Nem 11-00060

**Molecular and morphological characterisation of *Rotylenchus vitis* n.
sp. infecting grapevine in southern Spain**

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Received: 2011; revised: July 2011

Accepted for publication: July 2011

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Summary – *Rotylenchus vitis* n. sp., a new amphimictic species infesting soil and roots of commercial vineyards in southern Spain, is described. *Rotylenchus vitis* n. sp. is characterised by a truncate lip region with 7-9 annuli and continuous with the body contour, lateral fields areolated at pharyngeal region only, body without longitudinal striations, stylet length of 36-48 µm, vulva position at 47-57%, tail rounded to hemispherical with 13-21 annuli. Morphologically, this species is related to *R. cazorlaensis*, *R. capitatus*, *R. elegans*, *R. fabalus*, *R. iranicus*, *R. labiodiscus*, *R. montanus* and *R. troncapitatus*. The results of the phylogenetic analysis based on the sequences of the D2-D3 expansion regions of the 28S and ITS1-rRNA genes confirmed the species differentiation and the close molecular relationship between *R. eximius* and *R. montanus*, respectively.

Keywords - D2-D3 region, grapevine, molecular, morphology, morphometrics, new species, phylogeny, spiral nematodes, taxonomy.

Twelve out of 95 species of the genus *Rotylenchus* (viz. *R. brevicaudatus* Colbran, 1962, *R. breviglans* Sher, 1965, *R. buxophilus* Golden, 1956, *R. cazorlaensis* Castillo & Gómez-Barcina, 1987, *R. goodeyi* Loof & Oostenbrink, 1958, *R. incultus* Sher, 1965, *R. jaeni* Vovlas, Subbotin, Troccoli, Liébanas & Castillo, *R. magnus* Zancada, 1986, *R. mesorobustus* Zancada, 1986, *R. pumilus* (Perry in Perry, Darling & Thorne, 1959) Sher, 1961, *R. robustus* (de Man, 1876) Filipjev, 1936, *R. unisexus* Sher, 1965) have been reported in the Iberian Peninsula associated with natural environments, but also in agricultural crops such as olive orchards and olive nurseries, banana, pea, prickly pear, and several horticultural crops (Abrantes *et al.*, 1987; Peña Santiago, 1990; Nico *et al.*, 2002; Talavera & Navas, 2002; Castillo & Vovlas, 2005; Vovlas *et al.*, 2008). These data indicate the high diversity within this group in the Iberian Peninsula.

Nematode surveys in commercial vineyards in southern Spain revealed low to moderate soil infestations and root infections by an amphimictic population of a *Rotylenchus* species. Preliminary morphological observations indicated that this species appeared to be morphologically related to *R. cazorlaensis*, but did not fully fit with the description of any of the 95 nominal species described in this genus (Castillo & Vovlas, 2005; Atighi *et al.*, 2011). Detailed observations using light and scanning electron microscopy (SEM), and molecular characterisation analyses, indicated that these specimens should be assigned to a new species. Therefore, the objectives of this paper were: *i*) to verify the taxonomic status of this species, conducting detailed morphometric and molecular studies of this unknown *Rotylenchus* species, which is described herein as *R. vitis* n. sp.; and *ii*) to determine the molecular phylogenetic affinities of *R. vitis* n. sp. with closely related species using the rRNA gene sequences (D2-D3 of 28S and ITS1-rRNA).

Material and methods

NEMATODE POPULATION

Nematodes of *R. vitis* n. sp. used in this study were obtained from the rhizosphere and roots of grapevine plants in Montemayor (Córdoba province), southern Spain (37°39'44.71"N latitude 4°39'23.52"W longitude) at an altitude of 205 m a.s.l., and were collected with a shovel from the upper 30 cm of soil on January 2011 by J.

Martin-Barbarroja and G. León-Ropero (IAS-CSIC). The nematodes were extracted from rhizosphere soil samples by the centrifugal-flotation method (Coolen, 1979).

Specimens to be observed under light microscopy (LM) were killed by gentle heat, fixed in a solution of 4% formaldehyde + 1% propionic acid and processed to pure glycerin using De Grisse's (1969) method. Specimens were examined using a Zeiss III compound microscope with Nomarski differential interference contrast at up to $\times 1000$ magnification. Measurements were done using a *camera lucida* attached to this microscope. For line drawing, handmade drawings were scanned and imported to CorelDraw software version 12 and redrawn.

