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Description of *Meloidogyne fallax* n. sp. (Nematoda : Heteroderidae), a root-knot nematode from The Netherlands

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Summary – A root-knot nematode, *Meloidogyne fallax* n. sp., is described and illustrated from tomato (*Lycopersicon esculentum* Mill.) from The Netherlands; this new species is characterized by : female stylet dorsally curved, 14.5 µm long, with rounded to transversely ovoid knobs, slightly sloping posteriorly; perineal pattern with a moderately high dorsal arch with coarse striae, indistinct lateral lines and ventral region oval to angular; male stylet 19.6 µm long, with large rounded knobs, set off from the shaft; male head region slightly set off, round raised labial disc and distinct lateral lips present; second-stage juvenile 403 µm long, tail 49.3 µm long tapering gradually until distinct hyaline tail terminus 13.5 µm long with broadly rounded tip. So far, the known distribution in Europe is restricted to a small number of locations in the south-eastern part of The Netherlands.

Résumé – ***Description de* Meloidogyne fallax *n. sp. (Nematoda : Heteroderidae), un nématode galligène des Pays-Bas.*** – Un nématode galligène, *Meloidogyne fallax* n. sp., provenant de la tomate (*Lycopersicon esculentum* Mill.) aux Pays-Bas, est décrit et figuré; la nouvelle espèce est caractérisée par le stylet de la femelle long de 14.5 µm, incurvé dorsalement, avec des boutons basaux arrondis à ovoïdes transversalement, légèrement inclinés postérieurement; la figure périnéale avec une arche dorsale modérément haute avec des stries épaisses, des lignes latérales indistinctes et une région ventrale ovale à angulaire; le stylet du mâle long de 19.6 µm, avec de grands boutons basaux bien séparés de la partie cylindrique; la région céphalique du mâle légèrement séparée du reste du corps, avec un disque labial arrondi et des lèvres latérales distinctes; les juvéniles de deuxième stade longs de 403 µm avec une queue longue de 49.3 µm s'amincissant régulièrement jusqu'à une partie hyaline terminale longue de 13.5 µm et se terminant en une extrémité bien arrondie. Sa répartition en Europe est jusqu'à présent limitée à un petit nombre de sites dans le sud-est des Pays-Bas.

Key-words : *Meloidogyne fallax,* morphology, root-knot nematode, taxonomy, The Netherlands.

In 1992 a field plot experiment was conducted near Baexem, The Netherlands, to assess the host suitability of *Meloidogyne chitwoodi* Golden *et al.,* 1980 on different crops. *Zea mays* L., a good host for *M. chitwoodi* (O'Bannon *et al.,* 1982; Jepson, 1987) was recorded as a non- to poor host. A critical re-examination of second-stage juveniles, compared with *M. chitwoodi* paratypes, indicated differences in body, tail and hyaline tail terminus length. In the autumn of the same year, the Baexem population was studied biochemically. An unique malate dehydrogenase (MDH) pattern was detected (called *M. chitwoodi* B-type), deviating from *M. chitwoodi* phenotypes (Karssen, 1994; van Meggelen *et al.,* 1994). A potato root-knot nematode survey in 1993 revealed seven other isozyme deviating populations (B-types) in the Baexem region. After a detailed study, of all known B-type populations (Karssen, 1995), they were considered to be morphologically and biologically different from *M. chitwoodi* Golden *et al.,* 1980. The nematode also differs from other known root-knot nematodes. Because of these differences this nematode is here designated as a new species and described as *Meloidogyne fallax* n. sp. The species epithet refers to the misleading morphological resemblance to *M. chitwoodi.*

Materials and methods

A culture of *M. fallax* n. sp., derived from infected black salsify (*Scorzonera hispanica* L.) from Baexem, was maintained on tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) in a greenhouse and regularly checked for purity with isozyme electrophoresis (Karssen *et al.,* 1995). This culture was used for all morphologic and morphometric studies. Second-stage juveniles (J2) and males were extracted by periodically rinsing fresh samples of infected roots in a spray mist chamber. Adult females were hand picked from infected tomato roots.

