



Integrative taxonomy of the stunt nematodes of the genera *Bitylenchus* and *Tylenchorhynchus* (Nematoda, Telotylenchidae) with description of two new species and a molecular phylogeny

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Stunt nematodes are characterized by phenotypic plasticity, with overlapping morphology and morphometry leading to potential misidentification. Consequently, the application of integrative taxonomic approaches is useful to species delimitation based on a combination of different perspectives, e.g. morphology and DNA sequences. We conducted nematode surveys in cultivated and natural environments in Spain and the USA, from which we identified 18 known species of the family Telotylenchidae and two new taxa within the studied samples. These species were morphologically, morphometrically, and molecularly characterized. The results of light and scanning electron microscopic observations, and molecular and phylogenetic analysis also allowed two new species to be distinguished, described herein as *Bitylenchus hispaniensis* sp. nov. and *Tylenchorhynchus mediterraneus* sp. nov. The phylogenetic analysis was carried out using molecular data from nuclear ribosomal DNA genes [D2–D3 expansion segments of the large ribosomal subunit (28S), internal transcribed spacer (ITS), and partial small ribosomal subunit (18S)]. We also provide here a test of alternative hypotheses that confirms the monophyly of both *Tylenchorhynchus* and *Bitylenchus sensu* Siddiqi's classification but does not support Fortuner & Luc's conceptual view of *Tylenchorhynchus* as a large genus. Ancestral state reconstructions of several diagnostic morphological characters using a maximum parsimony approach showed congruence in morphological and molecular evolution for stylet knob inclination and tail tip annulation. Our analysis emphasizes some of the problems related to the taxonomy and phylogeny of nematodes of Telotylenchinae.

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ADDITIONAL KEYWORDS: 18S – Bayesian inference – cryptic species – D2–D3 expansion segments of the large ribosomal subunit (28S) – internal transcribed spacer – Shimodaira–Hasegawa test – stylet knob inclination – tail tip annulation.

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INTRODUCTION

The genus *Tylenchorhynchus* Cobb, 1913, and related genera represent a large group of plant-parasitic nematodes. These nematodes are known by the common name 'stunt nematodes'. Stunt nematodes are one of the largest groups of plant-parasitic nematodes within the order Tylenchida. There have been several attempts to classify them into different genera using various morphological characters, and different classifications have been proposed and developed depending on the authors' views of the taxonomic importance of these characters. Three classifications are currently most widely used: Fortuner & Luc (1987), Siddiqi (2000), and Geraert (2011). The use of molecular phylogenetic analysis allows the monophyly of different genera and higher rank taxa to be tested, and can improve upon the morphologically based classifications of these nematodes. For example, recently, the presence of deirids combined with the results of molecular phylogenetic studies (Subbotin *et al.*, 2006; van Megen *et al.*, 2009) were used by Sturhan (2011) as major arguments to exclude the subfamily Merliniinae from Telotylenchidae and re-establish the family Merliniidae Siddiqi, 1971. Stunt nematodes are obligate migratory root-ectoparasites of many plants, including various agricultural crops and native plants (Siddiqi, 2000). Several species have been reported to damage crops, e.g. *Tylenchorhynchus claytoni* Steiner, 1937, and *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986, on corn (Barker, 1974); *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971, and *Tylenchorhynchus nudus* Allen, 1955 on sorghum (Starr, 1992); and *Tylenchorhynchus clarus* on alfalfa (Noel & Lowensbery, 1978). Consequently, accurate and timely identification of *Tylenchorhynchus* spp. infecting crops is a prerequisite for designing effective management strategies, and reliable identification allows distinction between pathogenic and nonpathogenic stunt nematodes. Application of integrative taxonomic approaches is useful to species delimitation based on integration of different perspectives, e.g. morphology and DNA sequences (Dayrat, 2005). Integrative taxonomy has now been efficiently applied for nematodes (Neres *et al.*, 2010; Apolônio Silva De Oliveira *et al.*, 2012; Gutiérrez-Gutiérrez *et al.*, 2013a, b), other invertebrates (Schlick-Steiner *et al.*, 2010), vertebrates (Wiens & Penkrot, 2002), and plants (Marcussen, 2003).

Since the genera *Tylenchorhynchus* and *Bitylenchus* were established, many species have been identified and described. Allen (1955) published the first revision of the genus *Tylenchorhynchus* and 23 genera have been proposed since that date (Tarjan, 1973; Fortuner & Luc, 1987; Brzeski & Dolinski, 1998; Handoo, 2000; Siddiqi, 2000; Geraert, 2011; Ganguly, Lal & Rathour, 2013). These genera are considered to constitute the family Telotylenchidae or the subfamily Telotylenchinae