For SEM studies, fixed specimens were dehydrated in a graded ethanol series, critical point dried, sputter-coated with gold and observed with a JEOL JSM-5800 microscope (Abolafia *et al.*, 2002). Additional topotype specimens of *R. cazorlaensis* were also observed under SEM to confirm differences in lip region morphology.

DNA EXTRACTION, PCR, CLONING AND SEQUENCING

Nematode DNA from *R. vitis* n. sp. was extracted from single individuals using proteinase K as described by Castillo *et al.* (2003). Detailed protocols for PCR and sequencing were applied as described by Castillo *et al.* (2003). The following primers were used for amplification D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') for amplification of D2-D3 regions of 28S (Subbotin *et al.*, 2006); and TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') 5.8SM5 (5'-GGCGCAATGTGCATTTCGA-3') for amplification of the ITS1-rRNA (Vovlas *et al.*, 2008), and A (5'-AAAGATTAAGCCATGCATG-3'), 13R (5'-GGGCATCACAGACCTGTTA-3') and 18P-SSU_R_81 (5'-TGATCCWKC YGCAGGTTTAC-3') partial 18S (Boutsika *et al.*, 2004);. These sequences were amplified and used for sequence and phylogenetic analyses.

PCR products were purified after amplification with Geneclean turbo (Q-BIOgene SA, Illkirch Cedex, France) or QIAquick (Qiagen, USA) gel extraction kits, quantified using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and used for direct sequencing in both directions using the primers referred above. The resulting products were purified and run on a DNA multicapillary sequencer (Model 3100 genetic analyser; Applied Biosystems, Foster City, CA, USA) at the STABVIDA sequencing facilities (Monte da Caparica, Portugal). The newly obtained

sequences were submitted to the GenBank database under accession numbers JN032581-JN032583 as indicated on the phylogenetic trees.

PHYLOGENETIC ANALYSES

D2-D3 expansion segments of 28S and ITS1-rRNA newly sequences and sequences obtained from GenBank were used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen according to previous published data (Vovlas *et al.*, 2008) for D2-D3 expansion regions of 28S rRNA. The newly obtained and published sequences for each gene were aligned using ClustalW (Thompson *et al.*, 1997) with default parameters. Sequence alignments were manually edited using BioEdit (Hall, 1999). Phylogenetic analyses of the sequence data sets were performed with maximum likelihood (ML) using PAUP * 4b10 (Swofford, 2003) and Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). The best fitted model of DNA evolution was obtained using jModelTest v. 0.1.1 (Posada, 2008) with the Akaike Information Criterion (AIC). The Akaike-supported model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC were then used in phylogenetic analyses. BI analysis under TVM + I + G model for D2-D3 expansion segment of 28S rDNA and TVM + G for ITS1 region were run with four chains for 3.0×10^6 and 1.0×10^6 generations, respectively. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees were visualised using TreeView (Page, 1996). In ML analysis the estimation of the support for each node was obtained by bootstrap analysis with 100 fast-step replicates.

Results

*Rotylenchus vitis** n. sp.

*The species epithet refers to the Latin word *vitis* = vine, grapevine.

