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AS prepared the manuscript; RD performed morphological and morphometric characterization of a population of *Meloidogyne hapla*, prepared the manuscript; ŁF performed genetic identification of *Meloidogyne hapla*, prepared the manuscript; MD prepared the manuscript; WL collected the infected plants of paulownia

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Competing interests

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SHORT COMMUNICATION

First report of *Meloidogyne hapla* on *Paulownia tomentosa* in Poland

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Abstract

Meloidogyne spp. are serious pests on many economically important plants, including food crops, vegetables, ornamental trees, and shrubs. Galled roots of *Paulownia tomentosa* Steud were collected from a plantation in the 2018 growing season in Poland. Morphological, morphometric, and molecular studies revealed the presence of *Meloidogyne hapla* in root systems and root zones of empress trees. To our knowledge, this is the first record of the occurrence of this nematode species on *Paulownia tomentosa* in Poland, as well as in Europe.

Keywords

Poland; *Meloidogyne hapla*; *Paulownia tomentosa*; 28S rDNA

Introduction

The root-knot nematodes, *Meloidogyne* spp., includes worldwide distributed obligatory parasites of higher plants. Approximately 10 species of *Meloidogyne* are known to be serious pests of major food crops, vegetables, fruits, and ornamental plants in temperate, tropical, and subtropical regions [1–4].

One of the numerous host plants of root-knot nematodes is royal paulownia, *Paulownia* spp. This plant is native to East Asia and has been introduced to Europe and North and South America. The most common species within the genus are *P. fortunei*, *P. kawakamii*, *P. taiwaniana*, and *P. tomentosa*. Paulownia is a valued plant mainly for its high-quality wood, which is used for furniture, musical instruments, and dishes. Moreover, paulownia is known to be a good nectar producer and a fodder plant whose aboveground parts contain high levels of beneficial nutrients [5].

Out of a dozen root-knot nematode species that parasitize *Paulownia* spp., *M. incognita*, *M. javanica*, and *M. arenaria* [6–10] are most often mentioned as poor growth of paulownia plants has been observed in fields where these nematode species were identified.

The empress tree (*Paulownia tomentosa* Steud) has been cultivated for a long time in the southeastern region of Poland. In 2018, during a routine inspection of paulownia plantations, roots with outgrowths characteristic of the presence of *Meloidogyne* were observed. The population discovered was described using morphological, morphometric, and genetic methods. In this article, we provide the results of these investigations.

Material and methods

Soil samples with galled fragments of plant roots were collected from a 6-year-old plantation in Żoźnia, Poland (50°09'52.0"N, 22°15'54.7"E). Females with egg masses were manually dissected out of tissues. Perineal patterns and hatched juveniles of the J2 stage were mounted on temporary microscope slides according to Hooper et al. [11]. Morphological observations and morphometric analysis of females, males, and J2 juveniles were performed using an Axioskop 2plus light microscope with the Nomarsky differentiated contrast.

To confirm the morphological identification, the corresponding J2s ($n = 7$) were used for molecular characterization. Specimens were fixed in a DESS solution [12]. After washing in sterilized Milli-Q water, single nematode individuals were used for DNA extraction. DNA was isolated from specimens according to the nematode lysis procedure, as described by Holterman et al. [13]. The 28S rDNA regions were amplified using D2A (5'-ACAAGTACCGTGAGGGAAAGTT-3') and D3B primers (5'-TCG-GAAGGAACCAGCTACTA-3') [14]. To confirm that this nematode belonged to the species *M. hapla*, additional molecular tests were performed using species-specific primers. The PCR reaction was performed using the SCAR primer set JMV1 (5'-GGATG-GCGTGCTTCAAC-3') and JMV hapla (5'-AAAAATCCCCTCGAAAAATCCACC-3') species-specific to *M. hapla*. 5S-18S rDNA was amplified using the primer set 194 (5'-TTAACTTGCCAGATCGGACG-3') and 195 (5'-TCTAATGAGCCGTACGC-3') [15]. All PCR reactions were performed in a Veriti 96-well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplicons were visualized using a UV Illuminati after Midori Green (Nippon Genetics Europe, Duren, Germany) gel staining and gel electrophoresis. The 28S rDNA regions were sequenced using the Sanger method on an ABI 3500L genetic analyzer (Applied Biosystems). Sequences of 28S rDNA reported in this study have been deposited in GenBank with accession numbers MK213344–MK213350.

Results and discussion

Morphological observations revealed the presence of features typical of *M. hapla*. Female ($n = 12$) perineal patterns had low, rounded dorsal arches with wings on one lateral side and characteristic punctuations in the tail terminal area. The stylet had a cone slightly curved dorsally, with small, round knobs, set off from the shaft.

Male ($n = 8$) head regions were smooth, set off from body annulation, with a high and narrow head cup. The measurements of males comprised body length = 1,174 (961–1,404) μm , a (body length / maximal body diameter) = 43.2 (38.4–47.4), c (body length / tail length) = 110.7 (66.8–166.0), stylet = 17.6 (17.0–18.1) μm , dorsal gland orifice (DGO) = 4.5 (4.1–5.0) μm , c' (tail length / body width at anus level) = 0.6 (0.5–0.9), and spicules = 23 (21–28) μm .

