

First Report of the Spiral Nematode *Rotylenchus incultus* (Nematoda: Hoplolaimidae) from Cultivated Olive in Tunisia, with Additional Molecular Data on *Rotylenchus eximius*

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Abstract: Spiral nematode species of the genus *Rotylenchus* have been reported on olive (*Olea europaea* L.) in several Mediterranean countries (Castillo et al., 2010; Ali et al., 2014). Nematological surveys for plant-parasitic nematodes on olive trees were carried out in Tunisia between 2013 and 2014, and two nematode species of *Rotylenchus* were collected from the rhizosphere of olive cv. Chemlali in several localities of Tunisia (Tables 1,2). Twenty-two soil samples of 3 to 4 kg were collected with a shovel from the upper 50 cm of soil from arbitrarily chosen olive trees. Nematodes were extracted from 500 cm³ of soil by centrifugal flotation method (Coolen, 1979). Specimens were heat killed by adding hot 4% formaldehyde solution and processed to pure glycerin using the De Grisse's (1969) method. Measurements were done using a drawing tube attached to a Zeiss III compound microscope. Nematode DNA was extracted from single individuals and PCR assays were conducted as described by Castillo et al. (2003). Moderate-to-low soil populations of these spiral nematodes were detected (5.5–11.5, 1.5–5.0 individuals/500 cm³ of soil, respectively). This prompted us to undertake a detailed morphological and molecular comparative study with previous reported data. Morphological and molecular analyses of females identified these species as *Rotylenchus eximius* Siddiqi, 1964, and *Rotylenchus incultus* Sher, 1965. The morphology of *R. eximius* females (five specimens studied) was characterized by having a hemispherical lip region clearly off set, with four to five annuli, body without longitudinal striations, lateral fields areolated in the pharyngeal region only, stylet 32 to 36 µm long, and broadly rounded tail. The morphology of *R. incultus* females (51 females and 16 males; Table 2) was characterized by a hemispherical lip region with the basal annulus subdivided by irregular longitudinal striations, with three, rarely four annuli; stylet 21.5 to 27.5 µm long, female tail hemispherical with terminus regularly annulated; phasmids anterior to anus level (3–6 annuli above). The morphology of the isolated nematodes agreed with previous descriptions of *R. eximius* (Siddiqi, 1964; Castillo and Vovlas, 2005) and *R. incultus* (Sher, 1965; Castillo and Vovlas, 2005; Vovlas et al., 2008), respectively. A single individual was used for DNA extraction. Primers and PCR conditions used in this research were specified in Cantalapietra-Navarrete et al. (2013), and a single amplicon of 800, 1,100, and 450 bp was obtained and sequenced for D2 to D3, ITS1, and cytochrome c oxidase subunit I (*coxI*), respectively. Sequence alignments for D2 to D3 (KX669231–KX669233), ITS1 (KX669238–KX669240), and *coxI* (KX669244–KX669245) from *R. eximius*, showed 99% to 97%, 98% to 94%, 93% similarity to other sequences of *R. eximius* deposited in GenBank (EU280794–DQ328741, EU373663–EU373664, JX015401–JX015402, respectively). Similarly, D2 to D3 (KX669234–KX669237), ITS1 (KX669241–KX669243), and *coxI* (KX669246–KX669249) sequence alignments from *R. incultus*, showed 99%, 99% to 95%, 99% to 90% similarity, respectively, to other sequences of *R. incultus* deposited in GenBank (EU280797, EU373672–EU373673, JX015403, respectively). The best fitted model of DNA evolution was obtained using jModelTest v. 2.1.7 (Darriba et al. 2012) with the Akaike information criterion. BI analyses were performed under the general time reversible (GTR) with invariable sites and a gamma-shaped distribution of substitution rates (GTR + I + G) model for ITS1 and *coxI*. Phylogenetic analyses of ITS1 and *coxI* using Bayesian inference (BI) placed *R. eximius* and *R. incultus* from Tunisia in subclades that included all *R. eximius* and *R. incultus* sequences deposited in GenBank (Fig. 1), which agrees with previous results (Cantalapietra-Navarrete et al., 2013). Morphology, morphometry, and molecular and phylogenetic data obtained from these samples were consistent with *R. eximius* and *R. incultus* identification. To our knowledge, this is the first report of *R. incultus* in Tunisia. Consequently, all these data suggest that spiral nematode species of the genus *Rotylenchus* are predominant in olive as previously reported in other Mediterranean areas (Ali et al., 2014).

Key words: Bayesian inference, detection, new geographic record, phylogeny, spiral nematodes.

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TABLE 1. *Rotylenchus eximius* Siddiqi, 1964 and *R. incultus* Sher, 1965 sampled in olive cv. Chemlali in Tunisia and sequences used in this study.

