Nematology, 2004, Vol. 6(1), 59-72

Description of *Meloidogyne minor* n. sp. (Nematoda: Meloidogynidae), a root-knot nematode associated with yellow patch disease in golf courses

Gerrit KARSSEN [1,*], Robert Jan BOLK [2], Adriaan C. VAN AELST [3], Ineke VAN DEN BELD [1], Linda F.F. KOX [1], Gerard KORTHALS [2], Leendert MOLENDIJK [2], Carolien ZIJLSTRA [4], Richard VAN HOOF [4] and Roger COOK [5]

[1] *Plant Protection Service, Diagnostic Department, P.O. Box 9102, 6700 HC Wageningen, The Netherlands*
[2] *Applied Plant Research (PPO), P.O. Box 430, 8200 AK Lelystad, The Netherlands*
[3] *Wageningen University and Research Centre, Laboratory of Plant Cell Biology, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands*
[4] *Plant Research International B.V. (PRI), P.O. Box 16, 6700 AA Wageningen, The Netherlands*
[5] *Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion SY23 3EB, Wales, UK*

Received: 17 June 2003; revised: 29 October 2003
Accepted for publication: 4 November 2003

Summary – A relatively small root-knot nematode, *Meloidogyne minor* n. sp., is described and illustrated from tomato from the Netherlands. This new species is characterised by the following features: female with dorsally curved stylet, 14 μm long, with transversely ovoid knobs slightly sloping backwards from the shaft; perineal pattern rounded; male stylet 18 μm long, large transversely ovoid knobs slightly sloping backwards from the shaft; head region not set off, labial disc elevated, lateral lips prominent; and second-stage juvenile 377 μm long, with hemizonid posterior adjacent to excretory pore; tail 54 μm long; and a distinct hyaline tail terminus 16 μm long. Additionally, distinguishing information on isozymes, DNA, cytogenetics and host plants is presented. *Meloidogyne minor* n. sp. has so far been found on potato in the Netherlands and has been detected in the British Isles on several golf courses, associated with yellow patches, often together with *M. naasi*.

Keywords – cytology, DNA, Europe, golf greens, host plants, isozymes, morphology, potato, SEM, taxonomy.

In 2000, an unknown root-knot nematode was detected on heavily infected potato roots near Zeijerveld, The Netherlands in the same area where, in 1967, an undescribed *Meloidogyne* species had been found in a field of marigold. Re-sampling this potato field, formerly a 10-year-old pasture, also revealed many *Meloidogyne naasi* Franklin, 1965 second-stage juveniles. The unknown root-knot nematode was grown on tomato in the glasshouse and, following host plant, morphological, isozyme and DNA studies, confirmed to be an undescribed species.

During 2001 and 2002, we received several samples from golf courses in England, Wales and Ireland with many *Meloidogyne* root galls. These samples originated from golf courses with clear symptoms of yellow patch disease on creeping bent grass (*Agrostis stolonifera* var. *stolonifera* L.) and included *M. naasi* and, surprisingly, the same undescribed *Meloidogyne* species. Field applications of fertilisers and fungicides, and glasshouse experiments in which a nematicide was applied to cores with yellow and green grass, associated the root-knot nematodes with this yellow patch disease (Mark Hunt, pers. comm.) and further data, described in this study, confirmed they belonged to the same undescribed species.

The undescribed root-knot nematode is herein described as *Meloidogyne minor* n. sp., the name referring to the relatively small size of this harmful plant-parasitic nematode.

* Corresponding author, e-mail: g.karssen@minlnv.nl

Table 1. *Host and origin of the populations of five* Meloidogyne *species that were compared in this study with the two populations of* M. minor *n. sp.*

Species	Host	Origin
M. chitwoodi Golden *et al.*, 1980	*Triticum aestivum* L.	Smakt, The Netherlands
M. fallax Karssen, 1996	*Beta vulgaris* L.	Baexem, The Netherlands
M. hapla Chitwood, 1949	*Geranium* sp.	Horst, The Netherlands
M. javanica (Treub, 1885)	*Sagaretia* sp.	China
M. minor n. sp. (type population)	*Solanum tuberosum* L.	Zeijerveld, The Netherlands
M. minor n. sp.	*Agrostis stolonifera* L.	County Kildare, Ireland
M. naasi Franklin, 1965	unspecified lawn grasses	Amsterdam, The Netherlands

Materials and methods

Meloidogyne minor n. sp. was isolated from *Solanum tuberosum* L. roots, growing at a former old pasture near Zeijerveld, The Netherlands. This population was maintained on *Lycopersicon esculentum* Miller, in order to exclude any *M. naasi*, in the glasshouse at Applied Plant Research, Lelystad, The Netherlands, and used for this study. The methods used for extraction, fixation, preparation of permanent slides, light microscopy, scanning electron microscopy and isozyme electrophoresis are fully described by Karssen *et al.* (1998). See Table 1 for an overview of *Meloidogyne* populations used for comparison.

DNA was isolated from second-stage juveniles (J2) using the High Pure PCR Template Preparation Kit (Roche, Almere, The Netherlands) according to the instructions in the mammalian tissue protocol. The DNA was eluted with 40 μl TE (10 mM Tris-HCl (pH 8.0); 1 mM EDTA) and stored at -20°C. Primers 5367 (5′-TTG ATT ACG TCC CTG CCC TTT-3′) and 5368 (5′-TTT CAC TCG CCG TTA CTA AGG-3′) (Vrain *et al.*, 1992), annealing in the 18S and 26S ribosomal genes, respectively, were used for amplification of the internal transcribed spacer (ITS) region by the polymerase chain reaction (PCR). The 50 μl reaction mixture was composed as follows: 1 μl DNA, 1 μg each primer, 200 μM dNTP (Promega, Leiden, The Netherlands), 1 Unit HotStar *Taq* DNA polymerase (Qiagen, Westburg, Leusden, The Netherlands), 5 μl of 10× reaction buffer with 15 mM MgCl2 (Qiagen). The PCR was performed in a PTC200 thermocycler (MJ-Research: Biozym, Landgraaf, The Netherlands) with the following parameters: 15 min at 95°C, 35 cycles of 15 s at 94°C, 1 min at 58°C, and 45 s at 72°C, followed by a final extension for 10 min at 72°C and quickly cooled to room temperature. PCR products were electrophorised on 1.5% agarose gel along with a 100-bp DNA ladder (MBI Fermentas) to size the fragments. The DNA fragments were visualised by ethidiumbromide-staining and UV-illumination. PCR products of *M. hapla* Chitwood, 1949, *M. chitwoodi* Golden, O'Bannon, Santo & Finley, 1980, *M. fallax* Karssen, 1996 and *M. minor* n. sp. were directly sequenced using the Big Dye™ Terminator Kit (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) according to the manufacturer's protocol. Internal primers Melo FW (5′-CGC TGG TGT CTA GGT GTT GCT-3′) and Melo RV(5′-AAA TGA CCC TGA ACC AGA CG-3′) were used in addition to the original external primers in sequencing reactions to obtain complete sequences of complementary strands. The generated ITS fragment of *M. naasi* was purified from low melting point agarose gel and cloned into the pGEM-T vector (Promega) as described in Zijlstra (1997). The cloned insert was tested and sequenced as described in Zijlstra (2000). Sequence data were obtained by analysing the samples on an ABI 377-XL Automated DNA sequencer (Applied Biosystems). Contig assembly as well as the final alignment of consensus sequences were performed using the Lasergene software (DNA Star Inc., Madison, WI, USA). The sequences are deposited in GenBank (www.ncbi.nlm.nih.gov) under the accession codes AY81852 to AY81855 and AY302249. Sequence pair distances were calculated from a multiple alignment performed with the (weighted) Jotun Hein Method (Hein, 1990).

