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# Characterization of the root-knot nematode, *Meloidogyne ethiopica* Whitehead, 1968, from Slovenia

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**Summary.** A *Meloidogyne ethiopica* population was isolated from heavily infested tomato roots in a glasshouse situated in the village of Dornberk in Slovenia. This was the first finding of *M. ethiopica* in Europe. The species was identified by morphometrical analyses of second-stage juveniles, adult males and females and confirmed by isozyme electrophoresis stained for malate dehydrogenase and esterase. Identification of the species was completed by comparison of small subunit rDNA sequences with those from other *Meloidogyne* species. The sequences of *M. ethiopica* populations from Slovenia and Brazil showed a 99.7% of sequence similarity score and clustered together with high bootstrap support. In addition, biotests on different host plants were performed to determine host range of the species. Twenty-two new host plants for *M. ethiopica* were established in our tests. This polyphagous nematode can multiply on dicotyledonous as well as on monocotyledonous plants.

**Key words:** host plants, isozyme phenotypes *Meloidogyne ethiopica,* morphometrics, rDNA.

Root-knot nematodes (RKN) *Meloidogyne* spp. Goldi are obligate parasites that attack numerous higher plant species including monocotyledons, dicotyledons, herbaceous and woody plants (Eisenback & Hirschmann, 1991). The genus comprises more than 80 nominal species of which about ten are considered as agricultural pests. Four species, *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, are major pests with a world-wide distribution in agricultural areas (Karssen, 2002). Twenty RKN species have been detected in Europe so far, thirteen of them having been described from a European type locality (Karssen & Van Hoenselaar, 1998).

Until 2002, only *M. hapla* was reported from Slovenia. Recent studies revealed the presence of four RKN species in Slovenia: *M. arenaria*, *M. incognita*, *M. hapla* and *M. ethiopica* (Širca *et al.*, 2004a, b; Širca & Urek, 2004). A population of *M. ethiopica* was isolated in 2003 from heavily infested tomato roots of *Lycopersicum esculentum* cv. Belle grown in a glasshouse situated in the village of Dornberk in Slovenia. Tomato plants displayed ground symptoms of stunting and wilting.

*Meloidogyne ethiopica* was described by Whitehead (1968) from a single egg mass on a culture of tomato (type host) from the Mlalo region, Lushoto District, Tanga Province, Tanzania (type locality). In 2004, *M. ethiopica* was reported outside Africa, in Brazil and Chile (Carneiro *et al.*, 2004) and in Slovenia (Širca *et al.*, 2004b). The latter was also the first report of this species in Europe.

Morphologically, *M. ethiopica* is most similar to *M. arenaria* and *M. incognita.* In the original description, the perineal patterns of *M. ethiopica* were characterized as varying from *M. arenaria* type to "*acrita*" type of *M. incognita.* It seems possible that the nature of the perineal pattern of *M. ethiopica* might sometimes have made its accurate identification difficult or impossible, especially in the absence of other highly distinctive features of the female (Golden, 1992). This was the case in Chile where *M. ethiopica* may have been present on grapevine for many years, but it was incorrectly identified as *M. arenaria* or *M. incognita* on the basis of perineal patterns (Carneiro *et al.,* 2007).

In order to ensure precise species identification, a combination of different methods is necessary. Many studies have demonstrated that several RKN species can be differentiated using polyacrylamide-gel electrophoresis (PAGE) of certain enzymes (Dalmasso & Berge, 1978; Esbenshade & Triantaphyllou, 1985, 1990). Karssen *et al.* (1995) demonstrated isozyme phenotyping using very thin (0.4 mm) polyacrylamide slab gel electrophoresis

(PhastSystem, Pharmacia), where relatively small amounts of enzyme (single female content) can be analysed. This approach was shown to be very useful in RKN identification (Karssen *et al.*, 1995; Širca *et al.*, 2004b).

