

First Report of *Longidorus kuiperi* and *Rotylenchus eximius* from Coastal Sand Dunes in Crete, Greece

EMMANUEL A. TZORTZAKAKIS,¹ CAROLINA CANTALAPIEDRA-NAVARRETE,² ANTONIO ARCHIDONA-YUSTE,²
JUAN E. PALOMARES-RIUS,² AND PABLO CASTILLO²

Abstract: Plant-parasitic nematode species have been reported on several occasions from coastal sand dunes, including *Longidorus* and *Rotylenchus* species (Vovlas et al., 2008; De Luca et al., 2009; Mateille et al., 2014). In April 2016, 10 soil samples of 3 to 4 kg from the rhizosphere of *Tamarix smyrnensis* with different vegetation around (viz. *Elymus farctus*, *Lycium schweinfurthii*, *Crithmum maritimum*, and *Arthrocnemum* sp.) were collected for diagnosis of plant-parasitic nematodes. The area of sampling was a coastal sand dune near the archeological site of Komos, southwest of Crete, Greece. Low soil populations of a needle and a spiral nematode were detected (3 and 8 individuals/1,000 cm³ of soil, respectively), which prompted us to undertake a detailed morphological and molecular comparative study with previous reported data. Nematodes were extracted from soil with the wet sieving and decanting method (Cobb, 1918). Morphological and molecular analyses of females identified these species as *Longidorus kuiperi* Brinkman, Loof and Barbez, 1987, and *Rotylenchus eximius* Siddiqi, 1964. The morphology of *L. kuiperi* females (six specimens studied) was characterized by having a slender body; very broad lip region (27 ± 1.5 [25 to 30] µm in width); short, hemispherical tail; body length of (7.1 ± 0.8 [6.5 to 8.5] mm); vulva position at 47% to 55% of body length; odontostyle length of (105 ± 6.5 [90 to 115] µm); males very common (but less frequent than females [45% vs 55%]); tail region with 15 to 20 supplements and bulged terminal cuticle. The morphology of *R. eximius* females (four specimens studied) was characterized by having a hemispherical lip region clearly set off; with four annuli; body without longitudinal striations; lateral fields areolated in the pharyngeal region only; stylet 36 to 38 µm; and broadly rounded tail. The morphology of the isolated nematodes agreed with previous descriptions of *L. kuiperi* (Brinkman et al., 1987; De Luca et al., 2009), and *R. eximius* (Siddiqi, 1964; Castillo and Vovlas, 2005). A single individual was used for DNA extraction. Primers and polymerase chain reaction conditions used in this research were specified in Cantalapieira et al. (2013) and Archidona-Yuste et al. (2016), and a single amplicon of 800 and 1,100 bp was obtained and sequenced, respectively. D2-D3 (KX398055-KX398056) and ITS sequence alignments (751 and 648 bp, respectively) from *L. kuiperi* (KX398057) showed 98% to 99% similarity, differing in 4, and from 6 to 12 nucleotides, respectively, to other sequences of *L. kuiperi* deposited in GenBank from Italy and Spain (AM911623, AM905267-AM905270, respectively), with a query coverage of 99%. Similarly, D2-D3 sequence alignment from *R. eximius* (KX398058) showed 100% to 99% to another sequence of *R. eximius* deposited in GenBank from Italy and Spain (EU280794, DQ328741), differing in zero to three nucleotides, respectively, and a query coverage of 81%. Phylogenetic analyses using Bayesian Inference placed *L. kuiperi* in a highly supported (100%) clade that included all *L. kuiperi* sequences deposited in GenBank (Archidona-Yuste et al., 2016), and *R. eximius* in a highly supported (100%) clade that included all *R. eximius* sequences deposited in GenBank (Cantalapieira-Navarrete et al., 2013). All identification methods were consistent with *L. kuiperi* and *R. eximius*. To our knowledge, this is the first report of *L. kuiperi* and *R. eximius* in Greece. Consequently, all these data suggest that coastal sand dunes in Europe constitute environmental conditions optimal for colonization and development of *L. kuiperi*, as previously reported (De Luca et al., 2009). Similarly, *R. eximius* has been reported in several Mediterranean countries, including Italy, Morocco, Spain, and Tunisia (Castillo and Vovlas, 2005), and this report extend the geographical distribution of this species.

Key words: Crete, detection, *Longidorus kuiperi*, needle nematode, *Rotylenchus eximius*, spiral nematode.

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¹Department of Viticulture, Vegetable Crops, Floriculture and Plant Protection, Institute of Olive Tree, Subtropical Crops and Viticulture, N.A.G.R.E.F., Hellenic Agricultural Organization-DEMETER, P.O. Box 2228, 71003 Heraklion, Crete, Greece.

²Institute for Sustainable Agriculture (IAS), CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain.

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E-mail: pcastillo@ias.csic.es; p.castillo@csic.es.

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