For light microscope (LM) studies eggs, J2, and males were fixed at 70 °C, and mounted in TAF (Courtney *et al.,* 1955). Perineal patterns were cut from live young females in 45 % lactic acid and mounted in glycerin (Taylor & Netscher, 1974). Adult females were fixed in hot TAF, the head region was cut and mounted in TAF. Drawings were made with a drawing tube, and photographs and measurements were taken with a light microscope using differential interference contrast (DIC).

For scanning electron microscope (SEM) studies males and females were fixed in 3 % glutaraldehyde buf-

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fered with 0.05 M phosphate buffer (pH 6.8) for 1.5 h and post-fixed with 2 % osmium tetroxide for 2 h at 22 °C. The specimens were dehydrated in a seven-graded series of ethanol, critical-point dried with carbon dioxide, and sputter coated with a layer of 20 nm gold-palladium (Wergin, 1981). The nematodes were examined with a Jeol JSM 5200 scanning electron microscope operating at 15 kV accelerating voltage.

For preparation of type material females, males and J2 were fixed in hot FP [11 ml formalin (40 % formaldehyde), 1 ml propionic acid, 88 ml distilled water] and processed by a rapid glycerin-ethanol method (Seinhorst, 1959). The specimens were mounted in desiccated glycerin on Cobb slides.

Meloidogyne fallax n. sp.
= *Meloidogyne chitwoodi* B-type *in* Van Meggelen *et al.* (1994), Karssen (1995)

(Figs 1-8)

Measurements

See Table 1.

Description

Female : Body annulated, pearly white, globular to pear shaped, with slight posterior protuberance and distinct neck region projecting from the body axis at an angle of up to 90° to one side. Head region set off from body, marked with one or two annules. Head cap distinct but variable in shape; labial disc slightly elevated. Cephalic framework weakly sclerotized; vestibule extension distinct. Stylet cone dorsally curved and shaft cylindrical; knobs large, rounded to transversely ovoid, slightly sloping posteriorly from the shaft. Excretory pore located between head end and metacorpus levels. One or two large vesicles and several smaller ones located along the lumen lining. Pharyngeal glands variable in size and shape. Perineal pattern ovoid to oval shaped, sometimes rectangular; dorsal arch ranging from low to moderately high, with coarse striae. Tail terminus indistinct without punctations. Phasmids small and difficult to observe. Perivulval area devoid of striae. Lateral lines indistinct (LM), appearing as a weak indentation under SEM, increasing towards the tail terminus region and resulting in a relatively large area without striae. Ventral pattern region oval to angular shaped; striae moderately coarse.

Male : Body vermiform, slightly tapering anteriorly, bluntly rounded posteriorly. Cuticle with distinct transverse striae. Lateral field with four incisures ; outer bands irregularly areolated; a fifth broken longitudinal incisure is rarely present near mid-body. Head slightly set off, with a single post-labial annule (sometimes called head region) usually partly subdivided by a transverse incisure. Labial disc rounded, elevated and fused with medial lips. Prestoma hexagonal in shape with six inner cephalic sensilla adjacent to the rim. Medial lips crescent shaped with raised edges at lateral sides. Four cephalic sensilla small and marked by cuticular depressions on the medial lips. Amphidial openings appear as elongated slits between labial disc and medium sized lateral lips. Cephalic framework moderately sclerotized, vestibule extension distinct. Stylet cone straight; shaft cylindrical; knobs large and rounded, set off from the shaft. Pharynx with slender procorpus, metacorpus oval shaped with pronounced valve. Ventrally overlapping pharyngeal gland lobe variable in length. Hemizonid, 2-3 μm in length, two to four annules anterior to excretory pore. Testis usually long, monorchic, with reflexed or outstretched germinal zone. Tail short and twisted. Spicules slender, curved ventrally; gubernaculum slightly crescent shaped. Phasmids located anterior to cloaca.

