

Meloidogyne duytsi n. sp. (Nematoda: Heteroderidae), a root-knot nematode from Dutch coastal foredunes

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Summary – A root-knot nematode, *Meloidogyne duytsi* n. sp., is described and illustrated from *Elymus farctus* (Viv.) Melderis from Dutch coastal foredunes. This new species is characterized by the following characters: female with stylet slightly curved dorsally, 13.3 µm long, with large transversely ovoid knobs set off from the shaft, asymmetrical perineal pattern with a relatively low dorsal arch and coarse striae, lateral lines indistinct, and lateral wing present in most patterns, male with stylet 19.8 µm long, with large transversely ovoid knobs set off from the shaft, head region high and set off, labial disc slightly raised, lateral lips small, and tail conical with rounded tip, and second-stage juvenile 424 µm long, with hemizonid anterior to excretory pore, tail 70.4 µm long, tapering and slightly curved ventrally, and hyaline tail terminus distinct, 11.3 µm long. A previously unknown malate dehydrogenase pattern and a slow esterase isozyme band were detected. *M. duytsi* n. sp. was also found in mixture with *M. maritima* Jepson, 1987 on *Ammophila arenaria* (L.) Link. © Orstom/Elsevier, Paris

Résumé – *Meloidogyne duytsi* n. sp. (Nematoda : Heteroderidae), un nématode galligène des dunes côtières hollandaises – Un nématode galligène, *Meloidogyne duytsi* n. sp., parasitant *Elymus farctus* (Viv.) Melderis croissant sur les avant-dunes côtières hollandaises, est décrit et illustré. Cette nouvelle espèce est caractérisée par : femelle ayant un stylet long de 13,3 µm, légèrement incurvé dorsalement, boutons basaux grands et transversalement ovoïdes, bien séparés de la hampe ; empreinte périnéale asymétrique avec arche dorsale relativement basse, stries épaisses et lignes latérales indistinctes ; queue pointue. Mâle : région céphalique haute et séparée du reste du corps ; disque labial légèrement proéminent ; secteurs latéraux labiaux réduits ; queue conique à extrémité arrondie. Juvéniles de deuxième stade : longueur moyenne de 424 µm ; hémizonide antérieur au pore excréteur ; queue longue de 70,4 µm, effilée, légèrement incurvée ventralement à partie hyaline terminale distincte et longue de 11,3 µm. Un profil nouveau pour la malate deshydrogénase et une bande estérasique de type lent ont été détectés. *M. duytsi* n. sp. a également été collecté en mélange avec *M. maritima* Jepson, 1987 sur *Ammophila arenaria* (L.) Link. © Orstom/Elsevier, Paris

Keywords: foredunes, *Elymus farctus*, *Meloidogyne duytsi*, morphology, root-knot nematode.

In 1976, Sturhan reported the root-knot nematode *M. graminis* (Sledge & Golden, 1964) Whitehead, 1968 for the first time on *Ammophila arenaria* (L.) Link from foredunes in Europe. In 1978 and 1980, Cook found *M. graminis* in various locations in the UK on *A. arenaria* (see Jepson, 1987). Later, these populations were described as a new species: *M. maritima* Jepson, 1987. Brinkman (1985) reported the occurrence of *M. graminis* (attributed to *M. maritima* by Maas *et al.*, 1987) and an unknown *Meloidogyne* species on *A. arenaria*, sampled by Dr. K. Kuiper in foredunes on the Dutch island of Schiermonnikoog. A decline of the natural sand-stabilizing plant species *A. arenaria* is extensively studied by ecologists in the foredunes near Oostvoorne, The Netherlands. A disease complex of nematodes and fungi is thought to be responsible for the degeneration of *A. arenaria* (Van der Putten *et al.*, 1993; De Rooij-Van der Goes,

1995). In 1996, the senior author received *Elymus farctus* (Viv.) Melderis samples from foredunes near Oostvoorne for identification. The samples included an undescribed *Meloidogyne* species very different from *M. maritima* and other root-knot nematodes. This new species is here described as *Meloidogyne duytsi* n. sp.