It is known that *M. ethiopica* can parasitise several cultivated crops including tomato *Lycopersicon lycopersicum*, cowpea *Vigna unguiculata* L., bean *Vicia faba* L., black wattle *Acacia mernsii* de Wild, cabbage *Brassica oleracea* L. cv. Capitata, pepper *Capsicum frutescens* L., potato *Solanum tuberosum* L., pumpkin *Curcubita* sp. and tobacco *Nicotiana tabacum* L. (Whitehead, 1969), as well as lettuce *Lactuca sativa* L., soybean *Glycine max* L. Merril and sisal *Agave sisalana* Perrine (cited in Golden, 1992). Carneiro *et al.* (2004) isolated *M. ethiopica* from roots of kiwi fruit *Actinidia deliciosa*, grapevine *Vitis vinifera* and also watermelon cv. *Charleston Gray*. However, it seems that *M. ethiopica* is a typical polyphagous RKN species which can parasitise woody and herbaceous plants.

The present study provides comprehensive information on morphometrical characters and molecular features of *M. ethiopica* from Slovenia. Isozymes of malate dehydrogenase (MDH) and esterase (Est) phenotypes were analysed in order to confirm morphometrical identification. In addition, rDNA sequence of *M. ethiopica* from Slovenia and sequences from the NCBI databank were used for cluster analysis to assess phylogenetic relationship between different populations of *M. ethiopica* and closely related species. The study also presents information on new host plants for *M. ethiopica,* which were determined in differential host tests.

## MATERIAL AND METHODS

**Nematode populations.** Cultures of *M. ethiopica* were established from collected egg-masses of infested tomato roots grown in a glasshouse in Dornberk. The cultures were maintained on tomato cv. Volovsko srce planted in sterile sand and kept in a glasshouse at 20–25°C. After 7 weeks, the cultures were used for nematode isolation. Under a dissecting microscope, females were isolated from infested roots using a scalpel and a nematological needle and placed in 0.9% NaCl, which prevents females from bursting. Males and second-stage juveniles (J2) were isolated from the sand by decanting method (Hržič, 1973) followed by Baerman's funnel extraction.

Inoculum of eggs for host tests was prepared by shaking chopped galled roots from 2-month-old tomato plants in 1% sodium hypochlorite for 4 min to dissolve the gelatinous matrix surrounding root-knot nematode eggs (Hussey & Barker, 1973). The suspension of eggs was washed through 850, 250 and 32 µm banked sieves. The eggs on the lower sieve were washed with tap water to remove NaClO (Ehwaeti *et al.*, 1998).

**Morphometrical characterization.** Males and J2 were heat killed and fixed in triethanolamine-formalin (TAF) solution before analyses, while female parameters and perineal patterns were analysed on freshly isolated females. Nematode images and morphometrical analyses were performed using a microscope combined with a digital camera (Nikon), which was connected to a PC with software for image analysis (LUCIA). Different morphological parameters were measured for different life stages. Measurements were compared with the original description of the species from Tanzania (Whitehead, 1968) and *M. ethiopica* population from Brazil (Carneiro *et al.,* 2004).

**Isozymes.** Isolated females were rinsed with reagent-grade water and transferred to a 12 sample-well stamp placed on ice bath. Each female was placed in a separate sample well containing 0.5 µl of extraction buffer (20% sucrose, 2% Triton X-100, 0.01% Bromophenol Blue) (Esbenshade & Triantaphyllou, 1985) and squashed with a needle to release the body contents. Samples were loaded on two 12/0.3 sample applicators which were placed into the applicator arms of the PhastSystem equipment (GE Healthcare Life Sciences; Pharmacia). Freshly isolated *M. javanica* females (a culture obtained from the Plant Protection Service, Wageningen, The Netherlands) were used as a reference. Electrophoresis took place on a PhastGel gradient gel (8–25) with buffer system according to manufacturer's instructions. The program and conditions of electrophoresis were as described by Karssen *et al.* (1995). After electrophoresis, the gel was stained for enzymatic activity in a Petri dish at 37°C with different staining solutions. MDH staining solution contained 0.05 g β-NAD, 0.03 g nitro blue tetrazolium, 0.02 g phenazine methosulphate, 5.0 ml 0.5 M Tris pH 7.1, and 7.5 ml of a $Na_2CO_3$-malic acid mixture (10.6 g $Na_2CO_3$ + 1.34 g L-malic acid in 100 ml water) dissolved in 70 ml of reagent-grade water. The Est activity staining solution contained 100 ml 0.1 M phosphate buffer pH 7.3, 0.06 g Fast Blue RR salt, 0.03 g EDTA and 0.04 g α-naphthyl acetate dissolved in 2 ml acetone. The incubation for MDH lasted 5 min. After that, the gel was washed twice with distilled water and further stained for Est activity for 30 min. After isozyme phenotype patterns became clearly visible, the enzymatic reaction was stopped by rinsing the gel with distilled water and fixed for 5 min in a solution of 10% acetic acid, 10% glycerol and 80% distilled water.