Second-stage juveniles : Body moderately long, vermiform, tapering at both ends but posteriorly more than anteriorly. Body annules small but distinct. Lateral field with four incisures, not areolated. Head region truncate, slightly set off from body. Head cap low and narrower than head region. Cephalic framework weakly sclerotized, vestibule extension distinct. Stylet slender and moderately long, cone straight; shaft cylindrical; knobs distinct, rounded and set off from the shaft. Pharynx with faintly outlined procorpus and oval shaped metacorpus with distinct valve. Oesophageal gland lobe variable in length, overlapping intestine ventrally. Hemizonid distinct at the level of the excretory pore. Moderately sized tail, gradually tapering until hyaline tail terminus, with inflated proctodeum. Phasmids difficult to observe, small, slightly posterior to anus. A rounded hypodermis marks the anterior position of the smooth hyaline tail terminus; tail terminus ending in a broadly rounded tip. Terminus generally marked by faint cuticular constrictions.

Eggs (n = 30) : Length 89.7-103.6 μm (94.4 ± 3.39; SE = 0.62); width 34.1-44.2 μm (38.9 ± 3.17; SE = 0.58); length/width ratio 2.1-2.9 (2.4 ± 0.19; SE = 0.04).

Type material

Holotype : Female on slide WT 3127, collection of Agricultural University, Wageningen, The Netherlands. *Paratypes :* Two female perineal patterns and heads, two males and five J2's deposited at each of the following nematode collections : Agricultural University, Wageningen, The Netherlands (WT 3128-3130); Instituut voor Dierkunde, Rijksuniversiteit, Gent, Belgium; Rothamsted Experimental Station, Harpenden, U.K.

Type host, type locality and distribution

Described from roots of tomato (*Lycopersicon esculentum* Mill.). The nematodes were originally derived from infected roots of black salsify (*Scorzonera hispanica* L. cv. Lange Jan) from arable land one mile north of Baexem, province of Limburg, The Netherlands. The known dis-

Table 1. *Morphometrics of* Meloidogyne fallax *n. sp.* (*n = 30;* all measurements in μm).