Materials and methods

M. duytsi n. sp. was isolated from the roots of infected *E. farctus* plants, growing on foredunes on the beach plane in front of the coastal dunes. Before morphological and morphometric studies, the nematode was tested for purity with isozyme electrophoresis based on esterase and malate dehydrogenase of young egg-laying females (Karssen *et al.*, 1995). Adult females were hand-picked from infected roots, while males and second-stage juveniles (J2) were extracted

Table 1. Morphometrics of *Meloidogyne duytisi* n. sp. (mean + SD [range]; n = 30; all measurements in μm).

Character	Females	Males	J2
L	865.1 ± 108.3 (560.0-960.0)	1316.0 ± 173.6 (960.0-1680.0)	423.6 ± 13.7 (403.2-454.4)
Greatest body diam.	591.0 ± 115.9 (368.0-800.0)	34.4 ± 2.7 (27.2-36.7)	17.1 ± 0.6 (15.8-17.7)
Body diam. at stylet knobs	-	19.7 ± 0.7 (18.3-20.9)	-
Body diam. at excret. pore	-	30.1 ± 1.9 (25.3-32.9)	-
Body diam. at anus	-	-	12.3 ± 0.5 (11.4-13.3)
Head region height	-	5.4 ± 0.5 (4.4-6.3)	2.4 ± 0.3 (1.9-3.1)
Head region diam.	-	11.1 ± 0.5 (10.1-12.0)	6.0 ± 0.3 (5.7-6.3)
Neck length	270.9 ± 52.2 (192.0-336.0)	-	-
Neck diam.	118.4 ± 21.6 (80.0-160.0)	-	-
Stylet	13.3 ± 0.3 (12.6-13.9)	19.8 ± 0.3 (19.0-20.2)	11.1 ± 0.4 (10.7-12.0)
Stylet base-ant. end	-	-	15.1 ± 0.6 (13.3-15.8)
Stylet cone	-	10.3 ± 0.3 (9.5-10.8)	-
Stylet shaft and knobs	-	9.5 ± 10.1 (8.2-10.1)	5.9 ± 0.3 (5.7-6.3)
Stylet knob height	2.1 ± 0.3 (1.9-2.5)	3.0 ± 0.3 (2.5-3.2)	1.6 ± 0.3 (1.3-1.9)
Stylet knob width	4.7 ± 0.4 (4.4-5.1)	5.3 ± 0.3 (5.1-5.7)	2.7 ± 0.4 (1.9-3.2)
DGO	3.8 ± 0.4 (3.2-4.4)	4.0 ± 0.3 (3.8-5.1)	3.6 ± 0.3 (3.2-3.8)
Ant. end to metacarpus	-	74.1 ± 5.0 (63.2-87.2)	55.2 ± 2.0 (49.3-59.4)
Metacarpus length	38.4 ± 2.6 (34.8-41.1)	-	-
Metacarpus diam.	37.1 ± 2.9 (31.0-41.1)	-	-
Metacarpus valve length	12.0 ± 0.6 (11.4-13.3)	-	4.2 ± 0.4 (3.2-3.8)
Metacarpus valve width	9.6 ± 0.8 (8.9-12.0)	-	3.8 ± 0.2 (3.2-4.4)
Excretory pore-ant. end	37.5 ± 4.7 (30.3-47.4)	137.5 ± 11.8 (117.0-155.5)	79.4 ± 86.6 (69.5-86.6)
Tail	-	13.2 ± 1.5 (10.1-15.8)	70.4 ± 3.1 (65.1-76.5)
Tail hyaline part length	-	-	11.3 ± 1.0 (9.5-13.3)
Phasmids-post. end	-	8.6 ± 1.1 (5.7-10.1)	-
Spicule	-	25.9 ± 0.9 (24.0-27.2)	-
Gubernaculum	-	7.1 ± 0.4 (6.3-7.6)	-
Testis	-	692.2 ± 114.1 (379.2-853.2)	-
Vulva slit length	23.2 ± 2.2 (19.6-25.3)	-	-
Vulva-anus distance	21.5 ± 2.5 (15.8-26.5)	-	-
a	1.5 ± 0.2 (1.2-1.8)	38.2 ± 3.6 (30.3-45.8)	24.9 ± 1.1 (23.2-27.3)
c	-	100.6 ± 15.7 (76.2-130.8)	6.0 ± 0.2 (5.6-6.6)
c'	-	-	5.7 ± 0.3 (5.2-6.4)
T	-	52.6 ± 6.6 (38.7-65.8)	-
Body length/neck length	3.3 ± 0.7 (2.6-5.0)	-	-
Body length/ant. end to metacarpus valve	-	-	7.7 ± 0.3 (7.2-8.6)
Stylet knob width/height	2.3 ± 0.31 (1.8-2.7)	.8 ± 0.2 (1.6-2.3)	-
Metacarpus length/width	1.0 ± 0.1 (1.0-1.1)	-	-
(Excretory pore/L.) × 100	-	10.5 ± 1.0 (8.5-13.5)	18.8 ± 0.7 (16.3-19.9)