The relative migration rates (Rm) of the MDH (EC 1.1.1.37) and Est (EC 3.1.1.1) bands were calculated as a migration rate of Bromophenol Blue.

**DNA extraction, amplification and sequencing.** Genomic DNA was extracted from four males. Nematodes were transferred to a 1.5 ml Eppendorf tube in 1 µl of sterile water and stored at -80°C until DNA isolation. DNA was isolated with Wizard DNA purification kit (Promega) according to the manufacturer's instructions with minor modifications. Nematodes were homogenised with a micropestle in an ice cold mixture of 5 µl 1M EDTA (pH 8) and 25 µl nucleic lysis solution. Additional 5 µl of 1M EDTA (pH 8) and 25 µl of nucleic lysis solution were added to each tube before continuing according to the manufacturer's instructions. Extracted DNA was re-suspended in 10 µl of distilled water.

Two set of primers, 1A (5'-GGC GAT CGA AAA GAT TAA GCC–3') and 3B (5'-GGC GAT CGA TTG GCA AAT GCT TTC GC–3') described by Baldwin *et al.* (1997), and MelF (5'-TAC GGA CTG AGA TAT GGT–3') and MelR (5'–GGT TCA AGC CAC TGC GA–3') described by Tigano *et al.* (2005) were used for the amplification of 18S rDNA fragments. Both primer sets gave overlapping sequences. PCR reactions contained 1 µl of isolated DNA, 10 mM Tris-HCl pH 8.3, 25 mM $MgCl_2$, 2.5 mM of each of the dNTPs, 1 µM of each of the primers, 1U *Taq* DNA Polymerase (Promega) and distilled water up to 25 µl. The amplification was carried out in a thermocycler (A&B gene AMP PCR system 2700) using the following conditions: initial denaturation at 94°C for 2.5 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 s and elongation at 72°C for 1 min; followed by a final extension at 72°C for 2 min.

Sequences were obtained by direct sequencing of the PCR products. Quick Jet PCR purification kit (Genomed) was used for purifying the PCR products according to the manufacturer's instructions. ABI PRISM 310 DNA Sequencer and a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) were used for sequencing.

**rDNA sequence analyses.** Nucleotide sequences were assembled and analysed by the computer software Bioedit v. 7.0.5.2 (Hall, 1999). The rDNA sequence that we obtained and sequences of *M. ardenensis* (EU669946), *M. arenaria* (AY268118), *M. chitwoodi* (EU669932), *M. duytsi* (AF442197), *M. ethiopica* (AY942630), *M. exigua* (AY942627), *M. fallax* (AY593895), *M. floridensis* (AY942621), *M. graminicola* (AF442196), *M. hapla* (AY593893), *M. hapla* (AY593898), *M. incognita* (AY268120), *M. incognita* (EU669939), *M. javanica* (AY942626), *M. javanica* (EU669938), *M. maritima* (EU669945), *M. mayaguensis* (AY942629), *M. minor* (EU669937), *M. naasi* (AY593901), *M. paranaensis* (AY942622) and *Meloidogyne sp.* (AY942633) obtained from the NCBI Database, were aligned using Mega 4 software (Tamura *et al.*, 2007). The phylogenetic tree representing 18 *Melodoigyne* species and based on 18S rDNA sequences was constructed using the neighbour-joining method in Mega 4 software package. Sequences of *Globodera rostochiensis* (EU855120) and *Pratylenchus thornei* (EU669930) were used as out-groups.