Character	Females	Males	J2
L	491.3 ± 74.9 (404.1-720.3)	1171 ± 193.6 (736.2-1520.1)	403.2 ± 15.2 (381.4-435.2)
Greatest body diam.	361.6 ± 57.7 (256.2-464.1)	30.6 ± 2.1 (27.2-43.8)	14.3 ± 0.7 (13.3-16.4)
Body diam. at stylet knobs	-	17.7 ± 0.6 (16.4-19.0)	-
Body diam. at excr. pore	-	26.2 ± 1.7 (23.4-29.7)	-
Body diam. at anus	-	-	10.4 ± 0.4 (9.5-10.7)
Head region height	-	4.6 ± 0.3 (4.4-5.1)	2.7 ± 0.4 (1.9-3.2)
Head region diam.	-	10.7 ± 0.7 (9.5-12.0)	5.5 ± 0.3 (5.1-6.3)
Neck length	149.7 ± 33.0 (96.4-224.6)	-	-
Neck diam.	97.7 ± 23.6 (64.6-160.2)	-	-
Stylet	14.5 ± 0.4 (13.9-15.2)	19.6 ± 0.8 (18.9-20.9)	10.8 ± 0.4 (10.1-11.4)
Stylet base-ant. end	-	-	14.6 ± 0.7 (13.9-16.4)
Stylet cone	-	10.1-0.5 (9.5-12.0)	-
Stylet shaft and knobs	-	8.9 ± 0.5 (8.2-9.5)	5.5 ± 0.4 (5.1-6.3)
Stylet knob height	2.3 ± 0.3 (2.0-2.5)	3.0 ± 0.3 (2.5-3.2)	1.5 ± 0.3 (1.3-1.9)
Stylet knob width	4.2 ± 0.3 (3.8-4.4)	4.9 ± 0.4 (3.8-5.1)	2.3 ± 0.3 (1.9-2.5)
DGO	4.3 ± 0.5 (3.8-6.3)	4.4 ± 0.7 (3.2-5.7)	3.5 ± 0.3 (3.2-3.8)
Ant. end to metacorpus	-	65.4 ± 4.1 (58.8-72.7)	48.0 ± 3.5 (44.2-54.4)
Metacorpus length	41.9 ± 3.3 (34.8-47.4)	-	-
Metacorpus diam.	39.6 ± 3.8 (31.6-44.9)	-	-
Metacorpus valve length	12.0 ± 0.9 (10.1-13.9)	-	4.0 ± 0.3 (3.2-3.8)
Metacorpus valve width	94.4 ± 3.4 (89.7-103.6)	-	3.3 ± 0.2 (3.2-3.8)
Excretory pore-ant. end	22.5 ± 5.3 (12.6-32.9)	120.9 ± 11.4 (94.8-139.9)	69.1 ± 3.4 (63.2-77.1)
Tail	-	9.2 ± 1.4 (7.6-12.1)	49.3 ± 2.2 (46.1-55.6)
Tail terminus length	-	-	13.5 ± 1.0 (12.2-15.8)
Phasmids-post. end	-	11.7 ± 1.5 (9.5-15.2)	-
Spicule	-	26.6 ± 2.0 (22.1-29.7)	-
Gubernaculum	-	7.7 ± 0.5 (7.0-8.5)	-
Testis	-	496.5 ± 144.1 (316.3-695.2)	-
Vulva slit length	24.7 ± 1.8 (20.2-28.4)	-	-
Vulva-anus distance	15.9 ± 1.8 (12.6-19.0)	-	-
a	1.4 ± 0.3 (0.9-2.0)	38.2 ± 6.8 (21.2-53.5)	28.1 ± 1.7 (23.8-40.4)
c	-	127.8 ± 28.5 (82.7-201.7)	8.2 ± 0.5 (6.9-8.6)
c'	-	-	4.8 ± 0.3 (4.3-5.3)
T	-	42.4 ± 8.4 (24.4-62.1)	-
Body length/neck length	3.3 ± 0.9 (1.9-5.6)	-	-
Body length/ant. end to metacorpus valve	-	-	8.4 ± 0.7 (6.4-9.3)
Stylet knob width/height	1.9 ± 0.2 (1.5-2.2)	1.6 ± 0.2 (1.4-2.0)	-
Metacorpus length/width	1.1 ± 0.1 (0.9-1.2)	-	-
Excretory pore/L x 100	-	10.3 ± 1.9 (8.3-12.9)	17.2 ± 1.1 (16.1-19.3)

tribution of *M. fallax* n. sp. is restricted to the south-eastern part of The Netherlands close to the Belgian and German border region (Fig. 9). The distribution of *M. fallax* n. sp. occurs adjacent to the *M. chitwoodi* distribution pattern, suggesting a parapatric distribution.

Diagnosis and relationship

M. fallax n. sp. is characterized by a dorsally curved female stylet 14.5 μm (13.9-15.2) long with rounded set off stylet knobs. Oval shaped perineal pattern with coarse striae and moderately high dorsal arch. Male stylet length 19.6 μm (18.9-20.9) with prominent set off rounded knobs. The labial disc is elevated, crescent shaped medial lips raised at lateral side and distinct lateral lips. The J2's hemizonid is at the same level with the excretory pore. Tail and hyaline tail length 49.3 μm