defined by Siddiqi (1960) (Geraert, 2011). Several different concepts of the subfamily Telotylenchinae have been proposed and are presently in use: Fortuner & Luc (1987) recognized the following genera within the subfamily Telotylenchinae: *Tylenchorhynchus* (= *Bitylenchus*, *Telotylenchus* Siddiqi, 1960, and six other genera), *Trophurus* Loof, 1956, *Trichotylenchus* Whitehead, 1960, *Nagelus* Thorne & Malek, 1968, *Paratrophurus* Arias, 1970, *Merlinius* Siddiqi, 1970, *Triversus* Sher, 1974, and *Amplimerlinius* Siddiqi, 1976. According to Siddiqi (2000) the subfamily Telotylenchinae contains *Tylenchorhynchus*, *Bitylenchus* Filipjev, 1934, *Trophurus*, *Telotylenchus*, *Trichotylenchus*, *Paratrophurus*, *Histotylenchus* Siddiqi, 1971, *Quinisulcius* Siddiqi, 1971, *Telotylenchoides* Siddiqi, 1971, *Uliginotylenchus* Siddiqi, 1971, *Sauertylenchus* Sher, 1974, and *Neodolichorhynchus* Jairajpuri & Hunt, 1984. Geraert (2011) included nine genera in the subfamily Telotylenchinae: *Histotylenchus*, *Neodolichorhynchus*, *Paratrophurus*, *Quinisulcius*, *Sauertylenchus*, *Telotylenchus*, *Trichotylenchus*, *Trophurus*, and *Tylenchorhynchus*. Amongst these, the genus *Tylenchorhynchus* is well established and widely accepted by nematologists and contains the highest number of species, although its boundary definition is controversial. According to Geraert (2011) the genus *Tylenchorhynchus* includes 133 nominal species that are not easy to identify. Ganguly *et al.* (2013) compiled a check list of 144 nominal species of *Tylenchorhynchus* with *Bitylenchus*.

Bitylenchus was proposed as a subgenus of the genus *Tylenchus* by Filipjev (1934); however, it was later synonymized with *Tylenchorhynchus* (Filipjev, 1936). Jairajpuri (1982) resurrected *Bitylenchus* as a subgenus within *Tylenchorhynchus*, and Gómez Barcina, Siddiqi & Castillo (1992) and Siddiqi (2000) recognized it as a valid genus with six and 29 species, respectively, and provided detailed genus diagnoses. The genus *Bitylenchus* is differentiated from *Tylenchorhynchus* in having areolated outer bands of lateral fields, a large postanal intestinal sac containing intestinal granules and fasciculi, relatively more thickened cuticle at the female tail tip, and gubernaculum lacking a crest. Fortuner & Luc (1987) considered the genus *Bitylenchus* as a junior synonym of *Tylenchorhynchus*, arguing that it 'was defined using very secondary characteristics that are not known for many taxa, and that, when known, do not clearly differentiate this genus from *Tylenchorhynchus*'.

18S rRNA, internal transcribed spacer (ITS), and D2–D3 expansion segments of the 28S rRNA gene sequences have been shown to be useful diagnostic markers for the characterization of Telotylenchinae species and reconstruction of their phylogenetic relationships (Chen *et al.*, 2006; Subbotin *et al.*, 2006; Holterman *et al.*, 2009; van Megen *et al.*, 2009; Carta,

Skantar & Handoo, 2010). However, to date, a limited number of species of these genera has been sequenced and included in these analyses and testing of the validity of genera has never been comprehensively conducted. Likelihood-based statistical tests of competing evolutionary hypotheses (tree topologies) have been available for more than two decades. The Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) has some advantages over other tests (i.e. Kishino–Hasegawa or Templeton tests), as it simultaneously compares multiple topologies and corrects the corresponding *P*-values to accommodate the multiplicity of testing and may be applied to a posteriori hypotheses (Buckley *et al.*, 2001).

The objectives of this study were to: (1) carry out a detailed morphological and morphometric characterization of *Tylenchorhynchus* and *Bitylenchus* species and populations from cultivated and native plants from southern Spain and several states in the USA; (2) perform a molecular characterization of the species and populations using sequences of the D2–D3 expansion segments of the *28S rRNA* gene, the *ITS* of the rRNA gene, and the partial *18S rRNA* gene; (3) carry out a phylogenetic analysis within *Tylenchorhynchus*, *Bitylenchus*, and related genera based on sequences of the rRNA gene fragments; and (4) test the validity of the proposed genera of the Telotylenchinae using the SH test.

MATERIAL AND METHODS

NEMATODE POPULATIONS AND MORPHOLOGICAL STUDIES

Surveys for *Tylenchorhynchus*, *Bitylenchus*, and other Telotylenchidae were carried out in cultivated and natural environments in southern Spain and several states in the USA between 2010 and 2012. Nematode populations used in this study were obtained from several host-plants and localities and were characterized morphologically and identified to species level (Table 1). In addition, topotypes of *Paratrophurus striatus* Castillo, Siddiqi & Gómez-Barcina, 1989, were collected in the type locality and sequenced (Table 1). Populations from nontype localities analysed morphologically and molecularly in this study are proposed as standard and reference populations for each species given until topotype material becomes available and molecularly characterized.