**Host test.** To determine possible host plants, a glasshouse experiment was carried out with the emphasis on plants relevant to Slovenian conditions. The 26 tested plants including important vegetables were: barley *Hordeum vulgare,* bean *Phaseolus vulgaris* cv. Meraviglia di venezia nano, beet *Beta vulgaris var. conditiva* cv. Cylindria, broccoli *Brassica oleracea var. italica* cv. Corvet F1, cabbage *Brassica oleracea var. capitata* cv. Ljubljansko, carrot *Daucus carota* cv. Nantes, cauliflower *Brassica oleracea var. botrytis* cv. Snežna kepa, celery *Apium graveolens* cv. Praška, chicory *Cichorium intybus var. foliosum* cv. Castelfranco, cucumber *Cucumis sativus* cv. Eva, curled dock *Rumex patientia L.* cv. Crispus, eggplant *Solanum melongena* cv. Domači srednje dolgi, endive *Cichorium endivia* cv. Full heart, florence fennel *Foeniculum vulgare var. azoricum,* kale *Brassica oleracea var. subauda* cv. Železna glava, kohlrabi *Brassica oleracea var. gongylodes* cv. Hoffmanova rumena, lettuce *Lactuca sativa* cv. Leda, melon *Cucumis melo* cv. Ananas, onion *Allium cepa var. cepa* cv. Centurion F1, pea *Pisum sativum* cv. Senator, radish *Raphanus sativus var. radicula* cv. Saxa, spinach *Spinacia oleracea* cv. Matador, strawberry (*Fragaria x ananassa*), sunflower *Helianthus annuus*, sweet corn *Zea mays var. saccharata* cv. Gold cup F1 and tomato *Lycopersicon lycopersicum* cv. Volovsko srce.

The plants were planted in 16 cm diameter pots filled with 2220 g of fine sterilized sand (0.25 - 1.0 mm). Five seeds of each crop were planted per pot. The plants were grown in a glasshouse with daily temperatures ranging from 20 to 30°C. Water was added to 15% of the dry sand weight (Kutywayo & Been, 2006). Nutrients for hydroponic growth (Flora series) were used every time the plants were watered. Nutrient concentration depended on the stage of the plant development. Two month old plants were inoculated with an aqueous solution of 5,000 eggs of *M. ethiopica* per plant. The experiment was terminated 45 days after inoculation. Individual plant roots were washed free of sand and examined for the presence of galls and egg-masses.

