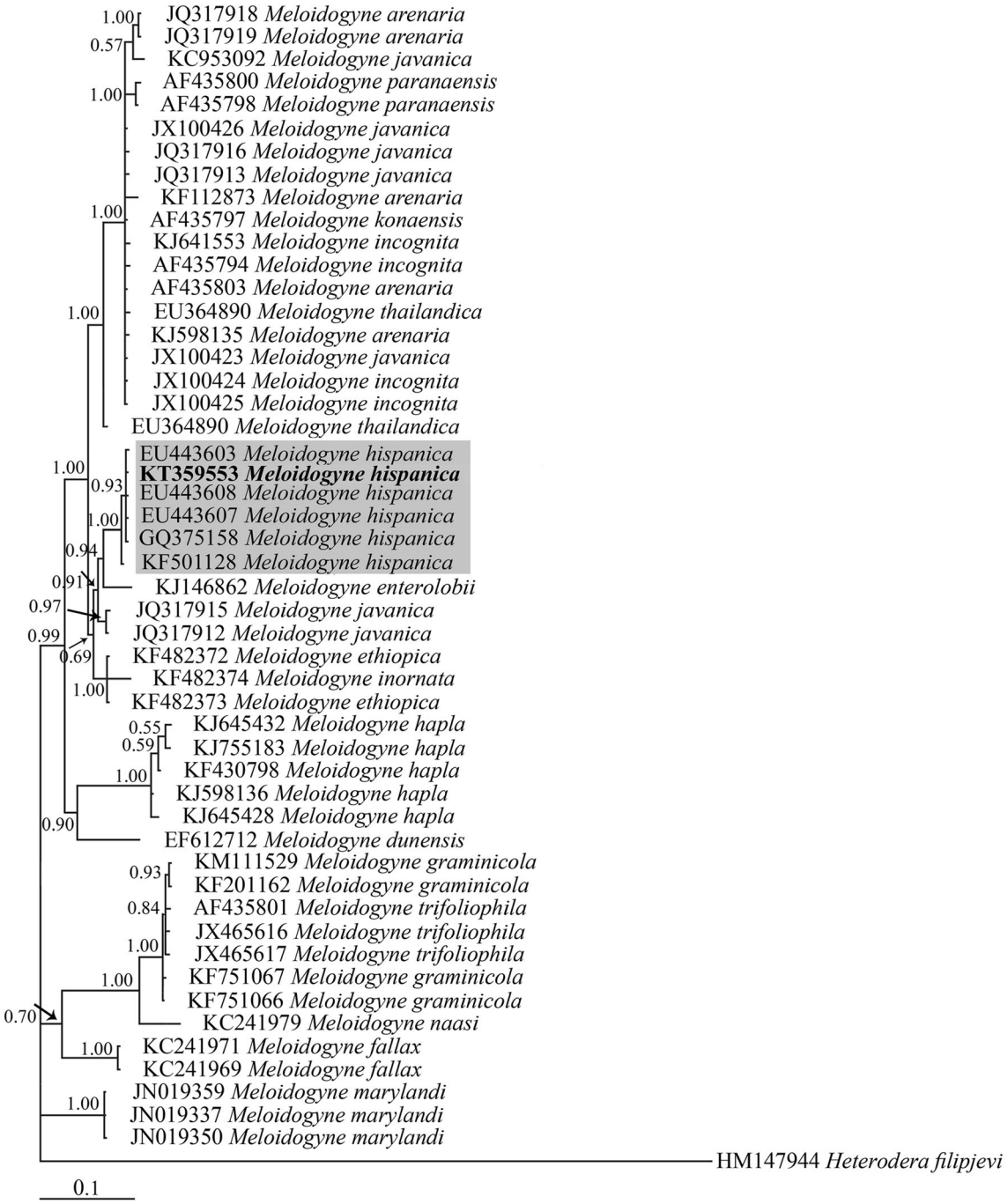

Fig. 2 The Bayesian tree inferred from known and newly sequenced *Meloidogyne* spp. from Iran based on the 28S rDNA region

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