To test host suitability, seedlings transplanted into pots of quartz sand were inoculated with a suspension of J2. The pots were held in a glasshouse at 20°C, for a period of 8 weeks. After this period, root material was separated from the soil and put in a mistifier for 6 weeks. The nematodes were counted and used to estimate the host status of different plant species.

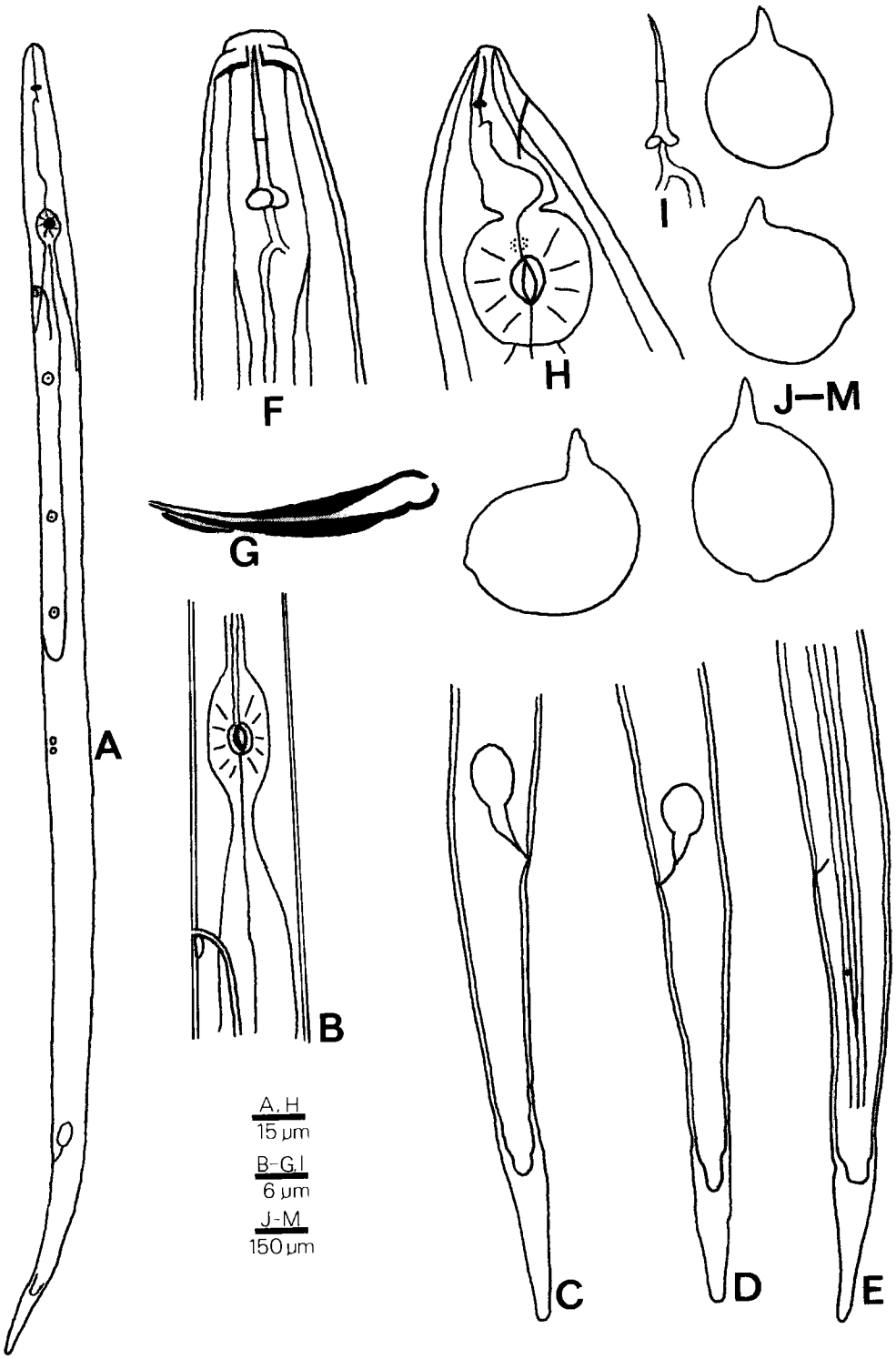

Fig. 1. Meloidogyne minor *n. sp. A-E: Second-stage juvenile. A: Body (lateral); B: Metacorpus region; C-E: Tails (lateral); F, G: Male. F: Head end (lateral); G: Spicule and gubernaculum (lateral); H-M: Female. H: Anterior head end (lateral); I: Stylet (lateral); J-M: Body shape.*

Table 2. *Morphometrics of adult stages and second-stage juveniles (J2) of* Meloidogyne minor *n. sp. Measurements are in μm and in the form: mean ± SD (range).*