Specimens were extracted from soil samples using the magnesium sulphate centrifugal flotation method (Coolen, 1979). Specimens for light microscopy (LM) were killed by gentle heat, fixed in a solution of 4% formaldehyde + 1% propionic acid, and processed to pure glycerine using Seinhorst's (1966) method. Nematode specimens were examined and measured in three

laboratories (USDA, USA; IAS-CSIC, Spain; and CDFA, USA) using a Leica Leitz DMRB compound microscope (Leica Microsystems, Wetzlar, Germany), Zeiss III compound microscope (Munich, Germany), or Olympus BX51 (USA), respectively, equipped with a Nomarski differential interference contrast. Line drawing illustrations were created using Adobe Illustrator (Adobe Systems Inc., San Jose, CA, USA) by tracing outlines of morphological structures from underlaid photomicrographs and referencing details from multiple specimens. Individual line drawings were then assembled into composite plates using Adobe InDesign. For scanning electron microscope (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, Liebanas & Peña-Santiago, 2002). Morphometric data were processed using STATISTIX 9.0 (NH Analytical Software, Roseville, MN, USA) and expressed as: mean ± SD (range).

DNA EXTRACTION, PCR ASSAYS, AND SEQUENCING

For molecular analyses, two live nematodes from each sample were temporarily mounted in a drop of 1 M NaCl containing glass beads. After taking measurements and photomicrographs of diagnostic characters, the slides were dismantled and DNA extracted. Nematode DNA was extracted from single individuals and PCR assays were conducted as described by Castillo *et al.* (2003). The D2–D3 expansion segments of *28S rRNA* were amplified using the D2A (5'-ACAAGTACCGTGAGGGA AAGTTG-3') and D3B (5'-TCGGAAGGAACCAGC TACTA-3') primers (Castillo *et al.*, 2003). The *ITS* region was amplified using the forward primer TW81 (5' GTTTCCGTAGGTGAACCTGC-3') and reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGT-3') as described in Subbotin *et al.* (2001). Finally, the partial *18S rRNA* was amplified using the primers G18SU (5'-GCTTGCTCAAAGATTAAGCC-3') and F18Ty11 (5' GTGCCAGCAGCCGCGTAATTCC-3') (Chizhov *et al.*, 2006).

PCR products were purified after amplification using ExoSAP-IT (Affymetrix, USB products) 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 or for cloning. The PCR products were cloned into the pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega, USA). Several clones of each sample were isolated using blue/white selection and subjected to PCR with the same primers. PCR products from each clone were sequenced in both directions. The resulting products were purified and run on a DNA multicapillary sequencer

Table 1. Nematode species and populations used in the present morphological and molecular study

