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# First Report of *Meloidogyne incognita* Infecting *Stachys byzantina* in Minas Gerais, Brazil

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## ABSTRACT

Lamb's ear (*Stachys byzantina* K. Koch) is a perennial herbaceous plant classified in Brazil as a non-conventional edible plant (NCEP) with gastronomic, ornamental, and pharmacological importance. In January 2024, plants from the Santa Rita Experimental Field of EPAMIG, in Prudente de Morais, Minas Gerais State, Brazil, showed leaf chlorosis, root galling, and reduced growth. Nematodes extracted from symptomatic roots were multiplied and used for pathogenicity tests and for morphological, morphometric, and biochemical characterisation. Pathogenicity tests confirmed nematode multiplication and symptom reproduction, with a mean reproduction factor of $23.33 \pm 1.08$. Morphological characterisation revealed perineal patterns with a high, squarish dorsal arch, morphometric measurements of second-stage juveniles, and biochemical analysis showing the esterase I2 phenotype ($R_m = 1.0$ and 1.05); together, these results were consistent with *Meloidogyne incognita*, representing the first report of this nematode parasitizing *Stachys byzantina* in Minas Gerais State, Brazil. This finding expands the known geographic distribution of this root-knot nematode and highlights the need for nematode management strategies in non-conventional crops.

Lamb's ear (*Stachys byzantina* K. Koch) is a perennial herbaceous plant of the Lamiaceae family and is classified in Brazil as a non-conventional edible plant (NCEP) with increasing interest due to its gastronomic potential and contribution to food biodiversity (Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Desenvolvimento Agropecuário e Cooperativismo 2013; Kinupp and Lorenzi 2014; Botrel et al. 2020). Its leaves are traditionally consumed fresh or processed and are valued for their sensory attributes and nutritional quality, including high fibre content (Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Desenvolvimento Agropecuário e Cooperativismo 2010a, 2010b; Botrel et al. 2020), as well as for their pharmacological properties (Asnaashari et al. 2010).

Despite growing incentives to promote its use in human nutrition, technical and scientific information on its cultivation under Brazilian soil and climatic conditions remains limited, particularly regarding crop management and pest and disease control (Silva et al. 2018). Nevertheless, *S. byzantina*, like other NCEP, plays an important role in diversifying food culture in different regions of Brazil (Madeira et al. 2013). However, the socioeconomic and nutritional importance of these plants can be compromised by plant-parasitic nematodes, especially *Meloidogyne* spp., recognised worldwide for their broad host range, aggressiveness, and destructive potential (Jones et al. 2013).

In Brazil, *Meloidogyne incognita* and *M. javanica* have been reported parasitizing lamb's ear only in Jaboticabal, São Paulo

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(Nascimento et al. 2021; Silva et al. 2024), highlighting the relevance of the present report. In January 2024, lamb's ear plants from the non-conventional vegetable bank of the Santa Rita experimental field at the Agricultural Research Company of Minas Gerais (EPAMIG), Prudente de Morais, MG (19°28′55″S, 44°09′18″W), showed leaf chlorosis, root galling, and reduced growth. Samples of symptomatic roots ($n = 5$) were collected in properly labelled polyethylene bags and sent to the Nematology Laboratory at the Viçosa Federal University (UFV) for diagnosis.

The *Meloidogyne* sp. population was multiplied on tomato (*Solanum lycopersicum* cv. Santa Clara) from a single egg mass and maintained under greenhouse conditions for 30 days at 25°C ± 5°C to obtain a pure population. This population was used for pathogenicity tests and for morphological, morphometric and biochemical identification. For pathogenicity evaluation on *S. byzantina*, seedlings ($n = 5$) grown in previously sterilised soil and maintained in 8-L polyethylene pots were inoculated with 20,000 nematode eggs. Sixty days after inoculation, nematodes were extracted from symptomatic roots using the maceration and centrifugal flotation method in sucrose solution (Boneti and Ferraz 1981), quantified using a Peters counting chamber under a light microscope (AX10, Carl Zeiss, Germany), and used to calculate the reproduction factor (RF = final population/initial population).

Morphological characterisation was based on the analysis of female perineal patterns ($n = 10$), according to Eisenback et al. (1980). Subsequently, second-stage juveniles (J2) of *Meloidogyne* sp. were obtained from a hatching chamber, following Cliff and Hirschmann (1985), for morphometric measurements ($n = 10$). Finally, biochemical identification was performed ($n = 15$), with four replicates, through the evaluation of female esterase isoenzyme phenotypes, according to Carneiro and Almeida (2001).

Perineal patterns were cut, cleaned in 45% lactic acid (Êxodo Científica), mounted in glycerin on glass slides according to Abrantes and Santos (1989), and examined under a light microscope (AX10, Carl Zeiss, Germany) at 1000× magnification. Morphometric characteristics of juveniles included body length, body width, stylet length, and dorsal gland orifice (DGO), expressed as mean ± standard deviation. For biochemical identification, females were removed from infected roots and transferred to microtubes containing 15 μL of extraction buffer (distilled water, 0.5 M Tris–HCl, pH 6.8, glycerol, and 0.5% (w/v) bromophenol blue), followed by maceration with a pestle. Protein extracts were subjected to vertical polyacrylamide gel electrophoresis, according to Ornstein (1964) and Davis (1964), with modifications for use in the Mini-Protean 3 Mini Vertical system. Previously characterised females of *Meloidogyne javanica*, exhibiting the J3 phenotype ($R_m = 1.0$), were used as a reference standard.