Character	Female holotype	Female	Male	J2
n		25	25	25
L	570	526 ± 71 (416-608)	1045 ± 54 (790-1488)	377 ± 8 (310-416)
a	1.4	1.6 ± 0.3 (1.1-2.3)	39.0 ± 4.2 (29.8-48.3)	28.4 ± 2.0 (23.9-32.4)
c	–	–	101 ± 21.3 (72.4-140)	7.0 ± 0.3 (6.2-7.6)
c′	–	–	–	5.7 ± 0.4 (4.5-6.3)
T	–	–	48.4 ± 12.3 (29.9-73.2)	–
Greatest body diam.	397	339 ± 55 (240-464)	26.9 ± 4.5 (21.5-31.6)	13.3 ± 1.3 (12.0-15.8)
Body diam. at stylet knobs	–	–	15.9 ± 2.2 (13.3-18.3)	–
Body diam. at excretory pore	–	–	23.6 ± 3.6 (20.2-26.5)	13.3 ± 0.9 (12.6-15.2)
Body diam. at anus	–	–	–	9.6 ± 1.2 (7.6-10.7)
Head region height	–	–	3.9 ± 0.7 (3.2-4.4)	2.0 ± 0.2 (1.9-2.5)
Head region diam.	–	–	9.6 ± 0.9 (8.9-10.7)	5.2 ± 0.4 (5.1-5.7)
Neck length	128	138 ± 41.5 (96-240)	–	–
Neck diam.	77	72 ± 13.2 (48-96)	–	–
Stylet	13.9	14.2 ± 1.1 (12.6-15.2)	17.8 ± 1.0 (17.1-19.0)	9.2 ± 0.9 (7.6-10.1)
Stylet base-ant. end	–	–	–	13.2 ± 0.9 (12.0-15.2)
Stylet cone	–	–	10.1 ± 0.6 (9.5-10.7)	–
Stylet shaft and knobs	–	–	7.7 ± 0.9 (6.9-8.8)	4.7 ± 0.6 (3.8-5.1)
Stylet knob height	1.5	1.7 ± 0.5 (1.3-1.9)	2.0 ± 0.3 (1.9-2.5)	1.3 ± 0.2 (1.2-1.4)
Stylet knob width	3.2	3.5 ± 0.5 (3.2-3.8)	4.2 ± 0.5 (3.8-5.1)	1.9 ± 0.3 (1.8-2.0)
DGO	–	4.1 ± 1.2 (3.2-6.3)	3.8 ± 0.4 (3.2-4.4)	3.0 ± 0.5 (2.5-3.2)
Ant. end to metacorpus	67	53 ± 10.7 (41-68)	61 ± 12.3 (38-71)	43 ± 3.1 (39-47)
Metacorpus length	32	35 ± 7 (27-46)	–	–
Metacorpus diam.	29	31 ± 7.3 (22-48)	9.0 ± 1.7 (7.6-12.0)	–

Table 2. *(Continued).*

Character	Female holotype	Female	Male	J2
Metacorpus valve length	11.2	11.5 ± 1.6 (9.5-13.3)	5.0 ± 0.7 (4.4-5.7)	3.3 ± 0.3 (3.2-3.8)
Metacorpus valve width	9.6	8.9 ± 1.2 (7.0-10.1)	3.6 ± 0.5 (3.2-3.8)	2.9 ± 0.5 (2.5-3.2)
Ant. end to end of gland lobe	–	–	–	179 ± 9.5 (166-198)
Excretory pore-ant. end	16.4	18.3 ± 7.8 (13.9-25.9)	114 ± 24.9 (87.9-137)	70.5 ± 6.6 (58.1-77.1)
Tail	–	–	10.5 ± 2.3 (8.2-12.6)	54 ± 6.2 (49-63)
Hyaline tail terminus	–	–	–	16.1 ± 3.9 (12.0-22.1)
Phasmids-post. end	–	–	2.6 ± 0.8 (1.9-3.2)	–
Spicule	–	–	25.6 ± 3.4 (22.8-28.4)	–
Gubernaculum	–	–	6.1 ± 0.6 (5.7-6.3)	–
Testis	–	–	529 ± 302 (316-876)	–
Vulva slit length	24.0	25.8 ± 2.5 (22.8-29.1)	–	–
Vulva-anus	13.8	15.3 ± 2.5 (12.6-17.1)	–	–
Body length/neck length	4.4	4.1 ± 0.7 (2.7-5.3)	–	–
Body length/ant. end to metacorpus	–	–	–	8.7 ± 0.5 (7.9-9.4)
Stylet knob width/height	2.1	2.2 ± 0.5 (1.7-3.0)	2.2 ± 0.2 (1.1-1.4)	–
Metacorpus length/width	1.1	1.2 ± 0.2 (0.9-1.7)	–	–
(Excretory pore/L) × 100	–	–	11.1 ± 1.4 (8.2-15.2)	18.7 ± 0.8 (17.0-20.1)

***Meloidogyne minor* n. sp.**
(Figs 1-7)

MEASUREMENTS

Females, males and second-stage juveniles

See Table 2.

Eggs (n = 25)

Length: 80.0-102.5 (90.6 ± 1.2, SE = 0.2) μm; diam.: 38.5-57.5 (47.2 ± 1.5, SE = 0.3) μm; length/width: 1.6-2.3 (2.0 ± 0.2, SE = 0.04).

DESCRIPTION

Female

Body relatively small, weakly annulated, pearly white, usually globose, sometimes elongated; neck region distinct, often bent; young females with a slight posterior protuberance. Head region set off from the body. Head cap distinct and highly variable in shape, labial disk elevated, lateral lips prominent; cephalic framework weakly sclerotised. Stylet cone slightly curved dorsally, shaft cylindrical; knobs transversely ovoid and slightly sloping backwards from shaft. Excretory pore located near stylet knob