by rinsing fresh root samples in a mist chamber for one week. Eggs, J2, males and females (head and perineal regions) were fixed at 70°C, and mounted in TAF (Courtney *et al.*, 1955). Measurements and photographs were taken with a light microscope using differential interference contrast. For the preparation of type material and for scanning electron microscope (SEM) observations, J2, males and females were prepared as previously described (Karssen, 1996). The nematodes were sputter coated with 4-5 nm Pt in a dedicated preparation chamber (CT 1500 HT, Oxford Instruments) and examined with a field emission electron microscope (Jeol 6300 F) at 5 kV.

***Meloidogyne duytsi** n. sp.**
(Figs 1-5)

MEASUREMENTS

Females, Males and J2: See Table 1.

Eggs (n = 30): Length = 99.2-116.8 µm (106.2 + 5.4; SE = 1.0); width = 44.8-52.4 µm (47.9 + 2.0; SE = 0.4); length/width = 2.0-2.5 (2.2 + 0.2; SE = 0.04).

DESCRIPTION

Female: Body relatively large, annulated, pearly white, globular shaped, neck region distinct and projecting sideways from the body axis, posterior protuberance not observed. Head region set off from body. Head cap distinct but rather variable in shape, labial disk slightly elevated. Cephalic framework weakly sclerotized. Stylet cone slightly curved dorsally, shaft cylindrical. Knobs large, transversely ovoid, and set off from the shaft. Excretory pore located half-way between metacarpus and head end. No vesicles observed near the lumen lining of the metacarpus. Pharyngeal glands rather variable in shape and size. Perineal pattern asymmetrical; dorsal arch relatively low, with coarse striae; one lateral wing, variable in size, present in most patterns. Tail terminus distinct and without punctations, with indistinct lateral lines. Phasmids small, located just above the covered anus; inter phasmidial distance 20.2 ± 2.7 µm. Ventral pattern region angular shaped with fine striae.

Male: Body vermiform, annulated and twisted. Four incisures present in lateral field; outer bands regularly areolated. Head set off from the body, one relatively high post-labial annule (or head region) present, transverse incisures not observed. Labial disc rounded, slightly elevated, fused with the submedial lips into a head cap. Submedial lips rounded, lateral edges slightly wider than the labial disc. Prestoma hexagonal in shape with six inner sensilla. Four cephalic sensillae present on the submedial lips and