(Figs 1-3)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body large, habitus slightly curved ventrally to open C-shaped. Lateral fields with four smooth equidistant lines, beginning anteriorly at 11-13th annulus as three lines forming two bands; after 19-22 annuli, central line dividing to form a third band. The three bands are 12.0 ± 1.2 (9-14) μm wide at mid-body, approximately one-fourth as wide as body diam. Regular areolation of lateral fields (external bands) observed in pharyngeal region. Cuticle 2.0-2.5 μm thick, clearly annulated, annuli 1.5-2.0 μm wide at mid-body. Body without longitudinal striations in any region. Labial region truncate, continuous with body, bearing 7-9 narrow annuli which are not divided longitudinally. Labial disc distinct under SEM, but not in lateral view under LM. Oral aperture oval, labial disc rectangular bordered by amphidial apertures. Oral disc clearly separated from first annulus of lip region, which is undivided. Labial framework well developed, 9.8 ± 0.8 μm (9-11) long, posterior margin at level of 8th or 10th body annulus. Stylet robust, 3.1-4.1 times longer than labial region diam. Basal knobs slightly flattened anteriorly, 8.5-10.0 μm wide, at level of 24th-30th annulus posterior to labial region. Dorsal pharyngeal gland opening 4.0-7.0 μm posterior to stylet base. Procorpus of pharynx cylindrical, with slight depression just anterior to median bulb, 52.4 ± 7.6 (40-69) μm long. Median pharyngeal bulb well developed, broadly oval, $20\text{-}26 \times 16\text{-}18$ μm , occupying 8-14 annuli, valvular apparatus 4.0-5.5 μm long, located at 47.5 ± 3.3 (43-53)% of pharyngeal length. Isthmus 19.8 ± 4.0 (14-26) μm long, encircled by nerve ring at mid-point. Pharyngeal glands sacciform, with three nuclei, overlapping intestine dorsally for 26-53 annuli. Nerve ring enveloping isthmus at middle, at 129 ± 8.5 (112-141) μm from anterior end. Excretory pore usually located near pharyngo-intestinal junction level, but varying from anterior to posterior of pharyngeal-intestinal valve. Hemizonid distinct, located at anterior to excretory pore, extending for *ca* 1.5-2.0 body

annuli width, just anterior to excretory pore, rarely posterior. Reproductive system with both genital branches equally developed; anterior branch 314 ± 65.6 (220-414) μm long, posterior branch 301 ± 50.1 (237-401) μm long. Vulva slightly posterior to mid-body, without a distinct epiptygma. Vagina with internal walls slightly sclerotised, 19.5 (17-21) μm long. Ovaries with a single row of oocytes, spermathecae functional, mostly oval, 30 (27-35) μm long and 20 (17-23) μm in diam., filled with rounded sperm 2.0 (1.5-2.5) μm in diam. Phasmids pore-like, 25.9 ± 4.9 (17-32) annuli anterior to level of anus or 37.4 ± 9.0 (19-46) μm anterior to anus. Tail short, of variable shape, from hemispherical to broadly rounded, with 13-21 annuli, terminus striated and cuticle at tail tip 7.4 ± 1.2 (5.5-10) μm wide.

Male

Common, but less abundant than female. Habitus slightly curved ventrally. Morphology similar to that of female, except for sexual dimorphism. Lip region as in female, but slightly more elevated in outline, 9.0 - 12.0 μm wide and 5.5 - 7.0 μm high. Stylet shorter than that of female. Stylet knobs less developed compared to that of female (6.5 - 8.0 μm wide). Testis single, anteriorly outstretched, 582 ± 148.7 (405-817) μm long. Tail conoid, 1.4 (1.0-2.1) times longer than that of female. Phasmid distinct, situated anteriorly at anus level. Bursa 76 ± 5.3 (68-83) μm long, completely surrounding tail which is tapering with a rounded-pointed tip. Gubernaculum prominent, with distinct titillae.

TYPE HOST AND LOCALITY

Rotylenchus vitis n. sp. was found in a clay soil and infecting grapevine plant roots (*Vitis vinifera* L.), in Montemayor, Córdoba province, southern Spain.

TYPE MATERIAL

Holotype female, 16 female and 12 male paratypes deposited in the Nematode Collection at the Institute for Sustainable Agriculture, CSIC, Córdoba, Spain. Another two female and one male paratypes deposited at each of the following collections: Istituto per la Protezione delle Piante (IPP) of Consiglio Nazionale delle Ricerche

(C.N.R.), Sezione di Bari, Bari, Italy; USDA Nematode Collection, Beltsville, MD, USA (collection number T-6105p) and WaNeCo, Plant Protection Service, The Netherlands, Wageningen. Specific D2-D3, ITS1 and 18S-rRNA sequences are deposited in GenBank with accessions numbers JN032581, JN032582, and JN032583, respectively.

DIAGNOSIS AND RELATIONSHIPS

Rotylenchus vitis n. sp. is a bisexual species assigned to the species group having more than six lip annuli, a truncate lip region, rounded female tail and stylet more than 35 µm long (Castillo & Vovlas, 2005). It is characterised by a truncate lip region with 7-9 annuli, continuous with body contour, lateral fields areolated at pharyngeal region only, body without longitudinal striations, stylet length of 36-48 µm, vulva position at 47-57%, tail rounded to hemispherical, with 13-21 annuli, and a specific D2-D3, ITS1-rRNA and partial 18S sequences.