Species	Reference		Location	Host	D2–D3 of 28S rRNA gene	ITS rRNA gene	ISS rRNA gene
	Sample code	GenBank accession no.					
<i>Dolichodorius mediterraneus</i>	AR31		Tarifa, Cádiz, Spain	Wild olive (<i>Olea europaea</i> ssp. <i>sylvestris</i>)	KJ461523	–	–
<i>Paratrophurus bhutanensis</i>	H152h		Niebla, Huelva, Spain	Fig tree (<i>Ficus carica</i>)	KJ461524	KJ461568	KJ461601
<i>Paratrophurus loffi</i>	CD362		Coto Rios, Jaén, Spain	Ash tree (<i>Fraxinus angustifolia</i>)	KJ461525	–	–
<i>Paratrophurus striatus</i>	CD338		Arroyo Frío, Jaén, Spain	Black poplar (<i>Populus nigra</i>)	KJ461526	–	–
<i>Telylenchus</i> sp.	CD201		Bank of June Lake, Mono County, California, USA	Unknown grasses	KJ461527, KJ461528	–	–
<i>Trophurus imperialis</i>	J071		Lebrjía, Sevilla, Spain	Grapevine (<i>Vitis vinifera</i>)	KJ461529	–	–
<i>Bitylenchus brevilineatus</i>	H42P		Villalba del Alcor, Huelva, Spain	Eucalyptus (<i>Eucalyptus globulus</i>)	KJ461533	–	KJ461603
<i>Bitylenchus hispaniensis</i> sp. nov.	OL32		Córdoba, Córdoba, Spain	Wild olive (<i>Olea europaea</i> ssp. <i>sylvestris</i>)	KJ461544	KJ461576	KJ461608
<i>Bitylenchus hispaniensis</i> sp. nov.	OL09		Castro del Río, Córdoba, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461545	KJ461577	KJ461609
<i>Bitylenchus hispaniensis</i> sp. nov.	OL76		Albánchez, Jaén, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461546	–	–
<i>Bitylenchus hispaniensis</i> sp. nov.	H162		Bonares, Huelva, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461547	–	–
<i>Bitylenchus hispaniensis</i> sp. nov.	ST16C		El Saucejo, Sevilla, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461548	–	–
<i>Bitylenchus hispaniensis</i> sp. nov.	M060		Montilla, Córdoba, Spain	Grapevine (<i>Vitis vinifera</i>)	*	–	–
<i>Bitylenchus hispaniensis</i> sp. nov.	OL46		Génave, Jaén, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	*	–	–
<i>Bitylenchus iphilus</i>	HATOR		Villamanrique de la Condesa, Sevilla, Spain	Cork oak (<i>Quercus suber</i>)	KJ461549	KJ461579	KJ461610
<i>Bitylenchus maximus</i>	419		Andújar, Jaén, Spain	Wild olive (<i>Olea europaea</i> ssp. <i>sylvestris</i>)	KJ461551	KJ461581	KJ461611
<i>Bitylenchus maximus</i>	H187		Niebla, Huelva, Spain	Wild olive (<i>Olea europaea</i> ssp. <i>sylvestris</i>)	KJ461552	KJ461582	KJ461612
<i>Bitylenchus ventrosignatus</i>	H085		Bollullós par del Condado, Huelva, Spain	Grapevine (<i>Vitis vinifera</i>)	KJ461567	KJ461596	KJ461617
<i>Tylenchorhynchus aduncus</i>	CON1		Comil de la Frontera, Cádiz, Spain	Sharp rush (<i>Juncus acutus</i>)	KJ461530	KJ461569	KJ461602
<i>Tylenchorhynchus aduncus</i>	AR16		Sanlúcar de Barrameda, Cádiz, Spain	Wild olive (<i>Olea europaea</i> ssp. <i>sylvestris</i>)	KJ461531	KJ461570	–
<i>Tylenchorhynchus agri</i>	CD304		Lake Worth, Florida, USA	Dwarf date palm (<i>Phoenix roebelenii</i>)	KJ461559	KJ461591	–
<i>Tylenchorhynchus agri</i>	CD712		Delray Beach, Florida, USA	Majesty palm (<i>Ravenea riularis</i>)	KJ461560, KJ475549	KJ461593	–
<i>Tylenchorhynchus annulatus</i>	CD252		Napa County, California, USA	Undetermined grasses	KJ461532, KJ475545	KJ461571	–
<i>Tylenchorhynchus clarus</i>	H152		Niebla, Huelva, Spain	Fig tree (<i>Ficus carica</i>)	KJ461534	KJ461573	KJ461604
<i>Tylenchorhynchus clarus</i>	H09		Chucena, Huelva, Spain	Cork oak (<i>Quercus suber</i>)	KJ461535	KJ461574	KJ461605
<i>Tylenchorhynchus clarus</i>	J228		Sanlúcar de Barrameda, Cádiz, Spain	Tamarisk (<i>Tamarix gallica</i>)	KJ461536	KJ461575	KJ461606
<i>Tylenchorhynchus clarus</i>	H163		Niebla, Huelva, Spain	Wheat (<i>Triticum aestivum</i>)	KJ461537	–	KJ461607
<i>Tylenchorhynchus clarus</i>	J059		Jerez de la Frontera, Cádiz, Spain	Sunflower (<i>Helianthus annuus</i>)	KJ461538	–	–
<i>Tylenchorhynchus clarus</i>	CD129		Merced County, California, USA	Alfalfa (<i>Medicago sativa</i>)	KJ461539, KJ461540	–	–
<i>Tylenchorhynchus clarus</i>	CD197		Calusa County, California, USA	Grapevine (<i>Vitis vinifera</i>)	KJ461541	–	–
<i>Tylenchorhynchus claytoni</i>	CD591		PPDC, CDFA, received from Mississippi, USA	Unknown plant	KJ461542, KJ461543	–	–
<i>Tylenchorhynchus leviterminalis</i>	CD303		PPDC, CDFA, received from Wilton, Connecticut, USA	Avocado (<i>Persea americana</i>)	KJ475546, KJ475547, KJ475548, KJ461550	KJ461580	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	VIDV		Jerez de la Frontera, Cádiz, Spain	Grapevine (<i>Vitis vinifera</i>)	KJ461553	KJ461584	KJ461613
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	OL57		Marchena, Sevilla, Jaén, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461554	KJ461585	KJ461614
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	HUEV		Huevar, Huelva, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461555	KJ461586	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	H038		Almonte, Huelva, Spain	Stone pine (<i>Pinus pinus</i>)	KJ461556	KJ461587	KJ461615
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	POM		Benalup-Casas Viejas, Cádiz, Spain	Grapefruit (<i>Citrus maxima</i>)	KJ461557	–	KJ461616
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	MONT		Montemayor, Córdoba, Spain	Grapevine (<i>Vitis vinifera</i>)	KJ461558	–	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	J221		Rota, Cádiz, Spain	Sunflower (<i>Helianthus annuus</i>)	*	–	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	M157		La Rambla, Córdoba, Spain	Grapevine (<i>Vitis vinifera</i>)	*	–	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	AR42		Tabernas, Almería, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461589	–	–
<i>Tylenchorhynchus mediterraneus</i> sp. nov.	H010		Chucena, Huelva, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461590	–	–
<i>Tylenchorhynchus thermophilus</i>	CD582		PPDC, CDFA, received from Indiana, USA	Unknown plant	KJ461561, KJ461562	–	–
<i>Tylenchorhynchus zece</i>	OL91		Alcalá la Real, Jaén, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461563	KJ461597	KJ461618
<i>Tylenchorhynchus zece</i>	H032		Manzanilla, Huelva, Spain	Grapevine (<i>Vitis vinifera</i>)	KJ461564	KJ461598	KJ461619
<i>Tylenchorhynchus zece</i>	M105		Montilla, Córdoba, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461565	KJ461599	–
<i>Tylenchorhynchus zece</i>	ST18C		Santaella, Córdoba, Spain	Olive (<i>Olea europaea</i> ssp. <i>europaea</i>)	KJ461566	KJ461600	–

*Sequenced population but not deposited in GenBank database as identical to other D2–D3 sequences in the species.

