First Report of the Root-Knot Nematode *Meloidogyne luci* on Tomato in Serbia

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Root-knot nematode (RKN) *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 was described from Brazil, Chile and Iran, parasitizing in various crops (Carneiro et al. 2014). It was later also described from Slovenia, Italy, Greece, Portugal, Turkey and Guatemala (review in Gerič Stare et al. 2017). It is considered an extremely damaging pest as it has a wide host range and infects numerous higher plants, including monocotyledons and dicotyledons as well as herbaceous and woody plants. This species was included in the European Plant Protection Organisation Alert List of harmful organisms. In Europe, *M. luci* has been detected in both greenhouse and field agricultural production (review in Gerič Stare et al. 2017). Furthermore, *M. luci* has been shown to survive winter in the field under continental and sub-Mediterranean climatic conditions (Strajnar et al. 2011).

In August 2021, an official survey for quarantine RKN in Serbia (Province Vojvodina) revealed in a greenhouse in the village of Lugovo (43^{0}43’32,562; 19^{0}08’55,168), near Sombor, yellowing, stunning and extensive root galls on tomato (*Solanum lycopersicum* L.) cultivar Diva F1 caused by an unknown *Meloidogyne* sp. (Fig. 1). As correct identification is essential for effective pest management program, the next step was to identify the nematode species.

Morphological characterization performed on freshly isolated females revealed perineal patterns similar to *M. incognita* (Kofoid and White, 1919) Chitwood, 1949. The shape was oval to squarish with the dorsal arch rounded to moderately high and without shoulders. The dorsal striae were wavy and continuous. The ventral striae were smooth and the lateral lines were weakly demarcated. The perivulval region was without striae (Fig. 2). The female stylet was robust with well-developed knobs and the stylet cone slightly curved dorsally. Although morphological characters was very variable, the nematode was suspected as *M. luci* based on comparison with originally described *M. luci* and *M. luci* populations from Slovenia, Greece and Turkey.

Identification was achieved with subsequent species-specific PCR and sequence analysis. The nematode was determined to belong to the tropical RKN group and the *M. ethiopica* group using two PCR reactions as described by Gerič Stare et al. (2019) (Figs. 3 and 4). Identification was confirmed by species-specific PCR of *M. luci* as described by Maleita et al. (2021), and a band of approximately 770 bp was obtained (Fig. 5). In addition, the identification was confirmed by sequence analyses. The region of mtDNA was amplified with primers C2F3 and 1108 (Powers and Harris 1993), cloned, sequenced (acc. no. OQ211107), and compared to other *Meloidogyne* spp. sequences from the Genbank. The determined sequence is 100% identical to an unidentified *Meloidogyne* sp. from Serbia, while the next highest scores are sequences of *M. luci* from Slovenia, Greece and Iran, all of which have 99.94% sequence identity. In phylogenetic tree, all *M. luci* sequences including the sequence from Serbia belong to a single clade.

Egg masses isolated from infected tomato roots were used to establish a nematode culture in greenhouse and they caused typical root galls on cultivar Maraton of tomato. The galling index assessed 110 days-post-inoculation was in the range 4-5 according to the scoring scheme (1-10) for field evaluation of RKN infestations (Zeck 1971).

To our knowledge, this is the first report of *M. luci* in Serbia. The authors hypothesize that climate change and higher temperatures could lead to much greater spread and damage to various agricultural crops in the field by *M. luci* in the future. National surveillance program for RKN in Serbia continued in 2022 and 2023. A management program to control the spread and damage from *M. luci* will be implemented in Serbia in 2023.

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“e-Xtra” supplementary file

J. Bačić, M. Pavlović, J.Kušić-Tišma, S. Širca, M. Theuerschuh, B. Gerič Stare.
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Plant Disease

Figure 1. Extensive root galls observed on tomato (*Solanum lycopersicum* L.) roots caused by root-knot nematode *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 during an official survey for quarantine root-knot nematodes in Serbia (Province Vojvodina) in August 2021 in the village of Lugovo (430 43’32,562; 190 08’55,168) near Sombor, Serbia.

Figure 2. Perineal pattern of female used for morphological characterization of *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 from Serbia. Scale bar 10 µm.