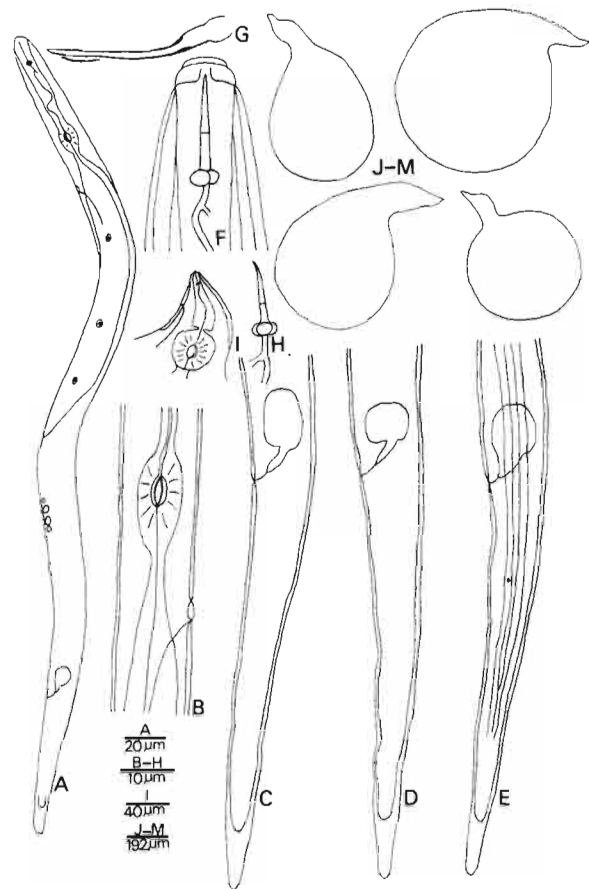


Fig. 1. *Meloidogyne duytsi* n. sp. Second-stage juvenile. A: Body; B: Metacarpus region; C-E: Tail variation. Male. F: Head end; G: Spicule. Female. H: Stylet; I: Head end; J-M: Variation of body shape.

marked by small cuticular depressions. Slit-like amphidial openings present between the head cap and the relatively small lateral lips. Cephalic framework moderately sclerotized; vestibule extension indistinct. Stylet with straight cone and cylindrical shaft; large transversely ovoid knobs, set off from the shaft. Pharynx with slender procorpus and oval-shaped metacarpus. Pharyngeal gland lobe ventrally overlapping the intestine, variable in length, two subventral gland nuclei present. Hemizonid, 4-5 µm long, 5 to 12 µm anterior to the excretory pore. Testis long, monorchic and usually with outstretched germinal zone. Tail twisted, relatively short, conical with rounded tip. Spicula slender, curved ventrally; two pores present on the spiculum tip. Phasmids located posterior to cloaca.

Second-stage juveniles: Body vermiform, moderately long and annulated. Lateral field with four incisures,

* The species name refers to Henk Duijts who found it near Oostvoorne for the first time and marked it as a species different from *M. maritima*.

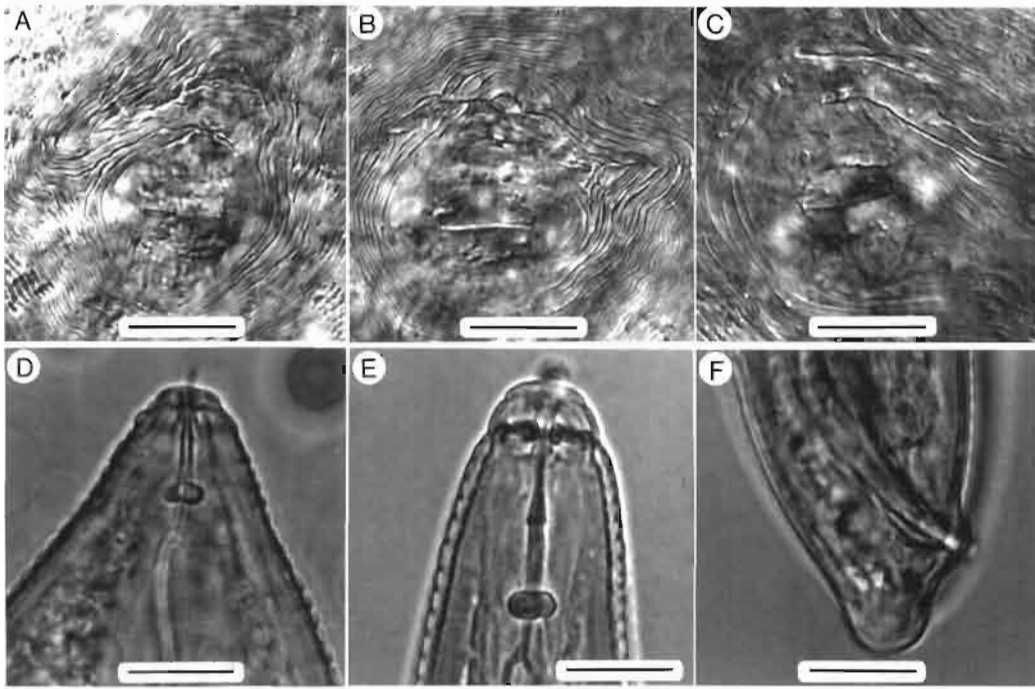


Fig. 2. LM photographs of *Meloidogyne duytsi* n. sp. Female. A-C: Perineal patterns; D: Head end (lateral view). Male. E: Head end (lateral view); F: Tail (lateral view). (Scale bars: A-C = 25 μ m; D-F = 10 μ m).