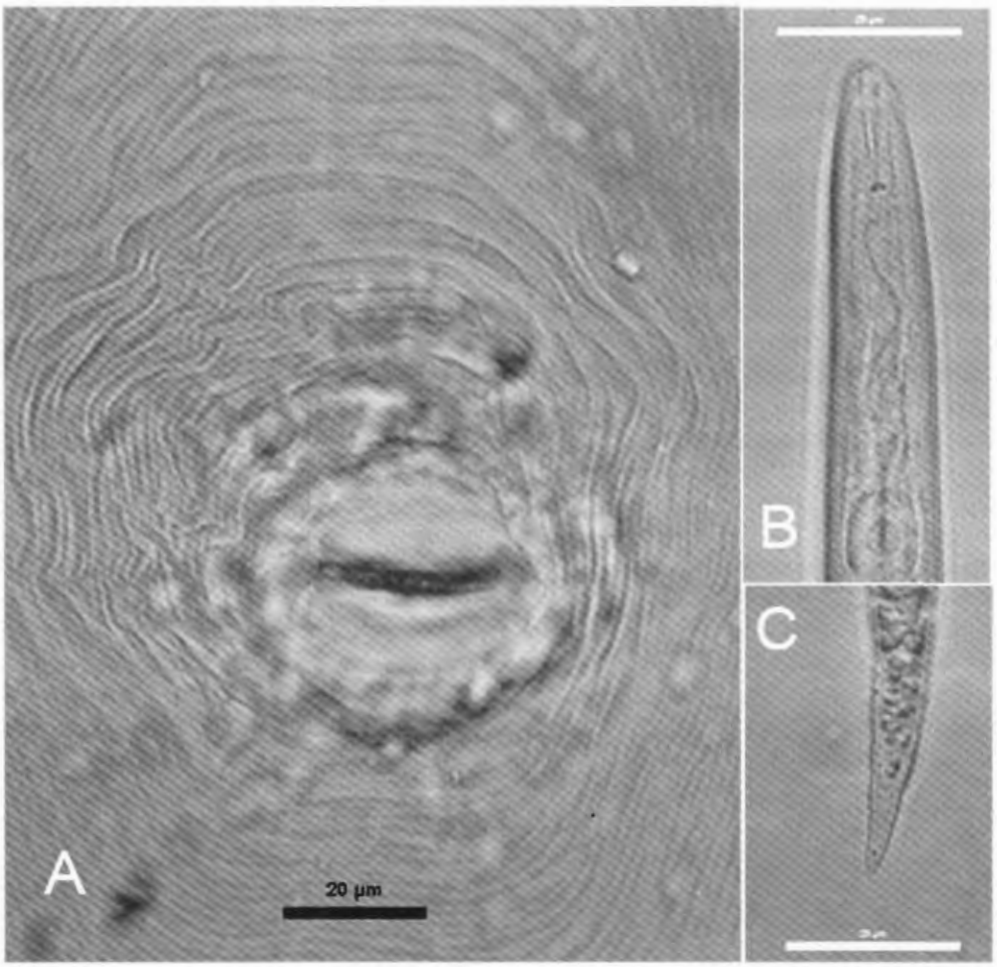


**Fig. 1.** Photographs of the morphometrical characters under light microscope of *Meloidogyne ethiopica* female (A) and second-stage juveniles (B, C) from Slovenia; A: Perineal patterns, B: Anterior end to metacorpus, C: Tail terminus (Scale bars: A - C = 20 µm).

## RESULTS

**Morphometrical identification.** Nematode species was determined by morphometrical analyses of the J2, adult males and females (Table 1, Fig. 1). Female neck long and usually well marked off from the rest of the body. Stylet knobs small. Stylet length from 11.8 µm to 18.6 µm. Excretory pore between dorsal pharyngeal gland orifice and metacorpus. Distance from anterior end to metacorpus from 37.8 µm to 79.8 µm; metacorpus length from 36.2 µm to 63.9 µm. Average metacorpus diameter 45.4 µm. Male body shape vermiform from 1176 µm to 1940 µm in length. Head cap overlapping slit-like amphid apertures, high, rounded. Stylet large, from 17.5 µm to 23.7 µm, robust, cone straight and pointed. Stylet knobs equal, rounded, with average knob height 3.0 µm and average knob width 4.3 µm. Stylet knobs usually with backward sloping anterior margins. Pharynx overlapped intestine dorsally, ventrally or laterally. Metacorpus oval with large valve. Distance from anterior end to metacorpus from 61.7 µm to 96.5 µm. Tail with average length 13.9 µm and tail terminus smoothly rounded without striations, phasmids located about half tail length behind cloaca. Spicules with short heads and length from 24.7 µm to 37.9 µm. Body lengths for the J2 between 321 µm and 375 µm. Stylet average length 13.6 µm. Stylet knobs rounded. Distance from anterior end to metacorpus from 41.8 µm to 55.7 µm. Tail smoothly tapering to subacute terminus with 44.4 µm average length.

**Isozymes.** Isozyme phenotype patterns were analyzed by calculating relative migration rates (Rm) (Fig. 2). Est phenotype pattern distinguished *M. ethiopica* (Rms: 0.60, 0.63 and 0.67) from *M. javanica* (Rms: 0.61, 0.66 and 0.69) as previously reported by

**Table 1:** Morphometrical data of *M. ethiopica* females, males and second-stage juveniles (J2) from Slovenia. All measurements in µm and formulated as mean ± standard deviation (minimum and maximum).