–, Not obtained or not sequenced.
CDFA, California Department of Food and Agriculture; D2–D3, expansion segments of the large ribosomal subunit (28S); ITS, internal transcribed spacer; PPDC, Plant Pest Diagnostic Center.

(Model 3130XL genetic analyser; Applied Biosystems, Foster City, CA, USA), using the BigDye Terminator Sequencing Kit v. 3.1 (Applied Biosystems) at the Stab Vida sequencing facilities (Caparica, Portugal), and Davis Sequencing (Davis, CA, USA). The newly obtained sequences were submitted to the GenBank database under the accession numbers indicated on the phylogenetic trees and Table 1.

PHYLOGENETIC ANALYSES

New D2–D3 expansion segments of *28S rRNA*, *ITS rRNA*, and partial *18S rRNA* sequences of species from the family Telotylenchidae and other published stunt nematode sequences from GenBank were used for phylogenetic reconstruction. Outgroup taxa for each data set were chosen according to previous published data (Subbotin *et al.*, 2006; van Megen *et al.*, 2009; Carta *et al.*, 2010). The newly obtained and published sequences for each gene were aligned respectively using the MAFFT program v. 7 (Katoh & Standley, 2013) with default parameters. Three sequence data sets were developed: (1) D2–D3 of *28S rRNA* alignment; (2) *ITS rRNA* alignment; (3) partial *18S rRNA* alignment. For hypothesis testing three additional data sets for each of the gene fragments with one sequence per species were also created. 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-fitting model of DNA evolution was obtained using jModelTest v. 2 (Darriba *et al.*, 2012) 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 a general time reversible of invariable sites and a gamma-shaped distribution (GTR + I + G) model for the D2–D3 expansion segment of *28S rRNA*, a transversal model of invariable sites and a gamma-shaped distribution (TVM + I + G) for *ITS*, and a transitional model of invariable sites and a gamma-shaped distribution (TIM1 + I + G) for the partial *18S rRNA* was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) analyses for 1×10^6 generations. The MCMC analyses 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) and bootstrap support (BS) are given on appropriate clades. Trees were visualized using

TreeView (Page, 1996). In the ML analysis, estimation of the support for each node was obtained by bootstrap analysis with 100 fast-step replicates. In order to test the alternative topologies, we used the SH test as implemented in PAUP (Swofford, 2003) using the RELL option, based on D2–D3 expansion segments of *28S*, *ITS*, and partial *18S rRNA* genes.

MORPHOLOGICAL MATRIX AND MAPPING OF MORPHOLOGICAL CHARACTERS

Several qualitative morphological characters (presence of tail tip annulation, shape of tail terminus, tail, stylet knobs, and lip region) proposed by Handoo (2000) for stunt nematode identification were traced over the ML tree reconstructed from the D2–D3 of the *28S rRNA* gene sequence alignment. The five characters consisted of the following states: (1) tail tip annulated/smooth; (2) shape of tail terminus bluntly pointed/bluntly rounded/hemispherical/round/filiform; (3) shape of tail clavate/conoid/cylindrical/subclavate/subcylindrical/pointed conoid /filiform; (4) stylet knob inclination anterior/lateral/posterior; (5) shape of lip region continuous/offset. The most representative value for each character was considered for the morphological matrix. The criterion of parsimony was used to optimize character state evolution on the ML tree using MES-QUITE 2.73 (Maddison & Maddison, 2010).

RESULTS

SPECIES IDENTIFICATION AND DELIMITING

Integrating traditional morphological taxonomic characters and molecular criteria, we distinguished 19 valid species within the studied samples: *Bitylenchus brevilineatus* (Williams, 1960) Jairajpuri, 1982; *Bitylenchus hispaniensis* sp. nov., *Bitylenchus iphilus* Minagawa, 1995; *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986; *Bitylenchus ventrosignatus* (Tobar-Jiménez, 1969) Jairajpuri, 1982; *Dolichodorus mediterraneus* Jiménez-Guirado, Murillo Navarro, Liébanas, Landa & Castillo, 2007; *Paratrophurus bhutanensis* (Ganguly, Lal & Procter, 2004) Andrassy, 2007; *Paratrophurus loofi* Arias, 1970; *Paratrophurus striatus* Castillo, Siddiqi & Gómez-Barcina, 1989; *Trophurus imperialis* Loof, 1956; *Tylenchorhynchus aduncus* de Guiran, 1967; *Tylenchorhynchus agri* Ferris, 1963, *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971; *Tylenchorhynchus clarus* Allen, 1955; *Ty. claytoni* Steiner, 1937; *Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982; *Tylenchorhynchus mediterraneus* sp. nov., *Tylenchorhynchus thermophilus* Golden, Baldwin & Mundo-Ocampo, 1995, *Tylenchorhynchus zae* Sethi & Swarup, 1968; and an unidentified *Telotylenchus* species. This *Telotylenchus* population could not be identified to species level because