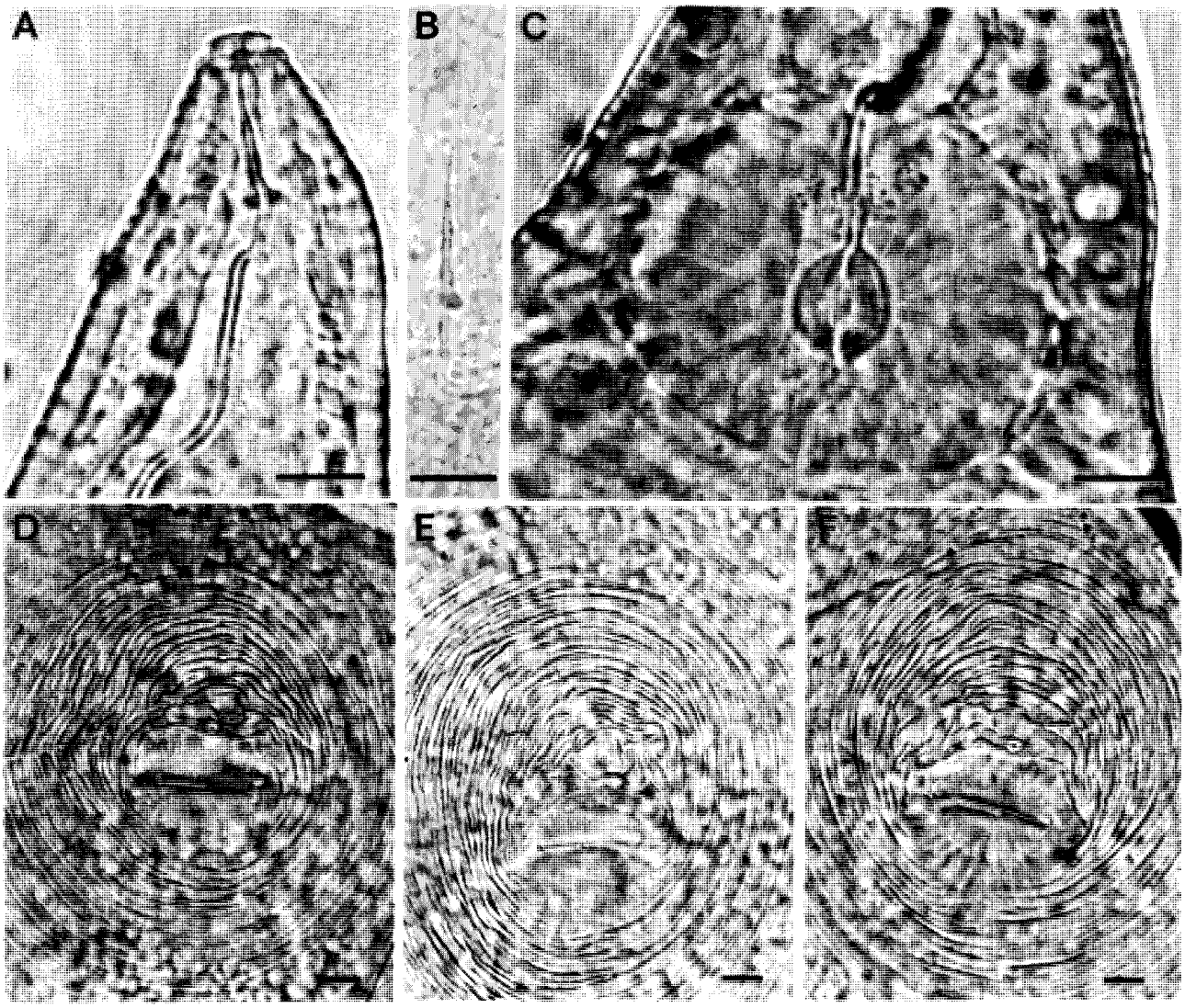

Fig. 2. *Light microscope photographs of* Meloidogyne minor *n. sp. Female. A: Head end (lateral); B: Stylet; C: Metacorpus; D-F: Perineal patterns. (Scale bars: A-F = 10 μm.)*

level. Several small vesicles observed near lumen lining of metacorpus. Pharyngeal glands variable in size and shape. Perineal pattern small, rounded with fine striae, dorsal arch low with coarse striae; tail remnant area distinct, without punctations; in some patterns weak lateral lines present; phasmids small, usually not visible, located above covered anus. Egg mass about five to six times larger than female body size.

Male

Body vermiform, annulated, usually not twisted, tail region curved. Four incisures present in raised lateral field, often with one or two incomplete incisures in middle near mid-body; outer bands irregularly areolated. Head not set off from body, one post-labial annule present often with one or two incomplete transverse incisures. Labial disc rounded, elevated, fused with anchor shaped submedial lips. Prestoma hexagonal in shape, surrounded by six inner sensilla. Four cephalic sensillae present on submedial lips, close to labial disc and marked by small slits. Slit-like amphidial openings present between labial disc and prominent lateral lips. Cephalic framework strongly sclerotised; vestibule extension distinct. Stylet with straight

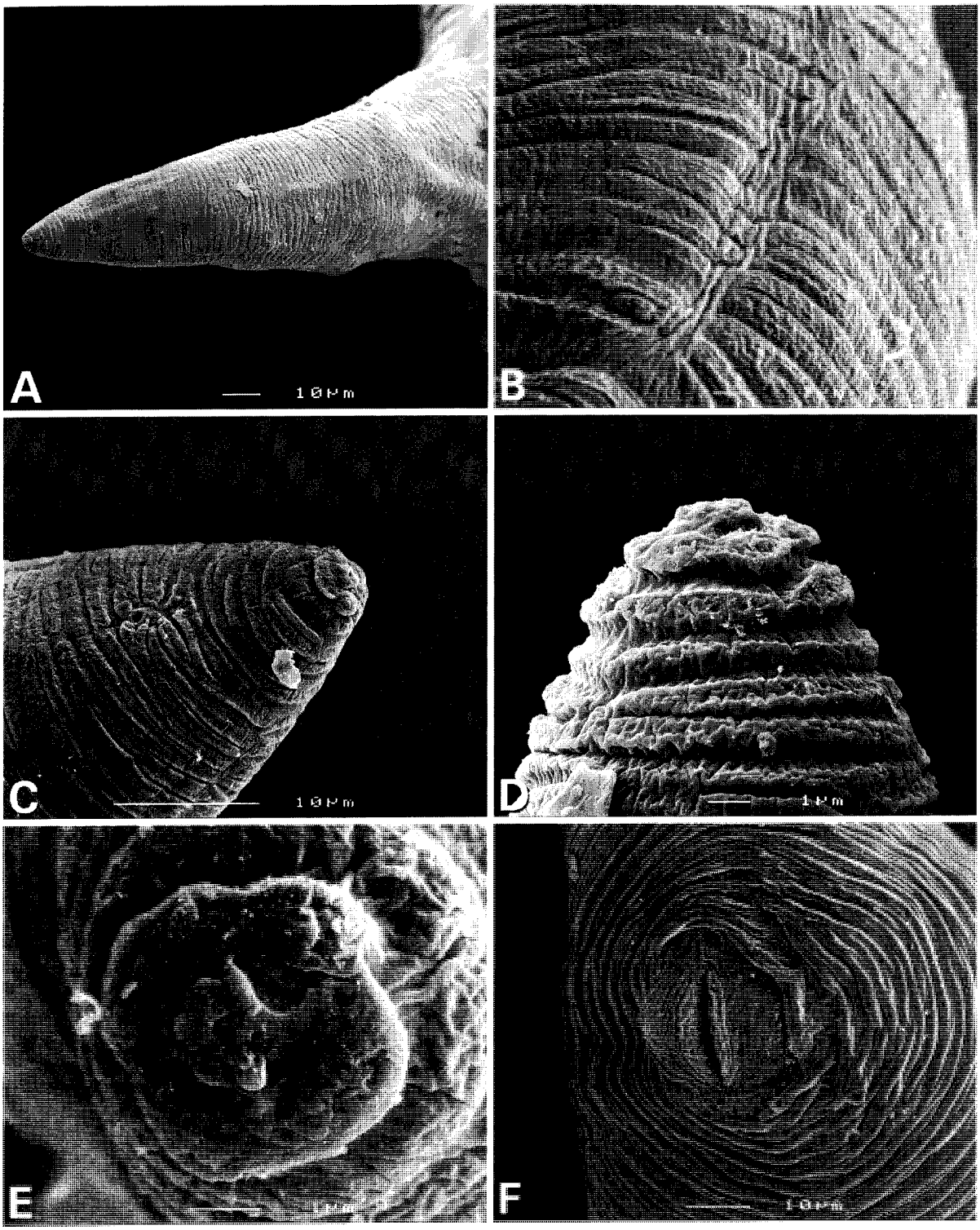

Fig. 3. *Scanning electron microscope photographs of* Meloidogyne minor *n. sp. Female. A: Neck region; B: Lateral field in neck region; C: Anterior end and excretory pore; D: Anterior end (lateral); E:* En face *view; F: Perineal pattern. (Scale bars: A, C, F = 10 µm; B, D, E = 1 µm.)*