Morphologically, *R. vitis* n. sp. can be distinguished from the most similar species by a number of particular characteristics resulting from its specific matrix code (A6,7, B4, C1, D4, E4, F2, G5, H2,3, I1,2, J1, K1 *sensu* Castillo & Vovlas, 2005). It is close to *R. cazorlaensis*, *R. capitatus* Eroshenko, 1981, *R. elegans* (Khan & Khan, 1982) Fortuner, 1987, *R. fabalus* Baydulova, 1984, *R. iranicus* Atighi, Pourjam, Pedram, Cantalapiedra-Navarrete, Palomares-Rius & Castillo, 2011, *R. labiodiscus* Wouts & Sturhan, 1999, *R. montanus* Vovlas, Subbotin, Troccoli, Liébanas & Castillo, 2008, and *R. troncapitatus* Scotto La Massèse & Germani, 1998. From *R. cazorlaensis* it differs by lip annuli (7-9 narrow annuli not divided longitudinally *vs* 6-7 annuli, irregularly divided into blocks, see Figures 3, 4), first lip annulus (undivided *vs* divided into six sectors, see Figures 3, 4), distance of orifice of dorsal pharyngeal gland to stylet base (4-7 *vs* 8.5-11.5µm), stylet length (38-48 *vs* 46.5-56.5 µm), spermatheca shape (oval *vs* spherical), phasmid position (17-32 annuli anterior to level of anus *vs* 0-4 annuli posterior to anus (Fig. 4); spicule length (29-35 *vs* 42-48 µm), and gubernaculum length (10-13 *vs* 19.5-25 µm). From *R. capitatus* it differs by body and stylet length (1478-2033, 39-48 *vs* 680-850, 26-29 µm, respectively), lip region shape (truncate with 7-9 annuli and continuous with body contour *vs* truncate with 7-8 annuli, slightly set off from body), and female tail shape (rounded to hemispherical *vs* conoid). From *R. elegans* it differs by body and stylet length (1478-2033, 39-48 *vs* 500-600, 22-25 µm,

respectively), and position of the phasmids (17-32 annuli anterior to level of anus vs at 5-8 annuli anterior to anus). From *R. fabalus* it differs by lip region shape (truncate with 7-9 annuli, continuous with body contour vs conoid, without annulation, continuous with body contour), stylet knob shape (slightly flattened anteriorly vs slightly sloping backwards), tail tip (regularly annulated vs smooth), phasmid position (17-32 annuli anterior to level of anus vs 0-2 annuli anterior to anus), and presence vs absence of males. From *R. iranicus* it differs by body length (1478-2033 vs 954-1237 μm), lip region shape (truncate with 7-9 annuli, continuous with body contour vs hemispherical, with 5-6, rarely 7 annuli and set off from body), pharyngeal glands overlap (43-85 μm long vs 3-16 μm), female tail shape (rounded to hemispherical with regularly annulated tip vs short, rounded, slightly conoid in some specimens, and typical smooth end). From *R. labiodiscus* it differs by body and stylet length (1478-2033, 39-48 vs 820-980, 33-37 μm , respectively), and position of phasmids (17-32 annuli anterior to level of anus vs located from five annuli anterior to one annulus posterior to anus level). From *R. montanus* it differs by lip region shape (truncate with 7-9 annuli, continuous with body contour vs hemispherical with 6-7 annuli), body and stylet length (1478-2033, 39-48 vs 913-1135, 33-37 μm , respectively), and presence vs absence of males. From *R. troncapitatus* it differs by body length (1478-2033 vs 940-1180 μm), lip region shape (truncate with 7-9 annuli, continuous with body contour vs truncate, with 7-10 annuli and set off from body), and phasmid position (17-32 annuli anterior to level of anus vs varying from four annuli anterior to four posterior to anus), and presence vs absence of males. Although *R. vitis* n. sp. is molecularly close to *R. eximius* Siddiqi, 1964 (see below), it morphologically differs body and stylet length (1478-2033, 39-48 vs 1200-1400, 38-41 μm , respectively), lip region shape (truncate with 7-9 annuli, continuous with body contour vs hemispherical, with four annuli and set off from body), and position of phasmids (17-32 annuli anterior to level of anus vs located near anus level).