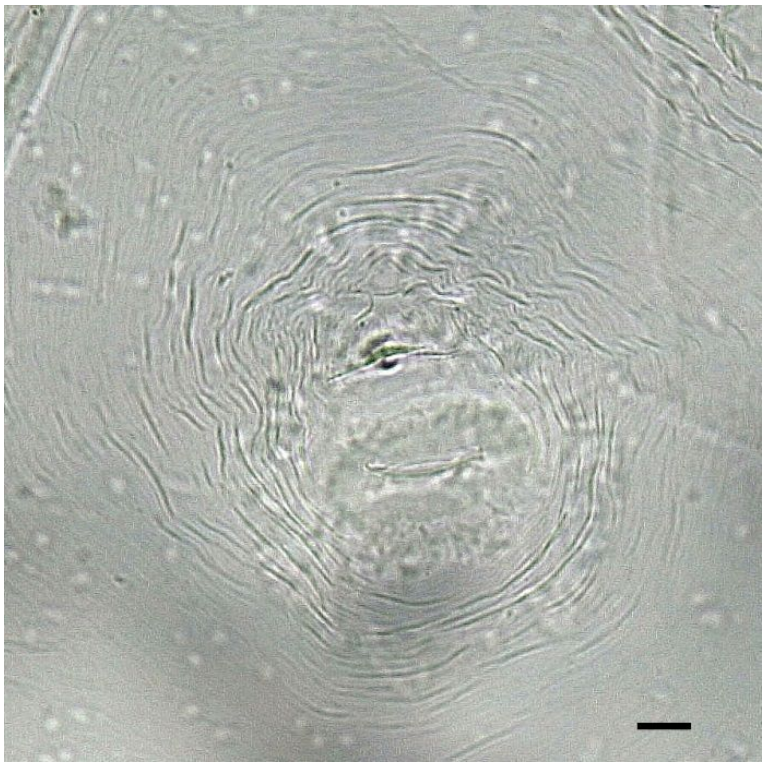


Figure 3. Determination of nematode belonging to tropical root knot nematode group (i.e. clade I of *Meloidogyne* spp.) using PCR reaction as described in Gerič Stare et al. (2019). Reaction with forward primer C2F3 (GGTCAATGTTCAGAAATTTGTGG; Powers and Harris, 1993) and a group specific reverse primer Mt575R (AGAACTTAAACTCTAAATAAC; Gerič Stare et al., 2019) yielded a 621 bp long amplicon specific for the tropical root knot nematode group. Samples on 1 % agarose gel, from left to right: molecular marker DNA 100 bp Plus by Thermo Scientific; *M. luci* from Lugovo, Sombor, Serbia; *M. chitwoodi* Golden, O`Bannon, Santos & Finley, 1980; *M. fallax* Karssen, 1996; *M. hapla* Chitwood, 1949; *M. luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 from Slovenia (positive control); water (negative control) and molecular marker.