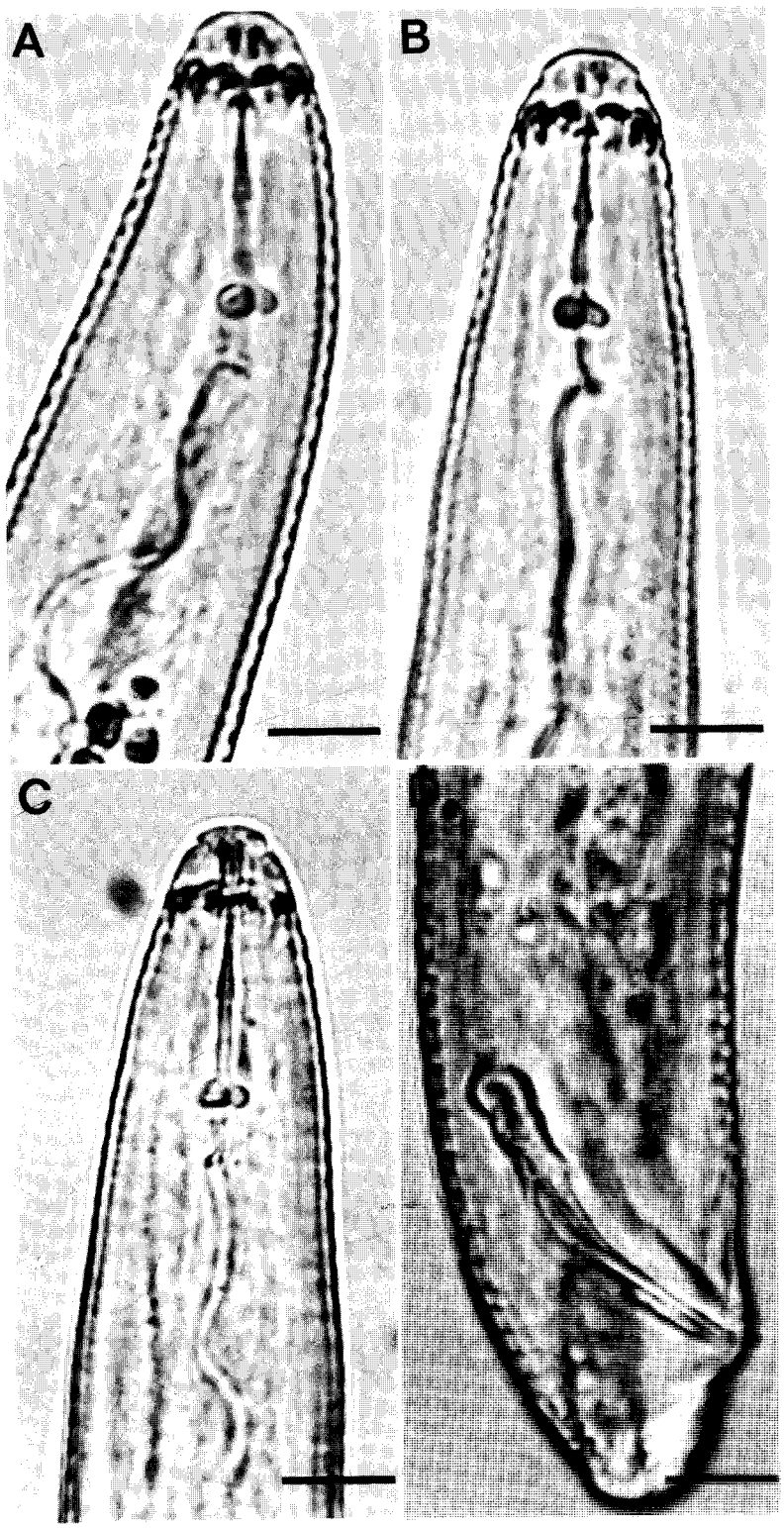

Fig. 4. *Light microscope photographs of* Meloidogyne minor *n. sp. Male. A, B: Head end (lateral); C: Head end (dorsal); D: Tail (lateral). (Scale bars: A-D = 10 μm.)*