PHYLOGENETIC POSITION OF *ROTYLENCHUS VITIS* N. SP. WITHIN THE GENUS AND OTHER HOLOLAIMIDAE

Amplification of the partial 18S, D2-D3 expansion segments of 28S rDNA and ITS1 rRNA from *R. vitis* n. sp. yielded a single fragment of approximately 1600, 750, and 800 bp, respectively. Similarity values from D2-D3 expansion segments of 28S and ITS1 rRNA among species of *Rotylenchus* are presented in Table 2. Interspecific

similarities for the D2-D3 sequence among *Rotylenchus* species retrieved from GenBank and *R. vitis* n. sp. varied from 44 to 58 nucleotides (8-18%). ITS1 rRNA from *R. vitis* n. sp. were similar from the aligned sequences of *Rotylenchus* species in a range from 99 to 151 nucleotides (25-33%). Since only six partial 18S rRNA sequences from *Rotylenchus* species are deposited in GenBank, it was not possible to carry out phylogenetic analyses with this gene. Similarity values from the partial 18S rRNA sequence of *R. vitis* n. sp. with those deposited in GenBank were high and ranged from 94% for *Rotylenchus* sp. (JH 2004, AY284608) to 97% for *R. uniformis* (EU306356).

The D2-D3 alignment consisted of 75 sequences with 556 bp in length. The 50% majority rule consensus phylogenetic tree generated from the D2-D3 alignment by BI analysis under the TVM + I + G model is presented in Figure 5. The tree topologies between ML and BI were congruent. *Rotylenchus* species represents ten moderately or highly supported lineages, seven of which were provided by Vovlas *et al.* (2008) and three additional lineages by Atighi *et al.* (2011) including one clade with *R. iranicus*, another with *R. conicaudatus*, and another with *R. buxophilus*. *Rotylenchus vitis* n. sp. formed a well supported clade with *R. eximius*, but occupied a paraphyletic position among the other genera of Hoplolaimidae (*Helicotylenchus*, *Hoplolaimus* and *Scutellonema*) included in the analysis (Fig. 5). This tree clearly showed the paraphyly of Hoplolaimidae and is in agreement with other authors (Subbotin *et al.*, 2007; Vovlas *et al.*, 2008; Atighi *et al.*, 2011).

The alignment generated for 26 ITS1-rRNA sequences of *Rotylenchus* samples was 800 bp long. After discarding ambiguously aligned regions from the alignment, the ITS1-rRNA data set included 429 bp. The 50% majority rule consensus phylogenetic tree generated from the ITS1-rRNA alignment by BI analysis under the TVM + G model is presented in Figure 6. The tree topologies between ML and BI were congruent. This tree was similar to that obtained by Vovlas *et al.* (2008) and Atighi *et al.* (2011). Morphological similarities associated with the phylogeny of *Rotylenchus* using ribosomal regions were difficult to assign in some species lineages (*i.e.*, *R. goodeyi* Loof & Oostenbrink, 1958, *R. incultus* and *R. laurentinus*), as well as the position of *R. unisexu*, which formed a well supported clade with *R. conicaudatus*, thereby agreeing with Atighi *et al.* (2011). We obtained similar results when including *R. vitis* n. sp., which formed a highly supported clade by BI analysis with *R. montanus* and *Rotylenchus* sp. SAS-2006. However, these species showed several morphological differences that made it difficult to establish a correspondence between morphological

characters and the phylogenetic trees inferred from the molecular data. Four (*R. cazorlaensis*, *R. jaeni*, *R. magnus*, *R. mesorobustus*) of the 12 *Rotylenchus* species recorded from the Iberian Peninsula have been described as new taxa. These data suggest that the Iberian Peninsula could be a centre of speciation within this genus.

In summary, molecular characterisation and phylogenetic analysis of D2-D3 region and ITS1-rRNA sequences and morphological and morphometric analyses clearly support the proposal of *R. vitis* n. sp. as a new species.

Acknowledgements

This research was supported by grant AGL2009-06955 from ‘Ministerio de Ciencia e Innovación’ of Spain and the European Social Fund. The authors thank J. Martín-Barbarroja, and G. León-Ropero from IAS-CSIC for excellent technical assistance, M. Pedram (Tarbiat Modares University, Tehran, Iran) for his help with line drawing, and B. Landa and J.A. Navas-Cortés (IAS-CSIC) for critical reading of the manuscript.