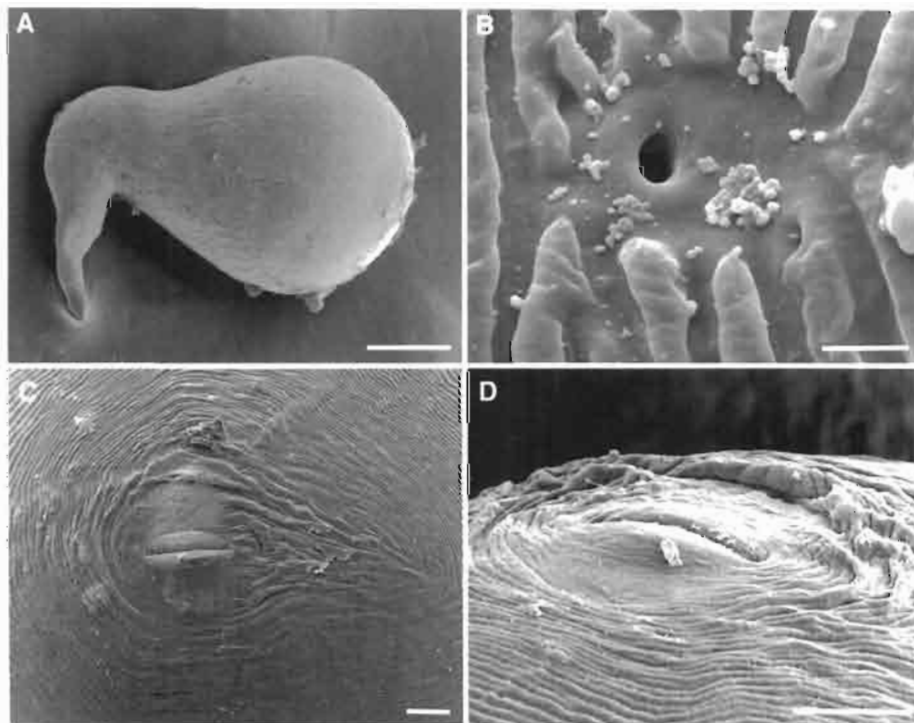


Fig. 3. SEM photographs of females of *Meloidogyne duytsi* n. sp. A: Body; B: Excretory pore; C: Perineal pattern; D: Perineal pattern (side view). (Scale bars: A-D, F = 1 μ m; E = 100 μ m).