the sample did not contain a sufficient number of females for morphological identification and determination of their taxonomic status. Although most of the samples taken from soil examined in this study were monospecific, one sample from Niebla, Huelva, Spain, on the rhizosphere of a fig tree contained a mixture of two species (Siddiqi 2000). The two new taxa were measured, described, and illustrated under LM and SEM (Figs 1–6, Tables 2–6), whereas brief descriptions and illustrations (Supporting Information Figs S1–S17) and morphometric values (Tables S1–S7) are given for the populations of the 15 previously described Telotylenchidae species.

SYSTEMATICS

GENUS *BITYLENCHUS* FILIPJEV, 1934

BITYLENCHUS HISPANIENSIS SP. NOV.

(FIGS 1–3, TABLES 2–3)

Holotype: Female extracted from soil samples collected from the rhizosphere of wild olive (*Olea europaea* ssp. *silvestris*), in Córdoba, Córdoba province, Spain, by P. Castillo, mounted in pure glycerine, and deposited in the USDA Nematode collection, Beltsville, Maryland (collection number T-416t).

Paratypes: Female paratypes extracted from soil samples collected from the rhizosphere of wild olive (*Olea europaea* ssp. *silvestris*), in Córdoba, Córdoba province, Spain, were deposited in the following nematode collections: the USDA Nematode collection, Beltsville, Maryland (collection numbers T-6233p to T-6248p); and the Nematode collection of the Department of Nematology, Landbouwhogeschool, Wageningen, the Netherlands (collection number OL32-4).

Etymology: The specific epithet refers to the geographical origin and is derived from the Latin *hispaniensis* = Spanish, from Spain.

Description of female: Body arcuate to open C shape after heat fixation, no longitudinal striae or ridges outside lateral fields. Body annuli distinct but fine, 1.0–1.5 μm wide around mid-body. Lateral fields originating at the level of the conus of the stylet and extending up to hyaline region of tail to tail terminus, with four incisures, outer two incisures areolated. Lip region high, rounded, almost continuous to body contour, 7.2 ± 0.3 (6.5–7.5) μm wide, 3.6 ± 0.3 (3.0–4.0) μm wide; with five to seven annuli. SEM observations show labial plate slightly squarish in outline, fused with labial sectors, bordering the amphidial apertures, small rounded labial disc and an oval oral opening surrounded by six labial papillae, labial disc with six raised papillae or prongs/lips (Fig. 3A, B). Stylet moderately strong, shaft and

conus equally long; knobs laterally to posteriorly directed. Dorsal gland orifice about 2.5 μm long behind stylet base. Median pharyngeal bulb oblong, basal bulb pyriform. Cardia well developed. Nerve ring located at 83.1 ± 5.6 (73.0–95.0) μm from anterior end. Hemizonid usually just two to three annuli anterior to excretory pore, 1.0–1.5 annuli wide. Lateral canals (intestinal fasciculi) present in the intestinal region, often extending to the tail terminus. Vulva a transverse slit slightly posterior to the middle of the body and distinctly protruding. Epiptygma absent. Reproductive system amphidelphic, didelphic; anterior and posterior ovaries equally developed. Spermatheca rounded, filled with rounded spermatozoa. Tail bluntly rounded hemispherical to clavate, tail terminus annulated. Phasmids located slightly anterior to middle of the tail. Postanal extension of intestine absent.

Description of male: Abundant, morphologically similar to female except for sexual characters, and body in posterior region usually more curved than in female. Gubernaculum well developed, half of the spicule length. Tail terminus conoid-pointed, bursa extending to the tail terminus.

Diagnosis: *Bitylenchus hispaniensis* sp. nov. is characterized by a median body length (548–790 μm), a high, rounded, almost continuous lip region with five to seven annuli, stylet moderately strong, 15.5–18.5 μm long with laterally to posteriorly directed knobs, lateral fields with four incisures, outer two crenate, lateral canals (intestinal fasciculi) present in the intestinal region, tail bluntly rounded with a hemispherical to clavate terminus, phasmids located slightly anterior to middle of the tail, and postanal extension of intestine absent. Specific D2–D3, *ITS*, and *18S rRNA* sequences were deposited in GenBank with accession numbers KJ461544–KJ461548, KJ461576–KJ461578, and KJ461608–KJ461609, respectively.

GENUS *TYLENCHORHYNCHUS* COBB, 1913

TYLENCHORHYNCHUS MEDITERRANEUS SP. NOV.