| Character | Female | Male | J2 |
|---|---|---|---|
| n | 30 | 30 | 30 |
| L | | 1452 ± 154.6 (1175.6 – 1940) | 347.6 ± 15.2 (320.5 – 374.7) |
| a | – | 41.2 ± 5.4 (33.0 – 52.5) | 24.3 ± 1.8 (21.2 – 27.1) |
| c | – | 106.9 ± 21.2 (75.7 – 156.5) | 7.9 ± 0.7 (5.8 – 9.6) |
| c' | – | – | 4.2 ± 0.6 (3.5 – 6.7) |
| Greatest body diam. | – | 35.5 ± 3.5 (29 – 43.3) | 14.37 ± 0.8 (12.4 – 15.7) |
| Stylet length | 14.9 ± 1.5 (11.8 – 18.6) | 21.3 ± 1.2 (17.5 – 23.7) | 13.6 ± 0.4 (12.6 – 14.3) |
| Stylet knob height | – | 3.0 ± 0.5 (2.2 – 3.7) | – |
| Stylet knob width | 3.8 ± 0.6 (2.4 – 4.8) | 4.3 ± 0.4 (3.3 – 4.99) | – |
| Body diam. at stylet knobs | – | 18.6 ± 0.9 (17.2 – 20.3) | 9.6 ± 0.6 (8.6 – 10.7) |
| DGO | 3.3 ± 0.7 (2.4 – 5.1) | 3.1 ± 0.5 (2.2 – 3.8) | 2.9 ± 0.5 (2.3 – 3.8) |
| Anterior end to metacorpus | 59.4 ± 9.9 (37.8 – 79.8) | 85.2 ± 8.97 (61.7 – 96.5) | 50.2 ± 2.5 (41.8 – 55.7) |
| Metacorpus length | 47.9 ± 6.8 (36.2 – 63.9) | – | – |
| Metacorpus diameter | 45.4 ± 8.1 (25.8 – 60.4) | – | – |
| Spicule length | – | 31.6 ± 3.4 (24.7 – 37.9) | – |
| Tail length | – | 13.9 ± 1.8 (9.7 – 18) | 44.4 ± 4.2 (37.6 – 58) |
| Tail terminus length | – | – | 10.6 ± 1.02 (8.1 – 12.9) |
| Body diam. at anus | – | – | 10.6 ± 0.6 (9.5 – 12) |

Carneiro *et al.* (2004), while MDH phenotype was the same for both species (Rm: 0.38) (Fig. 2).

**rDNA sequence analyses.** A sequence covering 1626 bp of the 18S rDNA region of *M. ethiopica* from Slovenia was obtained (acc. no. FJ559408). In the constructed phylogenetic tree (Fig. 3), our sequence clustered together (70% bootstrap support) with the sequences of *M. ethiopica* and *Meloidogyne* sp. from Brazil (Tigano *et al.*, 2005). The sequence identity score between the Slovenian and Brazilian *M. ethiopica* sequence was 99.7%.

**Differential host test.** From the 26 plant species tested in a host determination experiment the majority (25) serve as hosts for *M. ethiopica*. Galls and egg-masses were found on the roots of the following plants: barley, bean, beet, broccoli, cabbage, carrot, cauliflower, celery, chicory, cucumber, curled dock, eggplant, endive, florence fennel, kale, kohlrabi, lettuce, melon, onion, pea, radish, spinach, sunflower, sweet corn and tomato. No galls were found on the roots of strawberries, thus suggesting that they were unsuitable as a host for *M. ethiopica*.

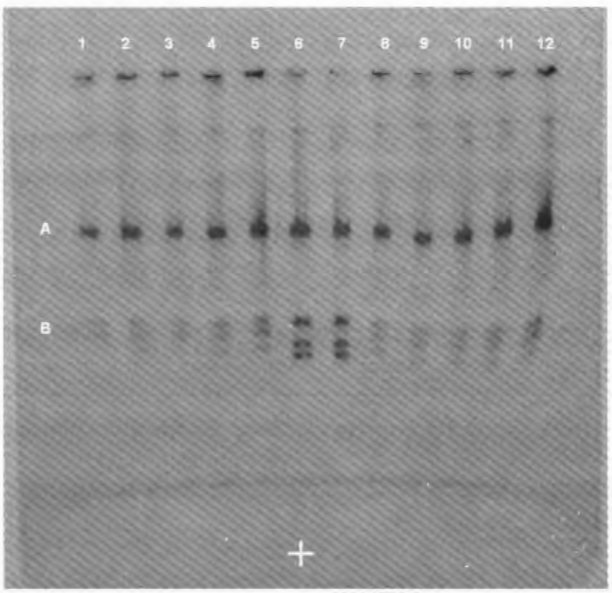


**Fig. 2.** Isozymes MDH (A) and Est (B) phenotype patterns of individual female of *Meloidogyne ethiopica* (lanes 1 - 5, 8 - 12) and *M. javanica* (lanes 6, 7) as a reference.