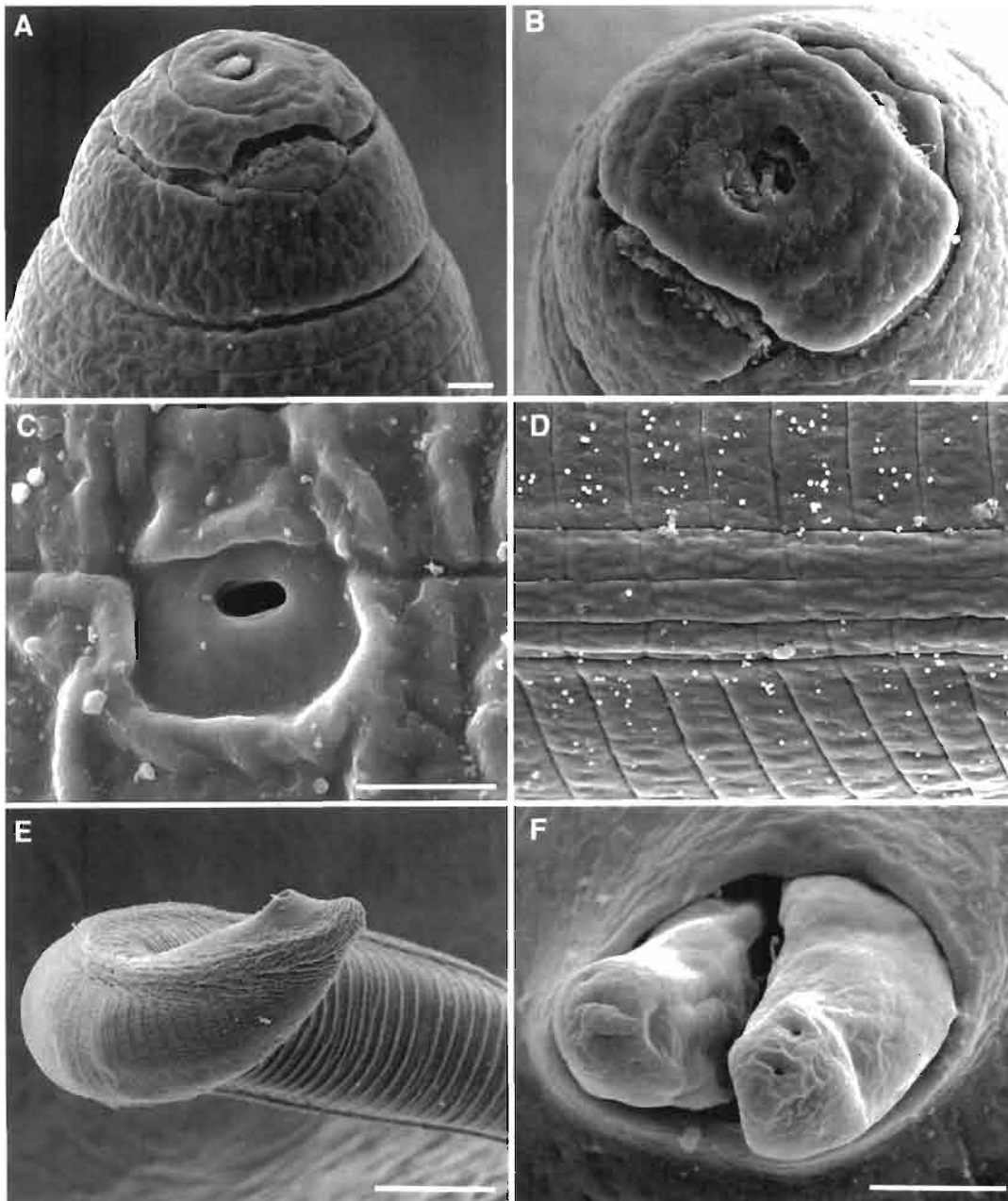


Fig. 4. SEM photographs of males of *Meloidogyne duytsi* n. sp. A: Cephalic region (lateral view); B: Cephalic region (face view); C: Excretory pore; D: Lateral field; E: Tail region (lateral view); F: Spicule. (Scale bars: A = 100 μ m, B = 1 μ m, C, D = 10 μ m).

weakly areolated. Head region truncate and slightly set off from the body. Cephalic framework weakly sclerotized, vestibule extension indistinct. Stylet moderately long, cone straight, shaft cylindrical; knobs rounded to transversely ovoid. Metacarpus ovoid, triradiate lumen with moderately sclerotized lining.

Pharyngeal gland lobe relatively long, well developed, ventral overlap of the intestine difficult to observe, three gland nuclei present. Hemizonid anterior, adjacent to the excretory pore. Tail slightly curved ventrally, relatively long, gradually tapering until distinct short hyaline tail terminus, rectum usually inflated.

Table 2. Relationships of *Meloidogyne duytsi* n. sp. with some species of the *Meloidogyne* "graminis"-group (all measurements in μm) *.