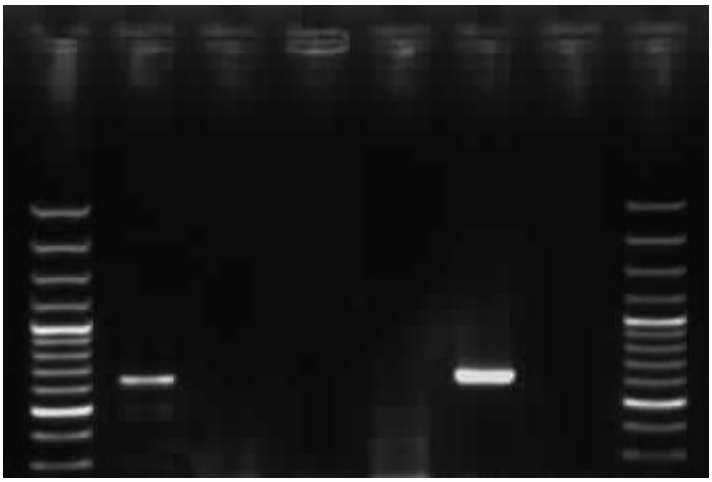


Figure 4. Determination of nematode belonging to *Meloidogyne ethiopica* group (i.e. group of three species: *M. ethiopica* Whitehead, 1968, *M. luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 and *M. inornata* Lordello, 1956) using PCR reaction as described in Gerič Stare et al. 2019. Reaction with primers Me309F (CTAATTTGGGTGAATTT) and Me549R (AATCAAAATCTTCTCCT) yielded a 241 bp long amplicon specific for *M. ethiopica* group. Samples on 1 % agarose gel, from left to right: molecular marker DNA 100 bp Plus by Thermo Scientific; *M. chitwoodi* Golden, O`Bannon, Santos & Finley, 1980*; M. fallax* Karssen, 1996*; M. hapla* Chitwood, 1949; *M. luci* from Lugovo, Sombor, Serbia; *M. luci* from Slovenia (positive control); water (negative control) and molecular marker.

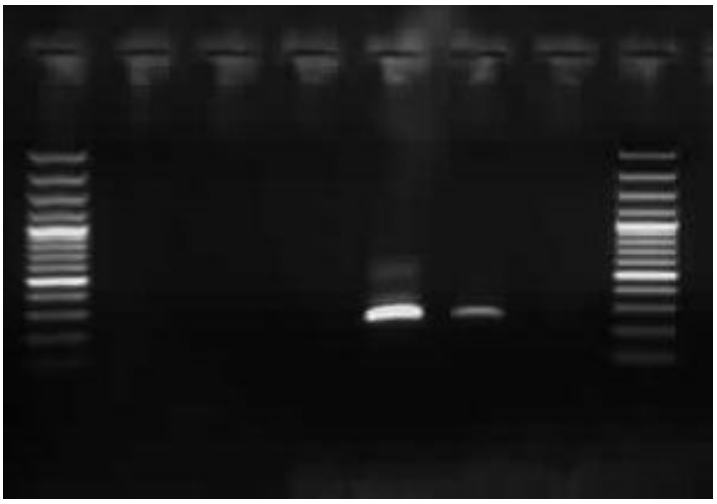
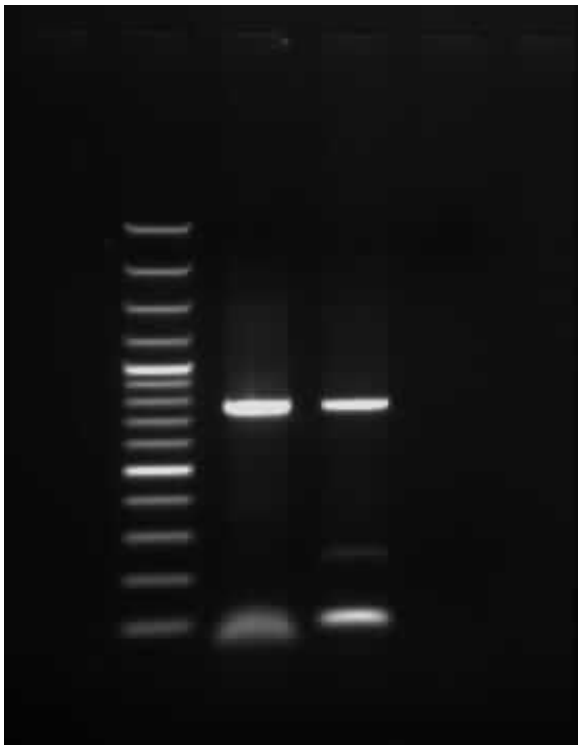


Figure 5. Identification of nematode as *Meloidogyne luci* Carneiro, Correa, Almeida, Gomes, Deimi, Castagnone-Sereno, and Karssen, 2014 using PCR reaction as described in Maleita et al. (2021). Reaction with primers Mlf (ACTCCTGCGACCTCATGGCATTTA) and Mlr (ACTCCTGCGAACACAACATTTACT) yielded a band of approximately 770 bp. Samples, from left to right: molecular marker DNA 100 bp Plus by Thermo Scientific; *M. luci* from Lugovo, Sombor, Serbia; *M. luci* from Slovenia (positive control); water (negative control).



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