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Table 1. *Morphometrics of Rotylenchus vitis n. sp. from the rhizosphere and roots of grapevines (Vitis vinifera L.) from Montemayor, Córdoba province, southern Spain. All measurements are in μm and in the form: mean $\pm$ s.d. (range).*

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	–	20	14
L	1867	1711 $\pm$ 130.1 (1478-2033)	1398 $\pm$ 116.9 (1222-1628)
a	42.4	35.1 $\pm$ 2.5 (31.8-40.0)	39.8 $\pm$ 2.8 (34.9-45.7)
b	17.6	15.8 $\pm$ 1.4 (13.8-19.0)	14.5 $\pm$ 1.4 (12.8-17.7)
b'	9.5	8.2 $\pm$ 0.9 (7.0-9.6)	7.4 $\pm$ 0.7 (6.5-9.0)
c	70.5	67.4 $\pm$ 10.0 (57.0-90.5)	40.7 $\pm$ 3.6 (36.7-48.2)
c'	0.7	0.7 $\pm$ 0.1 (0.4-0.8)	1.4 $\pm$ 0.1 (1.2-1.6)
V or T	51.0	50.7 $\pm$ 2.6 (47-57)	41.7 $\pm$ 10.3 (28.2-56.8)
G ₁	16	18.2 $\pm$ 3.0 (14.3-23.0)	–
G ₂	17	17.4 $\pm$ 1.8 (15.0-21.3)	–
Stylet	45.0	44.7 $\pm$ 2.7 (39-48)	39.0 $\pm$ 2.3 (36.0-45.0)
Stylet conus	21.5	20.4 $\pm$ 1.0 (18-22)	19.2 $\pm$ 0.9 (18.0-21.0)
DGO	4.5	5.2 $\pm$ 0.9 (4.0-7.0)	4.2 $\pm$ 0.3 (4.0-5.0)
O	10.0	11.8 $\pm$ 1.8 (9.1-15.2)	10.8 $\pm$ 0.6 (10.0-11.9)
Anterior end to centre of median bulb	106.0	109 $\pm$ 7.4 (97-120)	97 $\pm$ 4.7 (90-104)
Anterior end to excretory pore	149.0	150 $\pm$ 14.6 (114-170)	135 $\pm$ 5.1 (127-141)
Pharynx length	197.0	207 $\pm$ 17.9 (191-259)	189 $\pm$ 13.6 (173-215)
Pharyngeal overlap	50.0	59.0 $\pm$ 10.8 (43-85)	49.1 $\pm$ 9.4 (39-68)
Max. body diam.	44.0	48.5 $\pm$ 3.6 (43-54)	35.2 $\pm$ 2.6 (31.5-42.0)
Anal body diam.	36.5	36.0 $\pm$ 3.0 (29.5-40)	24.3 $\pm$ 2.1 (21-28)
Tail	26.5	25.9 $\pm$ 4.0 (17-31)	34.5 $\pm$ 2.7 (31-40)
Tail annuli	16.0	16.6 $\pm$ 2.4 (13-21)	–
Phasmid to terminus	72.0	61 $\pm$ 9.6	–

Rotylenchus vitis n. sp. from Spain

		(45-76)	
Spicules	—	—	32.2 ± 1.7 (29-35)
Gubernaculum	—	—	12.1 ± 0.9 (10.0-13.0)

Table 2. Similarity values (%) of rDNA sequences among *Rotylenchus* species. Above diagonal (in bold letters): D2-D3 expansion segments of 28S rDNA; below diagonal: ITS1 region.