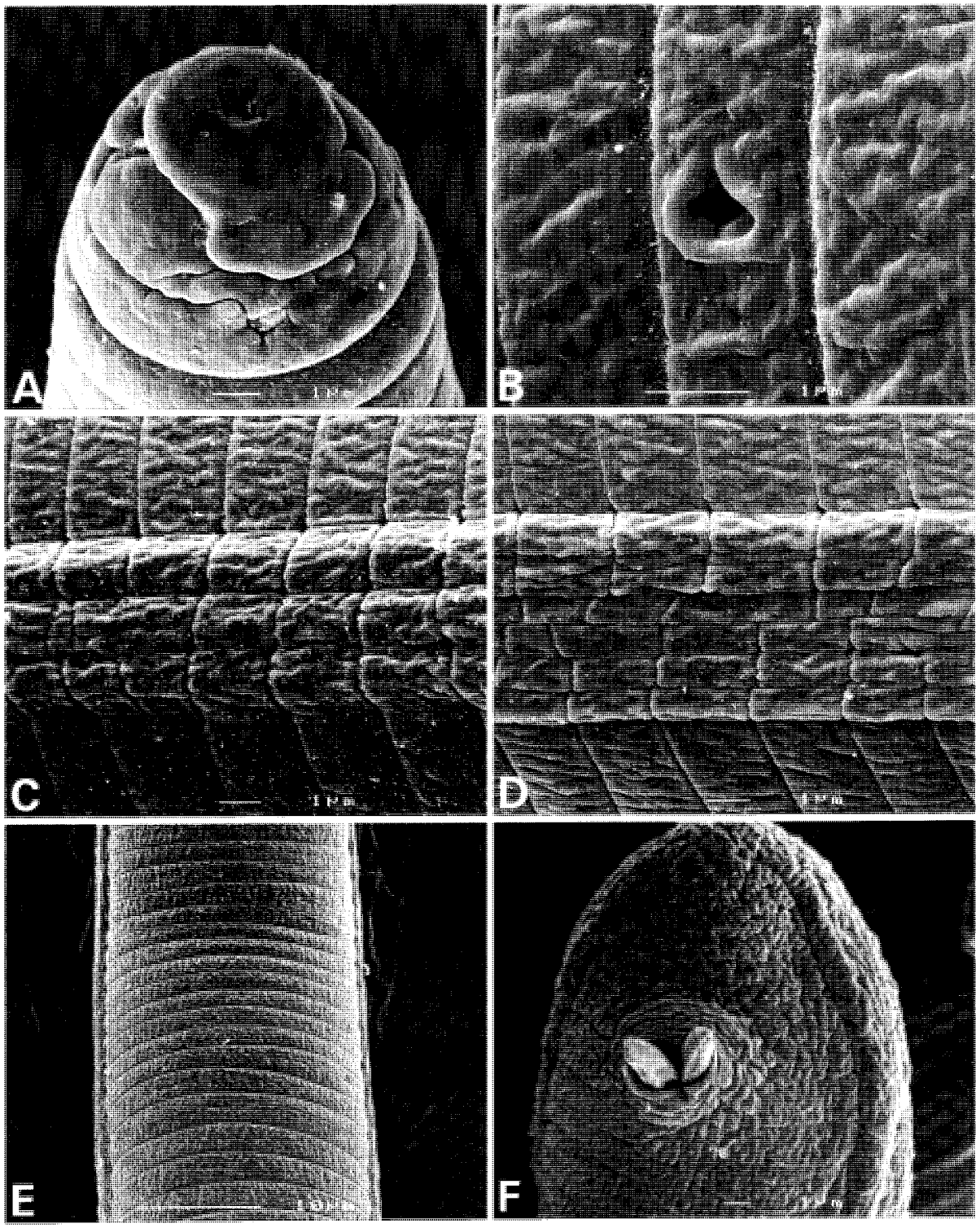

Fig. 5. *Scanning electron microscope photographs of* Meloidogyne minor *n. sp. Male. A: Anterior end; B: Excretory pore; C-D: Lateral field; E: Ventral body view near excretory pore level; F: Tail region (ventral). (Scale bars: A-D, F = 1 μm; E = 10 μm.)*

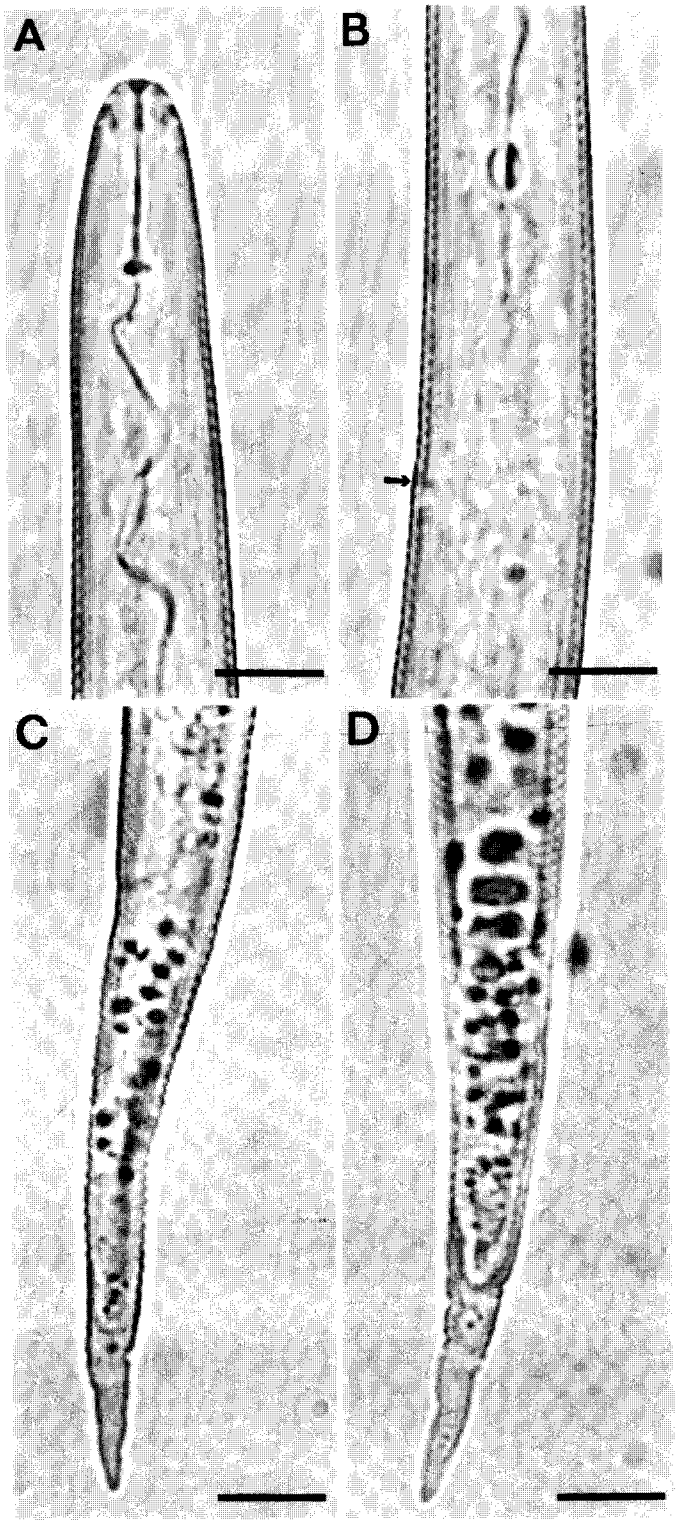

Fig. 6. *Light microscope photographs of* Meloidogyne minor *n. sp. second-stage juvenile. A: Head end (lateral); B: Metacorpus region (arrow, S-E pore); C, D: Tails (lateral). (Scale bars: A-D = 10 μm.)*

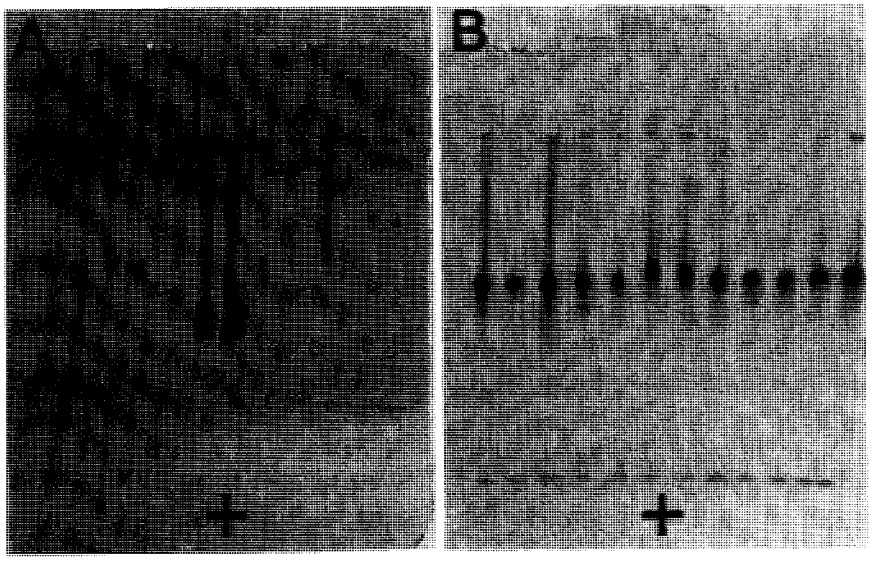

Fig. 7. *Isozyme patterns of* Meloidogyne minor *n. sp. (*M. javanica *used as a reference in two middle lanes). A: Esterase; B: Malate dehydrogenase.*

cone and cylindrical shaft; large transversely ovoid knobs, slightly sloping backwards from the shaft. Dorsal gland orifice close to stylet knobs. Pharynx with slender procorpus and oval-shaped metacorpus. Pharyngeal gland lobe ventrally overlapping intestine, two subventral gland nuclei present. Hemizonid 3.5-4.5 μm long, anterior to excretory pore. Testis very long, monorchic, with outstretched germinal zone. Tail usually curved ventrally, short, conical with bluntly rounded tip. Spicula and gubernaculum slender, curved ventrally; two small pores present on each spiculum tip. Phasmids small and located posterior to cloaca.