Juvenile specimens ($n = 25$) were characterized by the rounded head cup, a delicate stylet with small and rounded knobs set off from the shaft, a hemizonid anterior to the excretory pore, and the tail tip finely rounded and irregularly shaped. Measurements of J2s comprised body length = 362 (324–380) μm , $a = 25.0$ (21.6–29.7), $c = 7.8$ (7.2–9.6), stylet = 10.5 (10.0–11.5) μm , DGO = 4.6 (4.0–5.6) μm , tail = 46 (38–51) μm , and hyaline tail terminus = 13 (10–15) μm . Morphological and morphometric characteristics matched the variability range known for *M. hapla* [16,17].

The Basic Local Alignment Search Tool (BLAST) analysis for sequenced partial 28S rDNA (709 bp; MK213344–MK213350) showed a 99% similarity to the sequences of *M. hapla* deposited in GenBank (KU180679, KJ755183, KJ598136, KJ645433, GQ130139). The visualized PCR products were 440 bp and 700 bp, respectively, for the SCAR primer sets JMV1 / JMV hapla and 194/195. The obtained PCR products matched the DNA sequences of *M. hapla* [14].

The occurrence of single outgrowths on paulownia roots, as well as J2 specimens in the root zone indicate that this plant species supports the *M. hapla* population in the soil. Crops subsequent to paulownia may become infected by these nematodes and reduced yields or even wilting may occur as a consequence. This study should draw the

attention of agricultural practitioners and plant protection specialists to the interaction of paulownia cropping and *M. hapla* population density in the soil. To our knowledge, this is the first study of *M. hapla* parasitizing paulownia in Poland, as well in Europe.

References

1. Luc M, Sikora RD, Bridge J. Plant parasitic nematodes in subtropical and tropical agriculture. Wallingford: CABI Publishing; 2005. <https://doi.org/10.1079/9780851997278.0000>
2. Perry RN, Moens M, Star JL. Root-knot nematodes. Wallingford: CABI Publishing; 2009. <https://doi.org/10.1079/9781845934927.0000>
3. Evans K, Trudgill D, Webster JM. Plant parasitic nematodes in temperate agriculture. Wallingford: CABI Publishing; 1993.
4. Flis Ł, Dobosz R, Winiszewska G, Rybarczyk-Mydłowska K, Malewski T, Wasilewska-Nascimento B, et al. First report of the root-knot nematode *Meloidogyne incognita* on tomato in Cape Verde. *Plant Dis.* 2018;102(1):253. <https://doi.org/10.1094/PDIS-07-17-1020-PDN>
5. Barton IL, Nicolas ID, Ecroyd CE. Paulownia. *Forest Research Bulletin.* 2007;231:1–71.
6. Chunram C, Ahnon B, Darunee R, Raski DJ. A heavy infestation of *Meloidogyne incognita* was found on the roots of *P. kawakamii* and *P. mikado* in a nursery near Chiang Mai. *FAO Plant Protection Bulletin.* 1976;24(3):103.
7. Hsieh HJ. Study on paulownia diseases found in Taiwan. *Bulletin, Taiwan Forestry Research Institute.* 1983;388:1–24.
8. Kobayashi T, de Guzman ED. Monograph of tree diseases in the Philippines with taxonomic notes on their associated microorganisms. *Bulletin of the Forestry and Forest Products Research Institute.* 1988;35:99–200.
9. Perera MR, Bayliss KL, Jones MGK. New Australian record for infection of trees in paulownia plantations by root-knot nematodes. *Australas Plant Pathol.* 2005;34:419–420. <https://doi.org/10.1071/AP05041>
10. Kaur R, Brito JA, Dickson DW. A first report of *Paulownia elongata* as a host of *Meloidogyne* spp. in Florida. *Plant Dis.* 2007;91:1199. <https://doi.org/10.1094/PDIS-91-9-1199C>
11. Hooper DJ, Evans K. Extraction, identification and control of plant parasitic nematodes. In: Evans K, Trudgill DL, Webster JM, editors. *Plant parasitic nematodes in temperate agriculture.* Wallingford: CABI Publishing; 1993. p. 31–59.
12. Yoder M, de Ley IT, King I, Mundo-Ocampo M, Mann J, Blaxter M, et al. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology.* 2006;8:367–376. <https://doi.org/10.1163/156854106778493448>
13. Holterman M, Rybarczyk K, van den Elsen S, van Megen H, Mooyman P, Santiago RP, et al. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Mol Ecol Resour.* 2008;8:23–34. <https://doi.org/10.1111/j.1471-8286.2007.01963.x>
14. Nunn GB. Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences [PhD thesis]. Nottingham: University of Nottingham; 1992.
15. Adam MAM, Phillips MS, Blok VC. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathol.* 2007;56:190–197. <https://doi.org/10.1111/j.1365-3059.2006.01455.x>
16. Whitehead AG. Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four species. *Transactions of the Zoological Society of London.* 1968;31:263–401. <https://doi.org/10.1111/j.1096-3642.1968.tb00368.x>
17. Kornobis S. Diversity of *Meloidogyne hapla* Chitwood, 1949 population in Poland. *Russ J Nematol.* 2004;12:31–38.