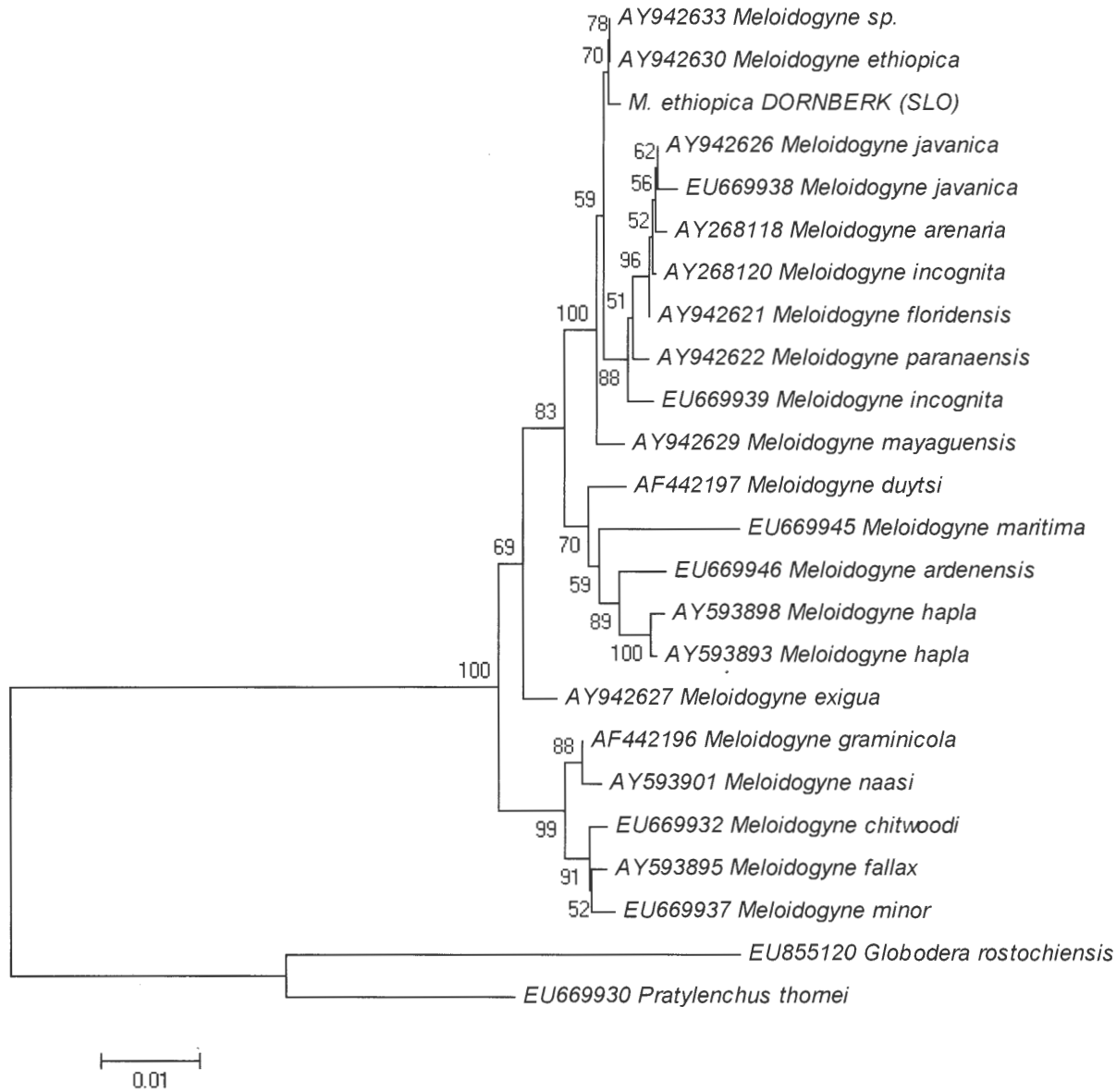


**Fig. 3.** The phylogenetic tree by neighbour-joining method based on 18S rDNA sequences of *Meloidogyne ethiopica* Dornberk (Slovenia) and other closely related *Meloidogyne* species (NCBI GenBank). Numbers next to branches are bootstrap values (>50%).