Second-stage juvenile

Body vermiform, relatively short, annulated; anterior part tapering behind stylet-knob level, posterior part slightly ventrally curved when heat relaxed. Lateral field with four incisures, areolation not visible. Head region rounded, not set off from body. Cephalic framework weakly sclerotised, vestibule extension distinct. Stylet small, cone straight, shaft cylindrical; knobs transversely ovoid and slightly sloping backwards. Metacorpus relatively large, ovoid, triradiate lumen with clear sclerotised lining. Pharyngeal gland lobe relatively long, ventral intestine overlap clearly visible, three gland nuclei present. Hemizonid posterior, adjacent to excretory pore, 2-2.5 μm in length. Tail straight, sometimes slightly curved ventrally, gradually tapering until finely pointed tail tip; rectum usually weakly inflated. Hyaline tail terminus distinct, relatively long and narrow, anterior hypodermal part rounded and relatively narrow, often one or two cuticular constrictions present on tail terminus. Phasmids posterior to anus, at about 33% of tail length, very small, located in ventral incisure of lateral field.

TYPE HOST AND LOCALITY

Material originally collected from the roots of *Solanum tuberosum*, grown near Zeijerveld, The Netherlands, but species described from a glasshouse culture on the roots of *Lycopersicon esculentum*.

OTHER LOCALITIES AND HOSTS

Other than the type locality, *M. minor* n. sp. has not been found so far in other Dutch locations. In the UK and Ireland, however, the situation is different, *M. minor* n. sp. being found in some 20 localities and always associated with golf courses and the grass *Agrostis stolonifera* var. *stolonifera* (L.).

TYPE MATERIAL

Holotype female: slide WT 3371, collection of Wageningen University and Research Centre, Wageningen, The Netherlands. Paratypes: two female perineal patterns and heads, two males and five J2 deposited at each of the following collections: Wageningen University and Re-

search Centre, Wageningen, The Netherlands (WT 3372-3374); Biology Department, University Gent, Gent, Belgium; Rothamsted Research, Harpenden, UK.

Diagnosis and relationships

Morphological characterisation

Meloidogyne minor n. sp. is characterised by a relatively small female body, stylet cone slightly curved dorsally, 14.2 (12.5-15) μm long, with transversely ovoid knobs slightly sloping backwards from the shaft, perineal pattern rounded with fine striae, dorsal arch low with coarser striae. Male stylet 17.8 (17-19) μm long, with transversely ovoid knobs slightly sloping backwards, labial disk elevated, lateral lips prominent. Second-stage juvenile body 377 (310-416) μm long, tail 54.1 (48.5-63) μm long and hyaline tail 16.1 (12-22) μm long; hemizonid posterior, adjacent to excretory pore; tail tip finely rounded.

Based on morphology, *M. minor* n. sp. could be confused with *M. chitwoodi* and *M. microtyla* Mulvey, Townshend & Potter, 1975 but differs from them by stylet knob shape, perineal pattern shape, male head shape and most juvenile characteristics. *Meloidogyne minor* n. sp. also differs in host range, isozyme patterns and at the DNA level from *M. chitwoodi* and *M. microtyla* (Townshend *et al.*, 1984; Esbenshade & Triantaphyllou, 1985; Ebsary, 1986; De Ley *et al.*, 2002). It is also very different from the other known European root-knot nematodes (Karssen & van Hoenselaar, 1998; Karssen, 2002): *M. ardenensis* Santos, 1968; *M. artiellia* Franklin, 1961; *M. duytsi* Karssen, van Aelst & van der Putten, 1998; *M. fallax*; *M. hapla*; *M. hispanica* Hirschmann, 1986; *M. kralli* Jepson, 1983; *M. lusitanica* Abrantes & Santos, 1991; *M. maritima* (Jepson, 1987) Karssen, van Aelst & Cook, 1998 and *M. naasi*.

Based on the definition of the '*graminis*-group' by Jepson (1987), *M. minor* n. sp. does not belong to the group of root-knot nematodes confined to hosts belonging to Poaceae and/or Cyperaceae, including typical golf course '*graminis*-group' members such as *M. graminis* (Sledge & Golden, 1964) Whitehead, 1968 and *M. marylandi* Jepson & Golden, 1987, as the female body is not markedly elongated, there is no clear posterior protuberance in adult females, it is not found in damp or wet conditions and, most importantly, it does not match most of the key morphological characters for this group (Jepson, 1987; Golden, 1989; Karssen *et al.*, 1998).

Table 3. *Sequence pair distances (% identity and divergence) of* Meloidogyne minor *n. sp.*, M. fallax, M. hapla, M. chitwoodi *and* M. naasi *calculated using the Jotun Hein alignment method.*

Species	% identity				
	M. chitwoodi	*M. fallax*	*M. hapla*	*M. minor*	*M. naasi*
M. chitwoodi	–	98.6	81.2	88.6	86.4
M. fallax	1.4	–	81.9	88.9	86.2
M. hapla	21.7	20.8	–	83.9	78.0
M. minor	12.4	12.0	18.1	–	84.5
M. naasi	15.0	15.2	26.1	17.3	–
	divergence				

Molecular and isozyme characterisation

ITS sequence homology analysis shows no match with ITS sequences from *M. chitwoodi*, *M. fallax*, *M. hapla*, *M. naasi* and other *Meloidogyne* species deposited in GenBank (Table 3). The Irish *M. minor* n. sp. population (Table 1) has the same ITS sequence as the type material from the Netherlands (results not included).

The species is also characterised by a N1a malate dehydrogenase (Mdh) pattern with two additional weaker bands after prolonged staining and one very slow weak VS1 esterase (Est) band (Fig. 7).

Biology and host range

Meloidogyne minor n. sp. reproduces by facultative meiotic parthenogenesis, with a haploid chromosome number of n = 17 (Hans van der Beek, pers. comm.).

Meloidogyne minor n. sp. failed to reproduce on marigold (*Tagetes patula* L.) and maize (*Zea mays* L.) but reproduced on carrot (*Daucus carota* L.), phacelia (*Phacelia tanacetifolia* Benth.), alfalfa (*Medicago sativa* L.), Italian ryegrass (*Lolium multiflorum* Lamk.), perennial ryegrass (*L. perenne* L.), oat (*Avena sativa* L.), lettuce (*Lactuca sativa* L.), tomato, vetch (*Vicia sativa* L.) and potato.