(FIGS 4–6, TABLES 4–6)

Holotype: Female extracted from soil samples collected from the rhizosphere of grapevine (*Vitis vinifera*), in Jerez de la Frontera, Cádiz, Spain, by P. Castillo, mounted in pure glycerine, and deposited in the USDA Nematode collection, Beltsville, Maryland (collection number T-640t).

Paratypes: Female paratypes extracted from soil samples collected from the rhizosphere of grapevine (*Vitis*

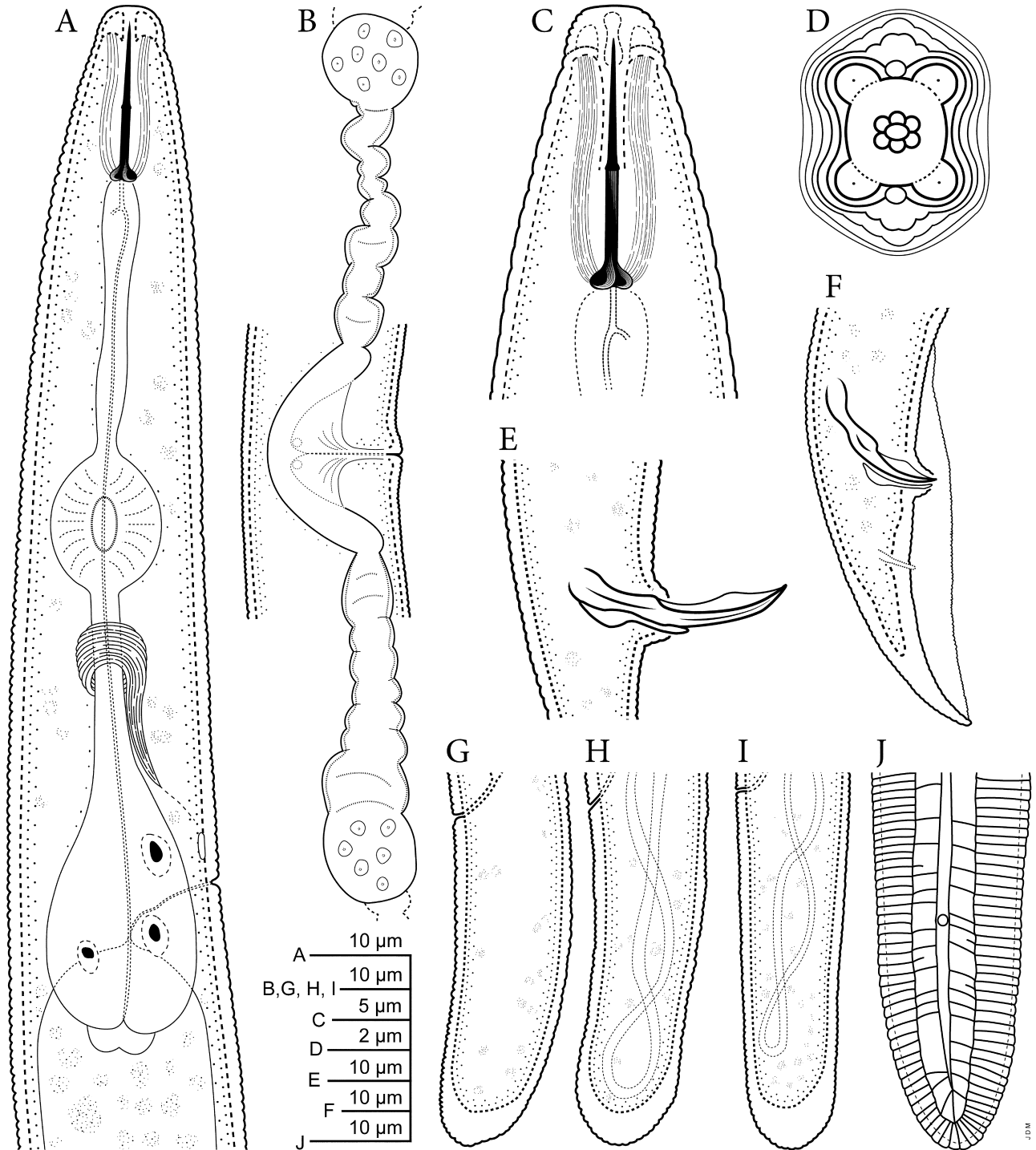


Figure 1. Line drawings of *Bitylenchus hispaniensis* sp. nov. A, female pharyngeal region; B, vulval region showing part of gonads and spermatheca; C, female lip region; D, details of lip region showing oral disc (*en face* view); E, F, male tails showing spicules and gubernaculum; G–J, female tails, with H and I showing intestinal fasciculi/sinuuous canals present and extending back to tail, and J with areolated lateral fields and phasmid in the middle.