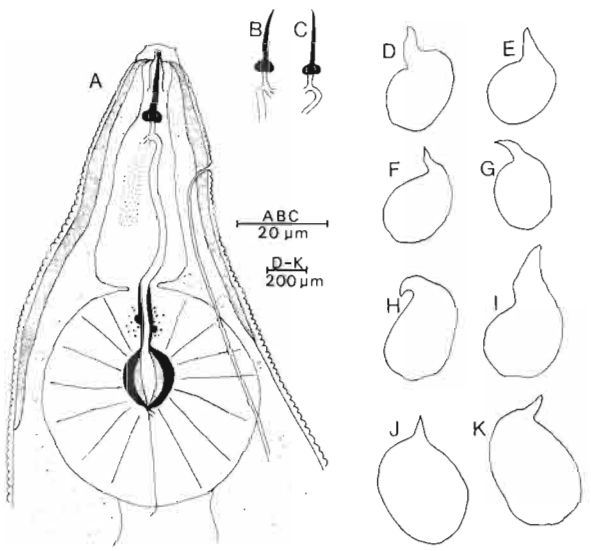

Fig. 1. Meloidogyne fallax *n. sp. females. A : Pharyngeal region (lateral view); B, C : Stylets (lateral view); D-K : Female body shapes.*

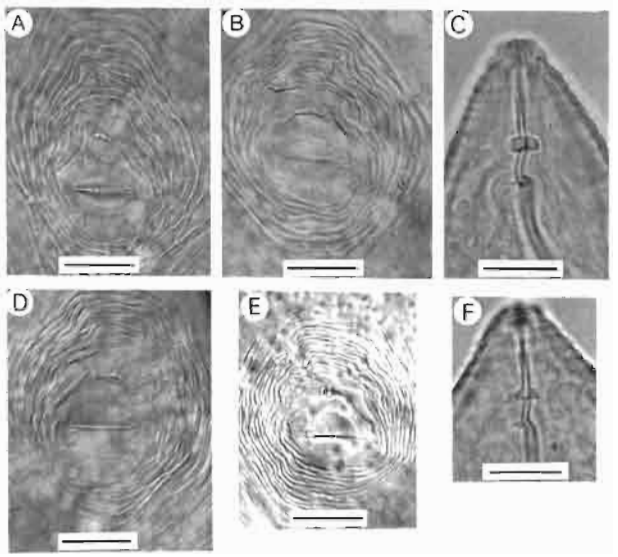

Fig. 2. *LM photographs of females of* Meloidogyne fallax *n. sp. (A-D) and* M. chitwoodi *(E-F). A, B, D, E : Perineal patterns; C, F : Head end (lateral view).* (Scale bars : A, B, D, E = 25 µm; C, F = 10 µm).

(46.1-55.6) and 13.5 µm (12.1-15.8), respectively. *M. fallax* n. sp. reproduces by facultative meiotic parthenogenesis, the haploid chromosome number is n = 18 (H. v.d. Beek, pers. comm.). *M. fallax* n. sp. is characterized by an unique malate dehydrogenase (MDH) pattern, not described by Esbenshade and Triantaphyllou (1987), and the lack of any major esterase (EST) band (Fig. 8). In combination these patterns are useful to dif-