Character	<i>M. duytsi</i> n. sp.	<i>M. maritima</i>	<i>M. kralli</i>	<i>M. naasi</i>	<i>M. graminis</i>	<i>M. sasseri</i>
Females						
n	30	1	20	25	20	60
Stylet length	13.3 (12.6-13.9)	13.5	13.3 (12.5-14.5)	13 (11-15)	12.4 (11.7-13.4)	14 13-15)
Knob shape	transversely ovoid	rounded to ovoid	transversely ovoid	rounded pear shape	transversely ovoid	rounded
Posterior protuberance	no	yes	yes	slight	yes	slight
Males						
n	30	20	5	25	20	45
Stylet length	19.8 (19.0-20.2)	21.2 (18.0-22.5)	18.8 (18.0-20.0)	18 (16-19)	18.3 (17.9-19.0)	20 (19-21.5)
Knob shape	transversely ovoid	transversely ovoid	transversely ovoid	rounded	rounded	rounded
Knob position	set off	set off	set off	posteriorly sloping	set off	posteriorly sloping
DGO	4.0	3.0	4.4	3.0	2.5	?
J2						
n	30	20	30	25	20	50
Body length	424 (403-454)	425 (388-486)	439 (408-476)	435 (418-465)	475 (420-510)	542 (490-575)
Tail	70.4 (65.1-76.5)	63.1 (59.4-68.4)	68.1 (61.0-78.0)	70 (52-78)	78.3 (68.0-88.0)	96.6 (85.0-107)
Hyaline tail	11.3 (9.5-13.3)	13.6 (9.0-17.1)	17.4 (14.5-21.0)	22.6 (17.1-27.0)	18.5 (14.0-22.4)	19.9 (16.0-23.0)
Hemizonid ref. to excr. pore	anterior	posterior	anterior	anterior	posterior	posterior
Vesicles in metacarpus	no	no	no	yes	no	yes

* Data from the original species descriptions.

Phasmids posterior to anus, small, located in ventral incisure of lateral field. One or two cuticular tail constrictions present.

TYPE MATERIAL

Holotype: Female on slide WT 3236, collection of Agricultural University, Wageningen, The Netherlands. Paratypes: Two female perineal patterns and heads, two males and five J2 deposited at each of the following collections: Agricultural University, Wageningen, The Netherlands (WT 3237-3239); University Gent, Zoology Institute, Gent, Belgium; Rothamsted Experimental Station, Harpenden, U.K.

TYPE HOST AND LOCALITY

Collected and described from the roots of *Elymus farctus* (Viv.) Melderis (sand twitch), grown near the so-called motorcar beach of the foredunes of Oostvoorne, The Netherlands (51°55' NL 5°6' EL), in a brackish environment.

DIAGNOSIS AND RELATIONSHIP

M. duytsi n. sp. is characterized by a relatively large female body, stylet slightly curved, 13.3 μm (12.6-13.9) long, with large transversely ovoid knobs set off from the shaft, perineal pattern asymmetrical with a low dorsal arch and coarse striae, and lateral wing present in most patterns. Male stylet 19.8 μm (19.0-

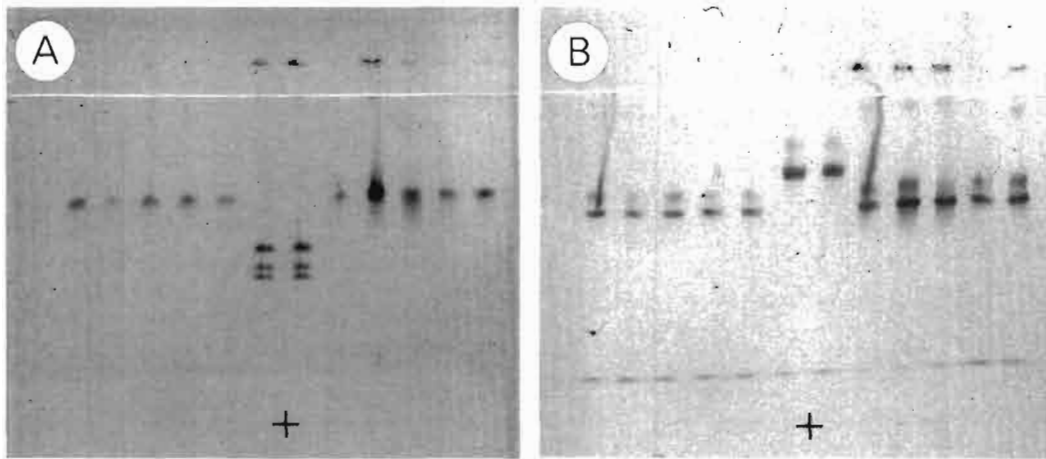


Fig. 5. Esterase (A) and malate dehydrogenase (B) isozyme patterns of *Meloidogyne duytsi* n. sp. (*M. javanica* was used as a reference and added to the two middle wells).