The pathogenicity test confirmed that the root-knot nematode was able to multiply in all inoculated plants and reproduce typical symptoms of chlorosis and gall formation (Figure 1), demonstrating a consistent association between the pathogen and the host, with a mean reproduction factor of RF = 23.33 ± 1.08. The perineal patterns (Figure 2A) were characterised by the presence of a high, squarish dorsal arch that often contains a distinct whorl in the tail terminal area, with striae smooth to wavy and sometimes zigzagged. The morphometric characteristics of juveniles (Figure 2B) showed a body length of 483.45 ± 12.50 μm, body width of 21.12 ± 0.44 μm, stylet length of 11.83 ± 0.77 μm, and dorsal oesophageal gland orifice to base of stylet (DGO) of 3.17 ± 0.35 μm. In addition, the esterase profile of the *Meloidogyne* sp. population exhibited the I2 phenotype (Figure 2C), characterised by a well-defined main band (Rm = 1.0) and a secondary band ($R_m = 1.05$), while females of *M. javanica* displaying the J3 phenotype ($R_m = 1.0$) were used as a reference standard (Carneiro et al. 2016).




**FIGURE 1** | Pathogenicity test of a *Meloidogyne* sp. population on lamb's ear (*Stachys byzantina*) 60 days after inoculation. Inoculated plants (1–5) exhibited leaf chlorosis and root galling. Lower panels show details of galled root systems. The mean reproduction factor was RF = 23.33 ± 1.08, confirming successful infection and multiplication of *Meloidogyne incognita*.

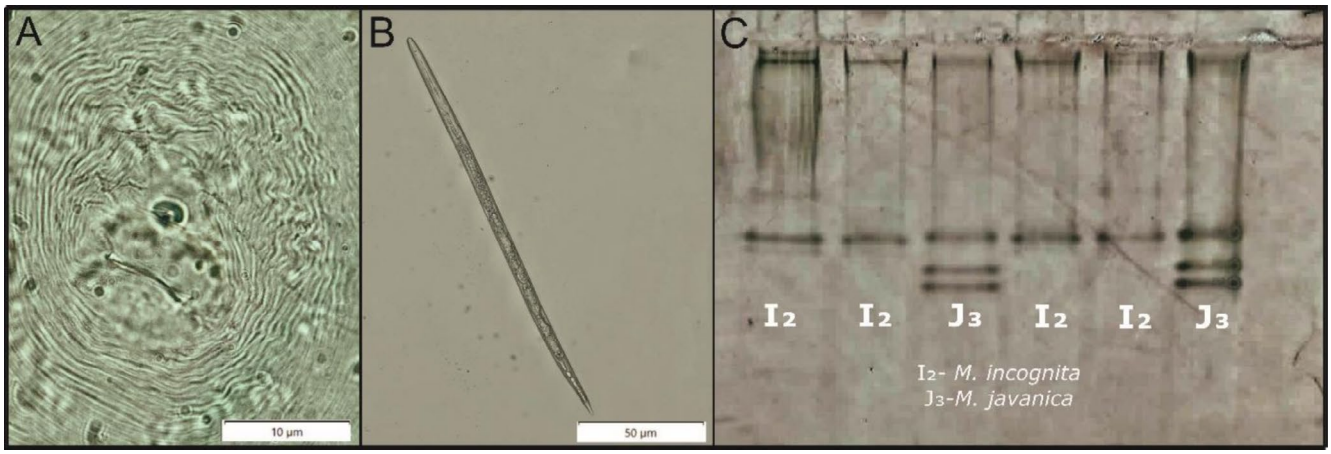



**FIGURE 2** | Morphological, morphometric and biochemical characterisation of the *Meloidogyne* sp. population. (A) Perineal pattern exhibiting a high, trapezoidal dorsal arch with thick striations, consistent with the identification of *M. incognita*. (B) Second-stage juvenile (C) Esterase phenotype $I_2$, with a well-defined main band ($R_m = 1.0$) and a secondary band ($R_m = 1.05$), characteristic of *M. incognita*. The $J_3$ phenotype, corresponding to *M. javanica*, is presented as a reference. Each esterase phenotype was obtained from 15 females. $R_m$ = migration ratio in relation to the slowest band ($R_m = 1.0$) of *M. javanica*.

The combined results of the pathogenicity test and the morphological, morphometric, and biochemical analyses were consistent and confirmed the identification of the nematode as *Meloidogyne incognita*. This study represents the first report of *M. incognita* parasitizing *S. byzantina* in Minas Gerais, Brazil, expanding the known geographic distribution of this root-knot nematode. The occurrence of this species in a plant cultivated for both ornamental and non-conventional purposes highlights the need for appropriate management strategies to prevent losses and limit pathogen dispersal. This report complements previous records from São Paulo State (Nascimento et al. 2021; Silva et al. 2024), reinforcing the relevance of *S. byzantina* as a host of *M. incognita* in different regions of Brazil. Future studies integrating molecular confirmation and monitoring of nematode populations are essential to support accurate diagnosis and effective management.

### Author Contributions

Conceptualization: Dalila Sêni Buonicontro, Marinalva Woods Pedrosa, and José Neto Rodrigues Santana; sample collection: Marinalva Woods Pedrosa, Kelly Isaltina Santos Silva and Juliana Maria de Oliveira; methodology: José Neto Rodrigues Santana, Amanda Pereira Honório and Kelly Isaltina Santos Silva; formal analysis and investigation: José Neto Rodrigues Santana, Amanda Pereira Honório, Maria Izabel de Almeida Leite, and Dalila Sêni Buonicontro; writing – original draft preparation: José Neto Rodrigues Santana; writing – review and editing: José Neto Rodrigues Santana, Maria Izabel de Almeida Leite, Dalila Sêni Buonicontro and Marinalva Woods Pedrosa; All authors read and approved the final manuscript.

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### Ethics Statement

The authors have nothing to report.

### Consent

The authors have nothing to report.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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