First detection of *Meloidogyne luci* (Nematoda: Meloidogynidae) parasitising potato in the Azores, Portugal

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Introduction

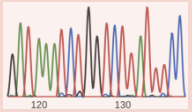
Potato, *Solanum tuberosum*, is the third most important crop in the world after rice and wheat, with more than 156 countries producing it[1]. In Portugal, this crop has a great social and economic importance, since it is grown throughout the country, including the archipelagos of Madeira and Azores[2]. The Root-knot nematodes (**RKN)** are one of the oldest known parasitic nematodes of plants and considered serious pests of economically important crops[3]. *Meloidogyne luci* was first described in 2014 from different plant species in Brazil, Chile and Iran. In Portugal, it was detected in 2013 in a potato field near Coimbra and has been recently found parasitising tomato, the ornamental plant *Cordyline australis* and the weed *Oxalis corniculata* [4]. The aim of the present study was to characterise morphological, morphometric, biochemical and molecularly the isolate of RKN *M. luci* found in the Azores Island.

Materials and Methods

Morphometric: During the 2019 National Survey in the Azores Island, soil samples were collected from the council of Santo António in Pico Island. Nematodes were extracted according to the protocol PM 7/119 (1) [5]. Using a bright-field light microscope nematodes were characterised morphologically and morphometric. For positive detections of *Meloidogyne* it was necessary to perform bioassays in order to obtain material (females, egg masses and males) for identification.

Biochemical: Young egg-laying females were handpicked from infected tomato roots and transferred to micro-haematocrit capillary tubes with 5 µL of extraction buffer and stored a -20°C until use. Proteins were separated by polyacrylamide gel electrophoresis (PAGE).

Molecular: The mtDNA COII/16S rRNA region was selected for molecular characterisation of the *M. luci* isolate from Azores Island. Total DNA was extracted from egg masses.



Amplicons were sequenced in both directions at STABVida, sequences were manually checked, edited and assembled and compared using the BLAST homology search. The multiple alignment was performed using ClustalW Multiple alignment in BioEdit. Phylogenetic analyses were conducted using MEGA X v10.1.

Results

A combination of morphological (Fig 1), biochemical (Fig 2) and molecular (Fig 3) analysis was used for identification. Results were compared with previous descriptions and found to be consistent. To our knowledge this is the first report of *M. luci* in the Island of Azores – Portugal, adding valuable information to the current location of this organism in the EPPO zone.

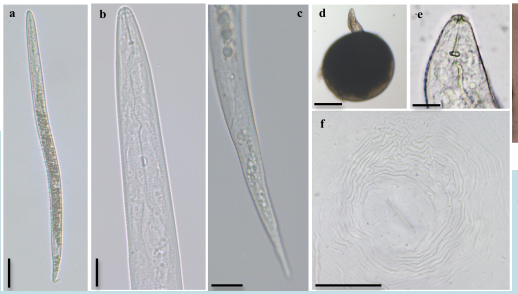


Fig1. *Meloidogyne luci* light microscope observations. Second-stage juvenile: **a-** whole specimen; **b-** anterior region; **c-** tail region; Female: **d-** egg-laying female, whole specimen; **e-** anterior end; **f-** perineal pattern. (bar = 20 µm)

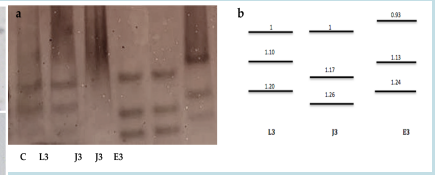


Figure 2. a. phenotypes of protein homogenates from one egg-laying female of *Meloidogyne* species: C- Positive control *M. luci*; 1- L3- *M. luci* Esterase (Azores); J3- *M. javanica*; E3- *M. ethiopica*. **b.** Relative mobility L3- *M. luci*; J3- *M. javanica*; E3- *M. ethiopica*.

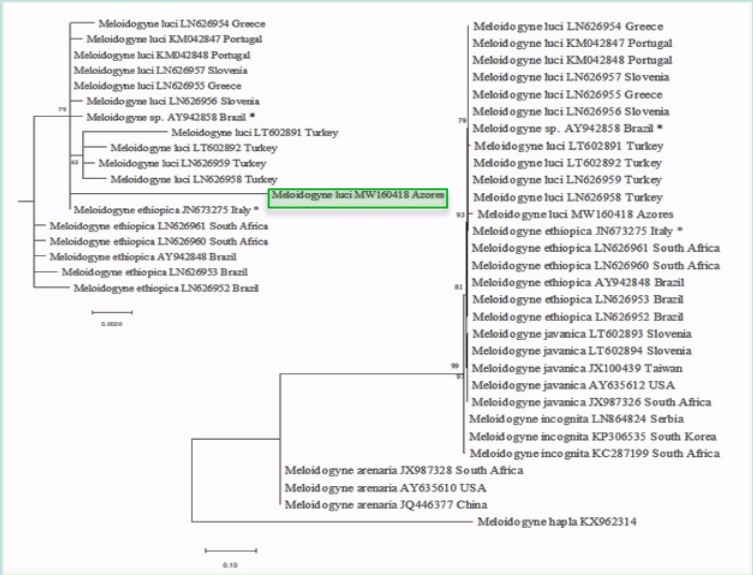


Figure 3. Phylogenetic relationships of *Meloidogyne luci* isolate collected from Azores-Portugal and *M. luci* isolates from other geographical regions, including other species of the *Meloidogyne*-group, based on the sequence alignment of the mtDNA region between COII and 16S genes. Maximum Likelihood method and Hasegawa-Kishino-Yano model with 1000 bootstrap replication. Bootstrap values are indicated at the nodes. The analysis involved 30 nucleotide sequences and there were a total of 1596 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

References: **1-FAO,** 2020. FAOSTAT. [http://www.fao.org/faostat/en/#data]. Accessed 16 October 2020; **2 Camacho, M.J.; Nóbrega, F.; Lima, A.; Mota, M.; Inácio, M.L.** Morphological and molecular identification of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in Portuguese potato fields. *Nematol.* 2017, 8, 883-889**; 3. Trudgill, D.L.; Blok, V.C.** Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annu. Rev. Phytopathol.* 2001, 39, 53-77; **4.Santos, D.; Correia, A.; Abrantes, I.; Maleita, C.** New Hosts and Records in Portugal for the Root-Knot Nematode *Meloidogyne luci*. *J. Nematol.* 2019, 51,1-4; **5. EPPO.** 2013. Standard protocol PM 7/119 (1). EPPO Bulletin 43:471–95.

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