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NEW DISEASE REPORT

First report of *Meloidogyne javanica* infecting Australian grass tree

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Xanthorrhoea glauca (grass tree, family Xanthorrhoeaceae) is commonly found in heathlands, woodlands and dry sclerophyllous forests. *Xanthorrhoea* is an instantly recognisable plant, endemic to continental Australia and associated offshore islands (Bedford, 1988). During import inspections of *X. glauca* originating from Australia, the growing media and roots were sampled and brought to İzmir Agricultural Quarantine Directorate Laboratory, Türkiye, in April 2021. Root-knot nematode (RKN) 2nd stage juveniles were detected from growing medium extractions using the Oostenbrink dish method. Symptomatic galls caused by *Meloidogyne* infestation were observed on the roots (Figure. 1, Figure. 2). RKN populations obtained from the roots of the plant were cultured on susceptible tomato cv. Rio Grande. After 60 days, egg masses formed in the roots were removed using a disection microscope. Tomato seedlings at the 2–4 true leaf stage were transplanted into pots containing sterilised sandy soil (80% sand, 5% loamy sand and 15% soil). One isolated egg mass was inoculated onto the root zone of each seedling. After 60 days this procedure was repeated to purify the nematode populations. Nematode females were collected from the roots for diagnostic studies. In addition, a confirmed *M. javanica* strain was produced following the described procedure to be used as a marker in polyacrylamide gel electrophoresis (PAGE) studies.

Permanent slides of perineal patterns were prepared following Hartman & Sasser (1985). Mature females of RKNs obtained from pure cultures were placed in 45% lactic acid for 25–30 minutes. The anterior of the females was dissected and the body contents removed. Five to ten perineal patterns were cut and mounted in glycerin on each slide Preparations were examined under a compound light microscope and compared with species descriptions and morphological keys (Figure. 3).

The esterase isoenzyme phenotypes of RKNs were analysed according to Esbenshade & Triantaphyllou (1985). PAGE was performed on five egg-laying females using a mini-protein II electrophoresis unit (Bio-Rad, USA). The females were placed (three females per tube) in 10 µL extraction buffer in sterile PCR tubes. Each was loaded into a well of a polyacrylamide gel consisting of a 4% stacking (pH 6.8) and a 8% separating sections (pH 8.8). A confirmed *M. javanica* strain was used in each gel as a reference. Under electrophoretic conditions, the gels were subjected to an electric current at 80 v for 15 min, then 200 v for 25–30 min or until the bromophenol blue dye moved approximately 0.5 cm from the base of the gel. Following electrophoresis, the gels were removed and stained for esterase activity using α-naphthyl acetate substrate. Gels were held in the dark for about 40 minutes. Esterase phenotype bands were assigned according to Maleita et al. (2016).

Meloidogyne javanica was identified by biochemical and morphological methods. This is the first report of *M. javanica* associated with *Xanthorrhoea glauca*.