Species	Sample code	Locality	D2–D3	ITS1	<i>coxI</i>
<i>R. eximius</i>	P03N68	Abida, Kairouan	KX669231	KX669238	KX669244
<i>R. eximius</i>	P11R34	Sbitla, Kasserine	KX669232	KX669239	*
<i>R. eximius</i>	P4AA10	Abida, Kairouan	KX669233	KX669240	KX669245
<i>R. incultus</i>	P11R08	Sbitla, Kasserine	KX669234	KX669241	KX669246
<i>R. incultus</i>	P1AA11	Chott-Mariem, Sousse	KX669235	-	KX669247
<i>R. incultus</i>	P04R35	Abida, Kairouan	KX669236	KX669242	KX669248
<i>R. incultus</i>	P03N70	Abida, Kairouan	KX669237	KX669243	KX669249

(-) Not obtained. (*) Sequenced population but not deposited in GenBank database because of their similarity with other of the species.

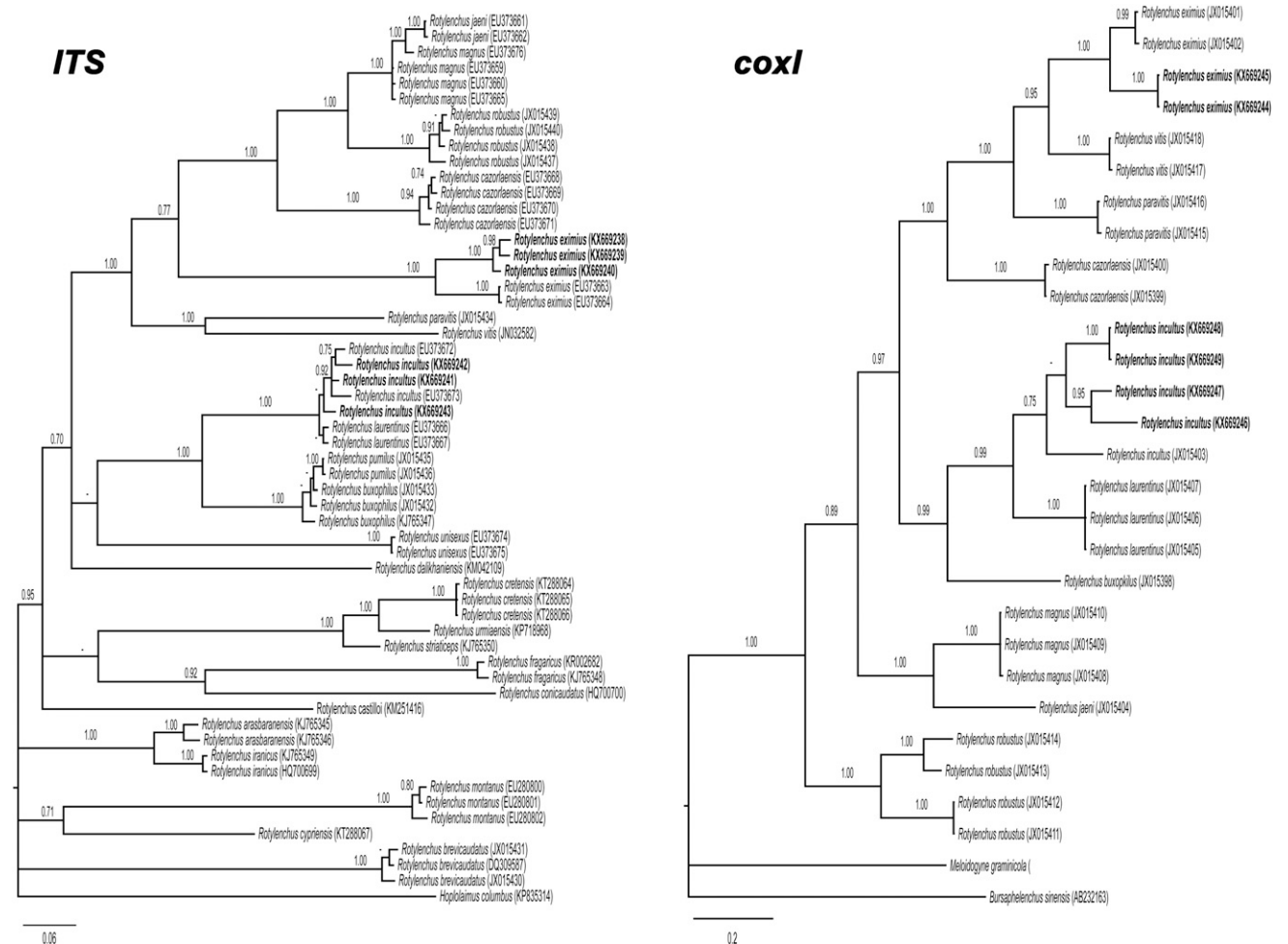


FIG. 1. Phylogenetic relationships within *Rotylenchus* species found in Tunisia and other species from GenBank. Bayesian 50% majority rule consensus trees as inferred from ITS1 and *coxI* sequences alignments under the GTR + I + G model. Posterior probabilities more than 0.70 are given for appropriate clades. Newly obtained sequences in this study are in bold. Scale bar = expected changes per site.