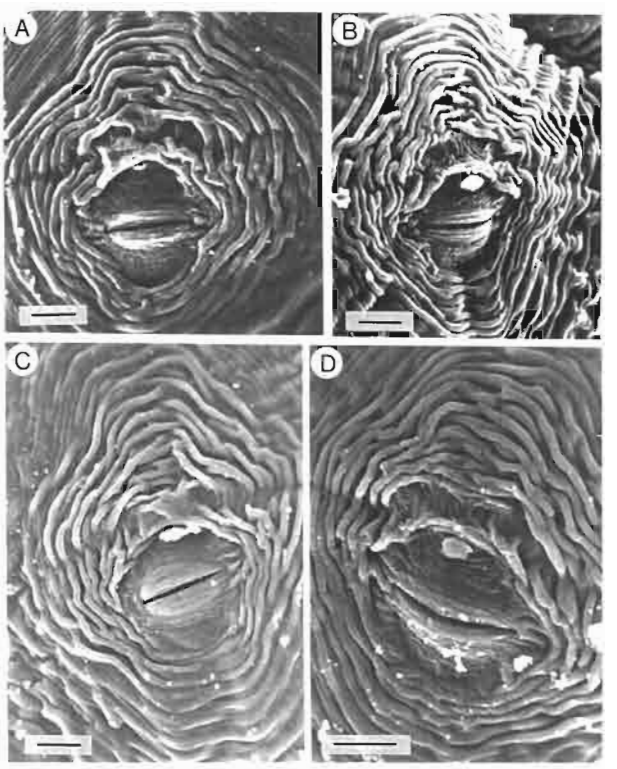

Fig. 3. *SEM photographs of* Meloidogyne fallax *n. sp. females. A-D : Perineal patterns.* (Scale bar = 10 µm).

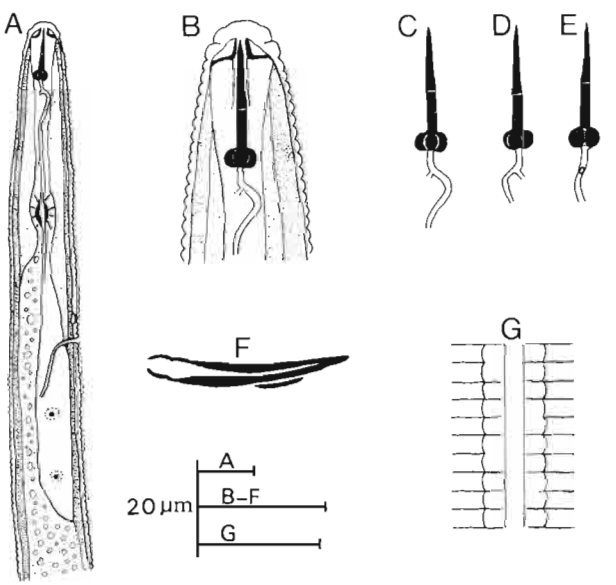

Fig. 4. Meloidogyne fallax *n. sp. males. A : Pharyngeal region (lateral view); B : Head end (lateral view); C-E; Stylets (lateral, lateral, ventral view, respectively); F : Spicule and gubernaculum (lateral view); G : Lateral field at mid-body.*

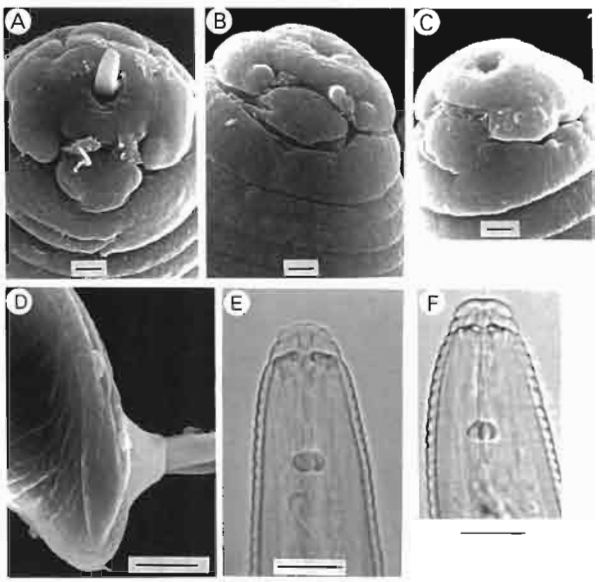

Fig. 5. *SEM (A-D) and LM (E-F) photographs of males of* Meloidogyne fallax *n. sp. (A, B, D, E) and* M. chitwoodi *(C, F). A : Cephalic region (face view); B, C : Cephalic region (lateral view); D : Tail (lateral view); E, F : Cephalic region (lateral view).* (Scale bars : A, B, C = 1 µm; D = 5 µm; E, F = 10 µm).