	<i>R. vitis n. sp.</i>	<i>R. agnetis</i>	<i>R. brevicaudatus</i>	<i>R. buxophilus</i>	<i>R. cazorlaensis</i>	<i>R. conicaudatus</i>	<i>R. eximius</i>	<i>R. goodeyi</i>	<i>R. incultus</i>	<i>R. iranicus</i>	<i>R. jaeni</i>	<i>R. laurentinus</i>	<i>R. magnus</i>	<i>R. montanus</i>	<i>R. robustus</i>	<i>R. uniformis</i>	<i>R. unisexus</i>
<i>R. vitis n. sp.</i>		90	-	90	92	89	92	88	88	87	92	89	92	88	92	82	89
<i>R. agnetis</i>	-		-	91	91	86	89	90	91	88	92	91	92	92	92	92	88
<i>R. brevicaudatus</i>	68	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>R. buxophilus</i>	-	-	-		92	88	89	95	96	87	92	96	92	91	93	92	90
<i>R. cazorlaensis</i>	68	-	73	-		88	90	92	92	87	95	92	95	90	94	94	90
<i>R. conicaudatus</i>	63	-	61	-	61		90	86	87	87	89	87	89	86	89	89	82
<i>R. eximius</i>	66	-	71	-	72	59		88	88	89	91	88	91	88	90	90	90
<i>R. goodeyi</i>	-	-	-	-	-	-	-		99	87	90	99	90	90	91	91	89
<i>R. incultus</i>	68	-	68	-	67	67	64	-		87	90	99	91	90	91	91	89
<i>R. iranicus</i>	68	-	68	-	68	68	65	-	72		88	87	88	87	88	88	86
<i>R. jaeni</i>	69	-	70	-	85	61	74	-	68	70		91	98	90	96	96	86
<i>R. laurentinus</i>	69	-	67	-	67	67	63	-	95	72	67		91	90	91	91	90
<i>R. magnus</i>	70	-	72	-	86	62	74	-	70	71	96	68		90	97	97	88
<i>R. montanus</i>	75	-	66	-	68	62	62	-	67	66	66	67	67		91	91	87
<i>R. robustus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-		99	89
<i>R. uniformis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		89
<i>R. unisexus</i>	67	-	68	-	67	65	68	-	68	73	68	68	69	67	-	-	21

Figure legends

Fig. 1. *Rotylenchus vitis n. sp.* A: Female pharyngeal region; B: Female anterior body region; C: Detail of pharyngeal gland; D: Detail of lateral fields at mid-body; E-G: Female tail regions; H: Male tail region.

Fig. 2. Photomicrographs of *Rotylenchus vitis n. sp.* A: Entire female; B: Female pharyngeal region; C-E: Female lip region; F, G: Details of pharyngeal glands; H, I: Detail of body at vulval region; J-M, Female tails; N, O: Male tail with bursa and copulatory apparatus; P: Male feeding on root of grapevine. Abbreviations: a = anus; ep = excretory pore; n = nucleus of pharyngeal glands; ph = phasmid; v = vulva. (Scale bars: A, P = 500 μ m; B = 50 μ m; C-E, H, J-O = 20 μ m; F, G = 50 μ m; I = 10 μ m.)

Fig. 3. Scanning electron microscope photographs of *Rotylenchus vitis n. sp.* A, B: Female lip region, lateral view; C-D, En face view showing oral aperture (oa) and labial disc (ld); E, F: Female tail region showing anus (a) and phasmid (ph); G, H: Male tail region showing spicules (sp). (Scale bars: A, B, C = 10 μ m; D = 5 μ m; E-H = 20 μ m.)

Fig. 4. Photomicrographs and scanning electron microscope photographs of *Rotylenchus cazorlaensis* Castillo & Gómez Barcina, 1987. A, B: Female lip region; C: Male lip region; D: Spermatheca; E-H: Female tails; I: Male tail with bursa and copulatory apparatus. J-N: Detail of female lip regions; O: vulval region; P: Female tail; Q: Male tail. Abbreviations: a = anus; ph = phasmid; v = vulva. (Scale bars: A-C = 50 μ m; D-I = 25 μ m; J, P = 20 μ m; K-N = 20 μ m; O, Q = 50 μ m.)

Fig. 5. The 50% majority rule consensus trees from Bayesian analysis generated from the D2-D3 of 28S rRNA gene dataset with the TVM + I + G model. Posterior probabilities more than 65% are given for appropriate clades; bootstrap values greater than 50% are given on appropriate clades in ML analysis. Newly obtained sequences are in bold letters.

Fig. 6. The 50% majority rule consensus trees from Bayesian analysis generated from the ITS-rRNA gene dataset with TVM + G model. Posterior probabilities more than 65% are given for appropriate clades; bootstrap values greater than 50% are given on appropriate clades in ML analysis. Newly obtained sequences are in bold letters.