20.2) long, with knobs transversely ovoid set off from the shaft, head region high, labial disc slightly raised, lateral lips small, and pointed tail. Second-stage juvenile body-, tail-, and hyaline tail 423.6 μm (403.2-454.4), 70.4 μm (65.1-76.5), and 11.3 μm (9.5-13.3) long, respectively, hemizonid anterior, adjacent, to excretory pore, and tail curved ventrally.

M. duytsi n. sp. is also characterized by a unique malate dehydrogenase (MDH) pattern and one very slow esterase (EST) band. According to the enzyme phenotype coding of Esbenshade and Triantaphyllou (1985), the patterns are named N2 (MDH) and VS1 (EST) respectively (Fig. 5).

M. duytsi n. sp. is related to the "graminis"-group (Jepson, 1987). Comparable species are: *M. graminis*, *M. maritima*, *M. kralli* Jepson, 1983, *M. naasi* Franklin, 1965 and *M. sasseri* Handoo, Heuttel & Golden, 1993. The species in the "graminis" group are morphologically very similar to one another (Jepson, 1987) and must be carefully compared. The most marked differences between *M. duytsi* n. sp. and related species are summarized in Table 2. *Meloidogyne sasseri* is included, as it is involved in the degeneration of the North American beach-grass *Ammophila breviligulata* Fern. (Seliskar & Huettel, 1993). Characteristics of second-stage juveniles, such as tail shape and hemizonid position, easily differentiate the par- or sympatrical species *M. maritima* and *M. duytsi* n. sp. The *M. maritima* populations from Oostvoorne and Haringvliet conform with the original description, except for some J2 morphometrics. For example J2 body length (459.3 + 13.3 μm [441.6-483.2], n = 25) of the *M. maritima* "Oostvoorne" population differs markedly from the original description

(Table 2). The J2 body length can also be used to separate *M. maritima* from *M. duytsi* n. sp.

M. duytsi induced no or only small galls on its hosts, *E. farctus* and *A. arenaria*, which makes it difficult to recognize root infection. A large egg mass protruding from the root is produced by each female. One or more males are present inside each egg mass.

DISTRIBUTION AND HOSTS

In addition to the type locality, *M. duytsi* n. sp. was also detected on *E. farctus* near foredunes north of the Haringvlietdam, which is 9 km southwards from the former site. *E. farctus* is a clonal, salt resistant dune grass that colonises beaches. The plant grows in an extremely dynamic and unpredictable environment. Plants may be washed away during storms. When embryonic dunes are formed, *Ammophila arenaria* (L.) Link (marram grass) may colonise and, finally, replace *E. farctus*. *M. duytsi* n. sp. has also been found in mixture with *M. maritima* Jepson, 1987 in the root zone of *A. arenaria* near Oostvoorne. *A. arenaria* is a native plant species from north-western Europe (Huiskes, 1979) that has been used since the 17th century to stabilize dunes in the Atlantic south-west coastal area of France. It also occurs in the Mediterranean and has been exported for sand stabilization to Australia, New Zealand, South Africa, and the west coast of North-America. As the hosts *E. farctus* and *A. arenaria* are very common beach grasses in the North Sea area, it is expected that *M. duytsi* n. sp. is widely distributed in West-European foredunes, par- or sympatrical with *M. maritima*.

Host suitability was studied in the greenhouse. *M. duytsi* n. sp. failed to reproduce on *Lycopersicon esculentum* Mill. cv. Moneymaker, *Brassica oleracea*

laciniata L. cv. Westlandse winterharde, *Zea mays* L. cv. Splenda, and *Lolium multiflorum* Lamk., but reproduced on *Triticum aestivum* L. cv. Minaret.

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