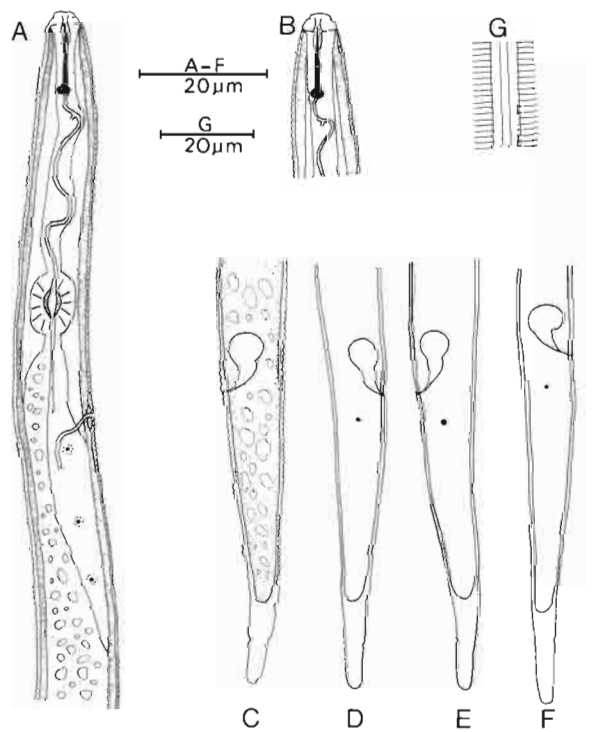

Fig. 6. Meloidogyne fallax *n. sp. second-stage juveniles. A : Pharyngeal region (lateral view); B : Head end (lateral view); C-F : Tail shape variation (lateral view) ; G : Lateral field at mid-body.*

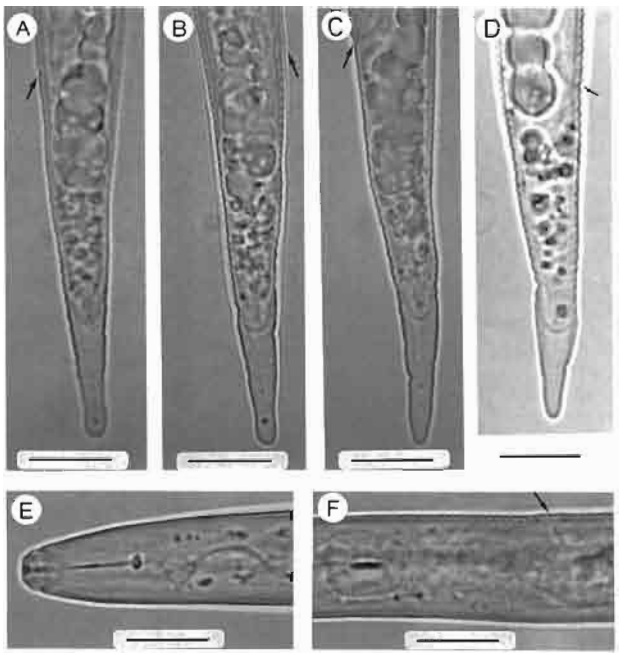

Fig. 7. *LM photographs of second-stage juveniles of* Meloidogyne fallax *n. sp. (A-C, E, F) and* M. chitwoodi *(D) (lateral view). A-D : Tail variation (arrow = anus); E : Head end; F : Meta- and postcorpus region (arrow = hemizonid).* (Scale bar = 10 µm).