# DISCUSSION

The discovery of *M. ethiopica* in Europe together with the results of this study shows that *M. ethiopica* can occur and parasitise plant species of moderate climatic zones. The species was reported from Africa: Ethiopia, Mozambique, South Africa, Tanzania, Zimbabwe, and from South America (Brazil and Chile). Our results on host determination support the hypothesis of the polyphagous nature of *M. ethiopica.* Whitehead (1969) reported reproduction on dicotyledonous plants while our results showed reproduction also on monocotyledonous plants. Members of the following families were successfully attacked: *Alliaceae, Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae,* Cichoriaceae, *Cucurbitaceae, Fabaceae, Poaceae, Polygonaceae* and *Solanacea.* Based on the data of potential hosts, this species can be classified in the group of the economically important *Meloidogyne* species. Additionally, further host determination experiments are underway. The control of this species could be very difficult, since it can also attack the woody plants

(Carneiro *et al.*, 2004). On the roots of the attacked plants small to large galls were observed as previously reported by Carneiro *et al.* (2004). *Meloidogyne ethiopica* caused poorly developed root systems distorted by small and large multiple galls and devoid of fine roots. The plants in Brazil also showed a reduction in plant growth and fruit size.

Morphologically, it is easy to confuse *M. ethiopica* with *M. incognita* or *M. arenaria* and, furthermore, all three species belong to the same cluster based on 18S rDNA sequences. Whitehead (1968) reported *M. ethiopica* to be close to *M. arenaria* from which it differs in the posterior cuticule from low arch 'arenaria' type to high arch 'incognita' type, the male head is more tapering and has two annules of equal length behind the head-cap on the sublateral head sectors, and the spicules are thicker walled, with strongly ridged shafts. In 1992, Golden found a useful diagnostic character for separating *M. ethiopica* from *M. arenaria* and *M. incognita* by comparing the nature of the female phasmids of all three species. Phasmids from *M. ethiopica* were large and distinct and with a conspicuous phasmidial canal while *M. incognita* and *M. arenaria* had small, indistinct phasmids and phasmidial canals that were difficult to observe. Similarly, in our study we observed large and distinct phasmids at perineal patterns of *M. ethiopica*.

While identification based exclusively on morphometrical characterisation might be difficult, Est isozyme patterns and analyses of 18S rDNA sequences provide a reliable confirmation of species identification. The biochemical identification of *M. ethiopica* from Slovenia showed the same Est and MDH phenotypes compared to phenotypes of *M. ethiopica* from Brazil (Carneiro *et al.*, 2004). The MDH phenotype however is not species-specific compared to the Est phenotype. In our experience, the Est phenotype pattern is a useful tool to differentiate *M. ethiopica* from other root-knot nematodes as suggested previously by Carneiro *et al.* (2004). Additionally, *M. ethiopica* from Slovenia seems to be very close to the Brazil isolate since 99.7% identity between the 18S rDNA sequences was obtained.

The origin of this nematode in Slovenia is unknown, because the infected plants of tomato were not imported from abroad. This fact suggests broader yet undocumented distribution.

Our further objectives are to determine whether *M. ethiopica* can survive in Slovenian (European) climate conditions, especially since most of the important vegetables grown in Slovenia were shown to be potential hosts.

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**P. Strajnar, S. Širca, B. Geric Stare, G. Urek.** Характеристика галлообразующих нематод вида *Meloidogyne ethiopica* Whitehead, 1968 из Словении.

**Резюме.** Из ризосферы сильно пораженных томатов в теплице селения Домберк в Словении была изолирована популяция галлообразующих нематод *Meloidogyne ethiopica*, что представляет собой первое сообщение об этом виде в Европе. Определение вида проведено по результатам морфометрического анализа личинок второй стадии, взрослых самцов и самок, и подтверждено результатами электрофоретического изучения ферментов: эстераз и малат-дегидрогеназ. Окончательное подтверждение определения вида было проведено сравнением нуклеотидных последовательностей участка малой субъединицы рибосомы с последовательностями других видов *Meloidogyne*. Последовательности популяций *M. ethiopica* из Словении и Бразилии показали сходство в 99.7% и образовывали единую группу с высоким уровнем bootstrap-поддержки. Были проведены эксперименты с целью выявления круга растений хозяев этого вида. Было показано, что двадцать два вида растений могут служить хозяевами для *M. ethiopica*, которая размножается как на однодольных, так и на двудольных растениях.