TABLE 2. Morphometrics of *Roflyenchus incultus* Sher, 1965 from olive cv. Chemlali in Tunisia.^a

Characters/ratios ^b	Abida, Kairouan (P3)		Abida, Kairouan (P4)		Sbitla, Kasserine (P11)		Choht-Mariem, Soussse (P1)	
	Female	Male	Females	Male	Females	Males	Females	Males
n	1	1	10	1	19	5	21	10
L	833 32.7	778 29.2	789 ± 34 (739-833) 28.6 ± 1.9 (26.2-31.8)	778 29.2	850 ± 65.5 (755-983) 31.2 ± 2.7 (27.1-36.6)	799 ± 55.4 (728-850) 34.0 ± 3.0 (29.6-36.2)	942 ± 67.7 (828-1,061) 34.3 ± 2.5 (29.6-40.0)	834 ± 81.2 (683-983) 37.3 ± 2.7 (32.4-41.0)
a	7.1	7.5	7.3 ± 0.3 (6.8-7.8) 41.4 ± 3.5 (36.8-47.8)	7.5	7.2 ± 0.7 (6.1-8.5) 47.4 ± 9.0 (30.3-60.2)	6.8 ± 0.3 (6.5-7.1) 33.1 ± 2.9 (29.7-36.4)	8.0 ± 0.8 (6.8-10.0) 53.8 ± 7.6 (41.1-71.3)	7.2 ± 0.8 (6.0-8.2) 31.4 ± 4.2 (24.6-38.6)
b	53.7	33.1	1.0 ± 0.1 (0.9-1.2)	33.1	0.9 ± 0.2 (0.7-1.3)	1.7 ± 0.1 (1.6-1.8)	0.9 ± 0.1 (0.7-1.2)	1.8 ± 0.2 (1.6-2.3)
c'	55.0	49.0	55.3 ± 1.3 (53.0-57.0)	49.0	54.8 ± 1.6 (52.0-57.0)	52.2 ± 11.9 (45.2-70.0)	54.1 ± 1.5 (51.0-57.0)	46.4 ± 3.5 (41.3-52.8)
V or T	23.5	24.0	24.7 ± 1.3 (22.0-26.5)	24.0	24.9 ± 1.3 (21.5-27.0)	23.4 ± 1.5 (22.0-25.5)	25.4 ± 1.1 (23.5-27.5)	22.8 ± 0.8 (21.5-23.5)
Stylet length	4.0	3.5	4.8 ± 0.5 (4.0-5.5)	3.5	4.3 ± 1.1 (2.5-6.0)	4.3 ± 0.4 (4.0-4.5)	5.6 ± 0.6 (4.0-7.0)	5.2 ± 0.6 (4.5-6.0)
Dorsal gland opening	17.0	14.6	19.5 ± 1.5 (17.0-21.6)	14.6	16.1 ± 3.8 (10.2-24.5)	18.5 ± 1.0 (17.8-19.2)	21.9 ± 2.6 (16.3-28.6)	22.6 ± 3.0 (19.2-27.3)
O	132.0	114.5	136.7 ± 14.3 (117-161)	114.5	139.9 ± 12.8 (122-173)	143.5 ± 22.6 (120-172)	144.4 ± 11.8 (130-169)	141.9 ± 16.9 (119-165)
Pharynx length	17.5	14.0	21.7 ± 3.7 (16.5-28.0)	14.0	22.2 ± 8.1 (13.5-46.0)	20.7 ± 6.1 (14.0-26.0)	22.5 ± 5.1 (14.5-30.0)	21.6 ± 7.4 (13.0-34.5)
Pharyngeal overlap	25.5	26.0	27.7 ± 2.0 (24.5-31.0)	26.0	27.2 ± 2.0 (24.0-33.5)	23.6 ± 2.5 (20.5-26.5)	27.5 ± 1.4 (25.0-31.0)	22.4 ± 1.0 (20.5-24.0)
Maximum body diameter	20.0	14.5	19.0 ± 1.0 (17.5-20.5)	14.5	20.3 ± 1.8 (16.0-24.5)	13.9 ± 0.6 (13.0-14.5)	18.9 ± 2.6 (16.0-22.0)	14.8 ± 1.5 (13.5-18.5)
Anal body diameter	15.5	23.5	19.3 ± 1.9 (16.5-22.5)	23.5	18.0 ± 3.4 (13.5-27.5)	24.3 ± 2.1 (21.5-26.5)	17.8 ± 2.6 (12.0-22.5)	26.9 ± 3.2 (22.0-31.5)
Tail	15.5	-	12.0 ± 2.0 (9-14)	-	10.7 ± 3.0 (6-15)	-	10.1 ± 2.8 (6-16)	-
Tail annuli	8	-	25.8 ± 4.4 (20.0-32.5)	-	22.9 ± 5.7 (15.0-33.5)	-	23.6 ± 5.8 (9.0-30.0)	-
Phasmid to terminus	15	-	-	-	-	30.8 ± 1.9 (29.0-33.5)	-	28.4 ± 2.2 (26.0-33.0)
Spicules	-	22.5	-	22.5	-	10.5 ± 0.6 (10.0-11.0)	-	12.6 ± 2.0 (9.5-16.0)
Gubernaculum	-	8.5	-	8.5	-	-	-	-

^a Measurements are in µm and in the form: mean ± SD (range).

^b Abbreviations are defined in Siddiqi (2000).