Discussion

Although *M. minor* n. sp. was described from tomato roots, it was originally isolated from potato roots. At the time of isolation only the roots were heavily infected *i.e.*, not the tubers. In additional glasshouse host plant tests using two different potato cultivars, the tubers were also heavily infected. The tuber symptoms are more or less comparable with those caused by *M. chitwoodi* and *M. fallax*, *i.e.*, numerous small pimple-like raised

areas apparent on the tuber surface with egg-laying females present just below the peel causing small dots of necrotic and brownish tissue. The only difference noticed, compared to the external tuber symptoms of *M. chitwoodi* and *M. fallax*, was that the pimple-like raised areas were more corky with *M. minor* n. sp. Further study is needed with different cultivars to ascertain if potato tubers become infested under field conditions and if this symptom difference is indeed useful for diagnosis.

It is clear that *M. minor* n. sp. appears to be a potentially dangerous plant-parasitic nematode capable of infesting most of the tested mono- and dicotyledonous plants. The samples examined from *A. stolonifera* var. *stolonifera* (creeping bent grass) golf greens were all collected because of the appearance of a yellow patch disease, known since 1999. The patches appear about 10 days after heavy spring rainfall from late May to early June and persist until October. On affected greens the patches appear in new positions each season. The symptoms have been observed in greens constructed with sand/peat (90/10%) sown with creeping bent cultivars. Greens affected include some on established courses and some on greens newly constructed on land formerly used for grass or arable farming. Field trials using plant nutrient solutions and fungicide treatments have not resulted in any improvement in the colour of affected greens (Mark Hunt and Kate Entwhistle, pers. comm.). In a glasshouse experiment, a single treatment of nematicide (oxamyl) applied to cores taken from affected and unaffected turf from three golf courses to control *M. minor* n. sp. resulted in improved grass growth and colour in affected cores. There were many more *M. minor* n. sp. J2 in the soil and roots of the yellow cores for all golf courses, densities averaging 3400, 2700 and 1700 J2/100 g dry soil in yellow cores while unaffected cores from the same greens had <100, 0 and 0 J2/100 g. Gall indices differentiated yellow and green cores and courses in the same way. The yellow patch disease and root galling have now been recorded in greens on some 20 golf courses (Kate Entwhistle, pers. comm.).

The presented malate dehydrogenase pattern, *i.e.*, a N1a type with two additional weaker bands, is rather unusual. A comparable type of Mdh pattern, the so-called hybridisation pattern, was published by van der Beek and Karssen (1997), as a result of a successful (F1) hybridisation between *M. chitwoodi* and *M. fallax*. Although the hatched F2 juveniles were small in number and not viable, it proved for the first time that interspecific hybridisation between two meiotic parthenogenetic species of root-knot nematodes is possible. Our Mdh pattern for *M. minor* n. sp. is comparable with this typical hybridisation pattern. Also, our esterase results show additional activity in some of the lanes in the so-called VF1 region, *i.e.*, the same position as *M. naasi* (Esbenshade & Triantaphyllou, 1985). This raises the intriguing possibility that *M. naasi* may be one parent of *M. minor* n. sp., as they are often found together and share also some morphological characters. More information is needed on the parents and the hybridisation process itself. Such hybridisation, with or without an additional polyploidisation, may be an important way of speciation within the genus *Meloidogyne*.

Acknowledgements

We thank Sigrid Arends (DLV, The Netherlands), Dr Hans van der Beek (WUR, The Netherlands) and Tine van Hoenselaar (PPS, The Netherlands) for cytogenetic and isozyme work, respectively; Dr Loes den Nijs (PPS, The Netherlands) for critical reading of this manuscript and Paul Fitter (National University of Ireland, Maynooth, Ireland) for providing some of the Irish populations. We are grateful to Mark Hunt (Headland Amenity Ltd, Great Chesterford, Essex, UK) and Kate Entwhistle (The Turf Disease Centre, Bramley, Hants, UK) for information about yellow patch and for providing populations from golf courses in UK and Ireland, and to Tony Mizen (IGER). The Institute of Grassland and Environmental Research, where part of this work was funded by Headland Amenity Ltd, is grant aided by the Biotechnology and Biological Sciences Research Council.

References

DE LEY, I.T., DE LEY, P., VIERSTRAETE, A., KARSSEN, G., MOENS, M. & VANFLETEREN, J. (2002). Phylogenetic analyses of *Meloidogyne* rDNA. *Journal of Nematology* 34, 319-327.

EBSARY, B.A. (1986). Species and distribution of Heteroderidae and Meloidogynidae (Nematoda: Tylenchida) in Canada. *Canadian Journal of Plant Pathology* 8, 170-184.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. (1985). Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17, 6-20.

GOLDEN, A.M. (1989). Further details and SEM observations on *Meloidogyne marylandi* (Nematoda: Meloidogynidae). *Journal of Nematology* 21, 453-461.

HEIN, J. (1990). Unified approach to alignment and phylogenies. *Methods in Enzymology* 183, 626-645.

JEPSON, S.B. (1987). *Identification of root-knot nematodes (*Meloidogyne *species)*. Wallingford, UK, CAB International, 265 pp.

KARSSEN, G. (2002). *The plant-parasitic nematode genus* Meloidogyne *Göldi, 1892 (Tylenchida) in Europe*. Leiden, The Netherlands, Brill Academic Publishers, 160 pp.

KARSSEN, G. & VAN HOENSELAAR, T. (1998). Revision of the genus *Meloidogyne* Göldi, 1892 (Nematoda: Heteroderidae) in Europe. *Nematologica* 44, 713-788.

KARSSEN, G., VAN AELST, A. & VAN DER PUTTEN, W.H. (1998). *Meloidogyne duytsi* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from Dutch coastal foredunes. *Fundamental and Applied Nematology* 21, 299-306.

TOWNSHEND, J.L., POTTER, J.W. & DAVIDSON, T.R. (1984). Some monocotyledonous and dicotyledonous hosts of *M. microtyla*. *Plant Disease* 68, 7-10.

VAN DER BEEK, J.G. & KARSSEN, G. (1997). Interspecific hybridization of meiotic parthenogenetic *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology* 87, 1061-1066.

VRAIN, T.C., WAKARCHUCK, D.A., LÉVESQUE, A.C. & HAMILTON, R.I. (1992). Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15, 563-573.

ZIJLSTRA, C. (1997). A fast PCR assay to identify *Meloidogyne hapla*, *M. chitwoodi* and *M. fallax*, and to sensitively differentiate them from each other and from *M. incognita* in mixtures. *Fundamental and Applied Nematology* 20, 505-511.

ZIJLSTRA, C. (2000). Identification of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* based on SCAR-PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology* 106, 283-290.