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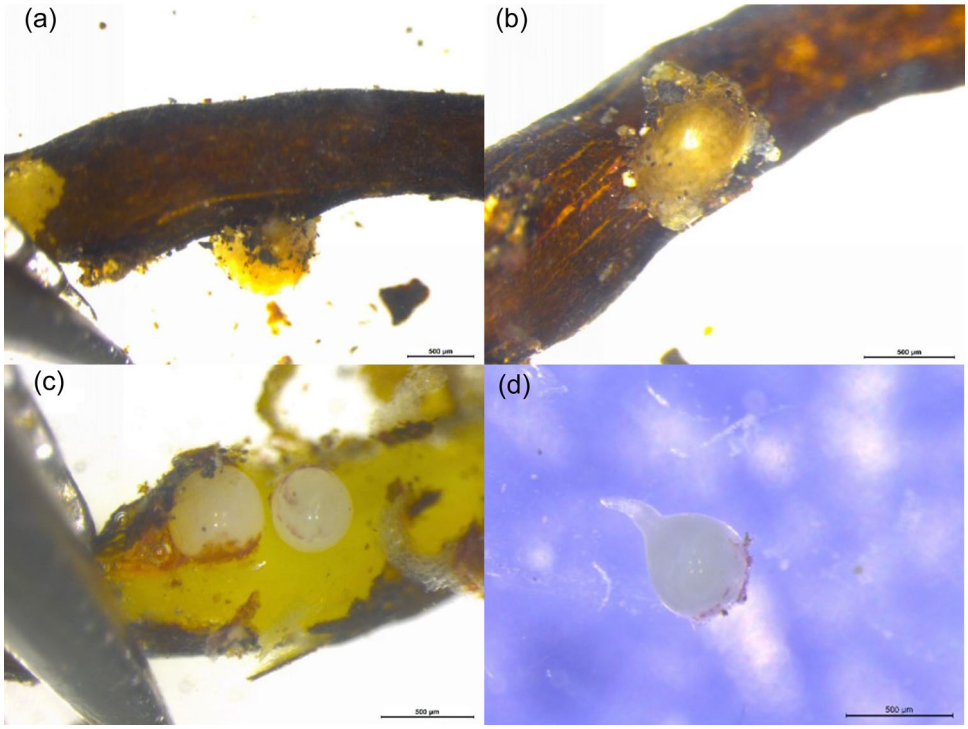


FIGURE 1 Binocular microscope photos of *Meloidogyne javanica* on grass tree root: (a, b) egg mass of *M. javanica*, and (c, d) female of *M. javanica*.



FIGURE 2 Light microscope photos of (a) 2nd stage juvenile and (b) anterior and (c) posterior of 2nd stage juvenile.

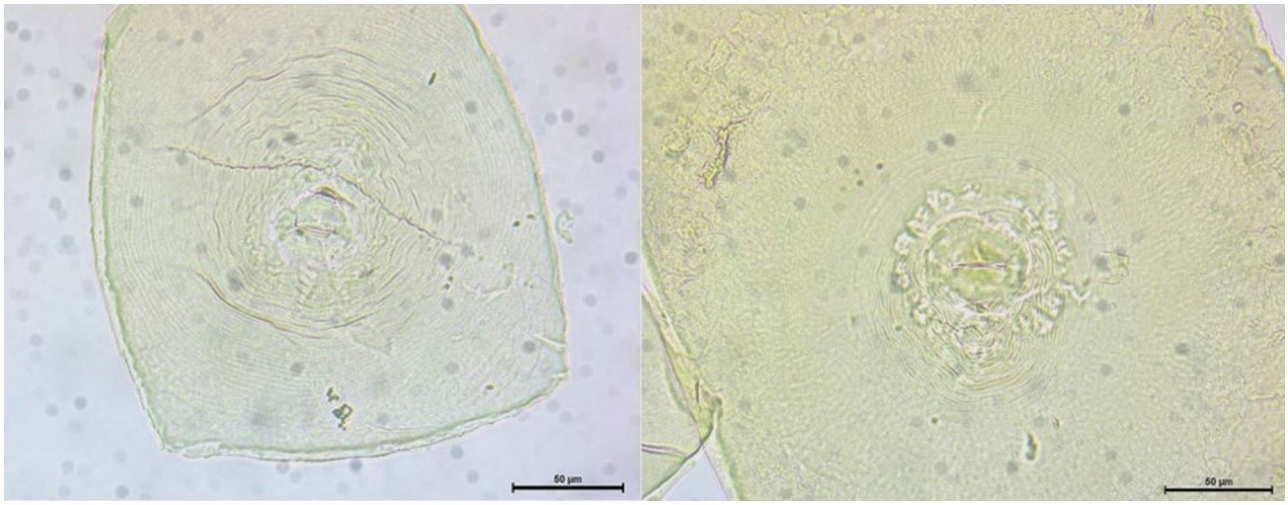


FIGURE 3 Light microscope photos of female perineal patterns of *Meloidogyne javanica*.

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