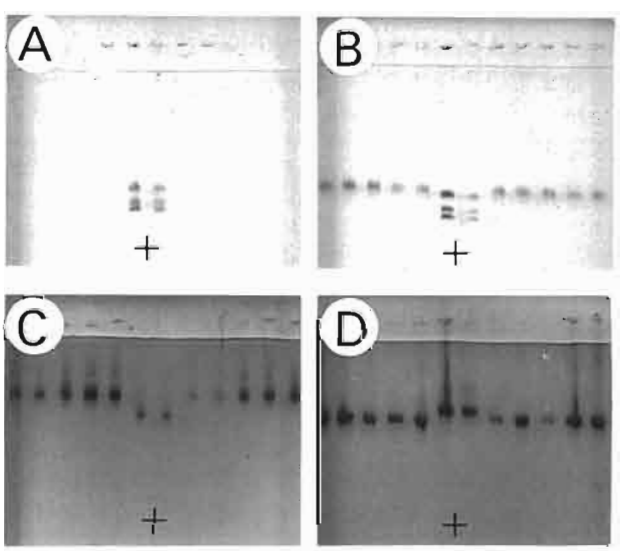

Fig. 8. *Esterase (A-B) and malate dehydrogenase (C-D) isozyme patterns of* Meloidogyne fallax *n. sp. (A, C) and* M. chitwoodi *(B, D)* (see also van Meggelen *et al.,* 1994).

ferentiate *M. fallax* from other known *Meloidogyne* species. Beside the mentioned difference in isozyme patterns between *M. chitwoodi* and *M. fallax* n. sp., biochemical differentiation was also confirmed by

Fig. 9. *Distribution pattern of* Meloidogyne fallax *n. sp. in The Netherlands.* (Black dot with star is type locality).

restriction analysis of ribosomal (ITS) DNA (Zijlstra *et al.,* 1995).

Meloidogyne fallax n. sp. differs from the morphologically close related *M. chitwoodi* Golden *et al.,* 1980 by greater female and male stylet length, absence of small, irregular outlined male and female stylet knobs (Eisenback & Hirschmann, 1991), male labial disk elevated, longer juvenile body-, tail- and hyaline tail length, different hyaline tail shape, hemizonid position, esterase and malate dehydrogenase patterns (Figs 2, 5, 7, 8); from *M. hapla* Chitwood, 1949 by the absence of fine, smooth striae, rounded and flattened dorsal arch and tail area punctations in the female perineal pattern, broader J2 tail and tail terminus with distinct hyaline part, shorter female and male stylet length, and the absence of small rounded stylet knobs; from *M. artiellia* Franklin, 1961 and *M. ardenensis* Santos, 1968 by much greater J2 body-, tail- and hyaline tail length and by hemizonid position relative to excretory pore; from *M. naasi* Franklin, 1965 by smaller J2 body-, tail- and hyaline tail length and the absence of vesicles in the juvenile metacorpus. The presence of vesicles or vesicle-like structures in the metacorpus of female *Meloidogyne* species was reported as " unique " with the description of *M. chitwoodi* Golden *et al.,* 1980, although first reported in *M. kikuyensis* De Grisse, 1961 and *M. oryzae* Maas *et al.,* 1978. These structures are also described in *M. hispanica* Hirschmann, 1986, *M. maritima* Jepson, 1987, *M. konaensis* Eisenback *et al.,* 1995, *M. fallax* n. sp. and some *M. hapla* populations from The Netherlands (Karssen, unpubl.). Therefore these vesicles are not useful as a discriminating character for *M. chitwoodi* identification.

Remarks

Host preference studies showed that *Phaseolus vulgaris* L. cvs Iprin, Strike and Groffy, *Zea mays* L., *Potentilla fructicosa* L., and *Erica cinerea* L. were good hosts for *M. chitwoodi* but not for *M. fallax* n. sp. On the other hand *M. fallax* n. sp. reproduced well on *Oenothera erythrosepala* Borb., *Phacelia tanacetifolia* Bentham, *Dicentra spectabilis* (L.) Lem. and *Hemerocallis* cv. Rajah, while *M. chitwoodi* reproduced not or poorly (Brinkman, Goossens & Van Riel, pers. comm.).

Acknowledgments

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Erratum

In the following publication:

KARSSEN, G. – Description of *Meloidogyne fallax* n. sp. (Nematoda: Heteroderidae), a root knot nematode from The Netherlands. *Fundam. appl. Nematol.*, 19 (6): 593-599 (1996),

Table 1 (p. 595) has to be corrected in the following way:

instead:	
– Female metacorpus valve width	94.4 ± 3.4 (89.7-103.6)
read:	
– Female metacorpus valve width	9.7 ± 0.6 (8.2-11.4)