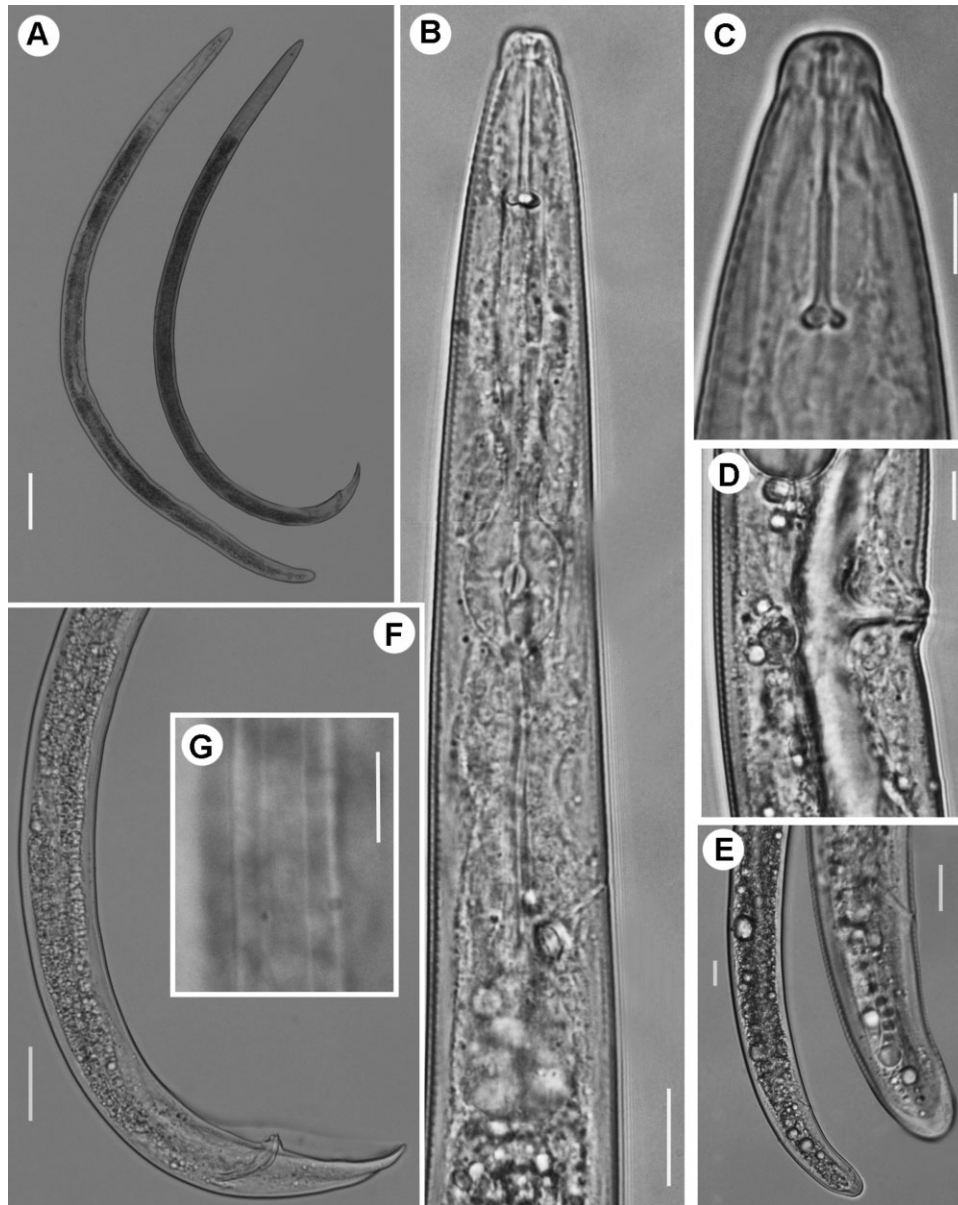


Figure 2. Photomicrographs of *Bitylenchus hispaniensis* sp. nov. A, whole body of female and male; B, female pharyngeal region; C, female lip region; D, vulval region; E, female tails; F, male tail; G, lateral fields at mid-body. Scale bars: A = 50 μ m, B, E = 10 μ m; C, D, G = 5 μ m; F = 20 μ m.

vinifera), in Jerez de la Frontera, Cádiz, Spain, were deposited in the following nematode collections: the USDA Nematode collection, Beltsville, Maryland (collection numbers T-6249p to T-6264p); and the Nematode collection of the Department of Nematology, Landbouwhogeschool, Wageningen, the Netherlands (collection number VIDV-5).

Etymology: The specific epithet refers to the geographical origin and is derived from the Latin *mediterraneus* = from the Mediterranean Basin.

Description of female: Body ventrally arcuate after fixation, no longitudinal striae or ridges outside lateral fields. Body annuli distinct but fine, 1.0–1.5 μ m wide around mid-body. Lateral fields originating at the level of the conus of the stylet and extending up to hyaline region of tail to tail terminus, with four incisures, areolated throughout the body. Lip region hemispherical, offset by a constriction, with five to seven annuli; lip sclerotization weakly developed. The *en face* view (SEM) observations revealed a labial disc flattened laterally, dorsally and ventrally, resulting in a squarish

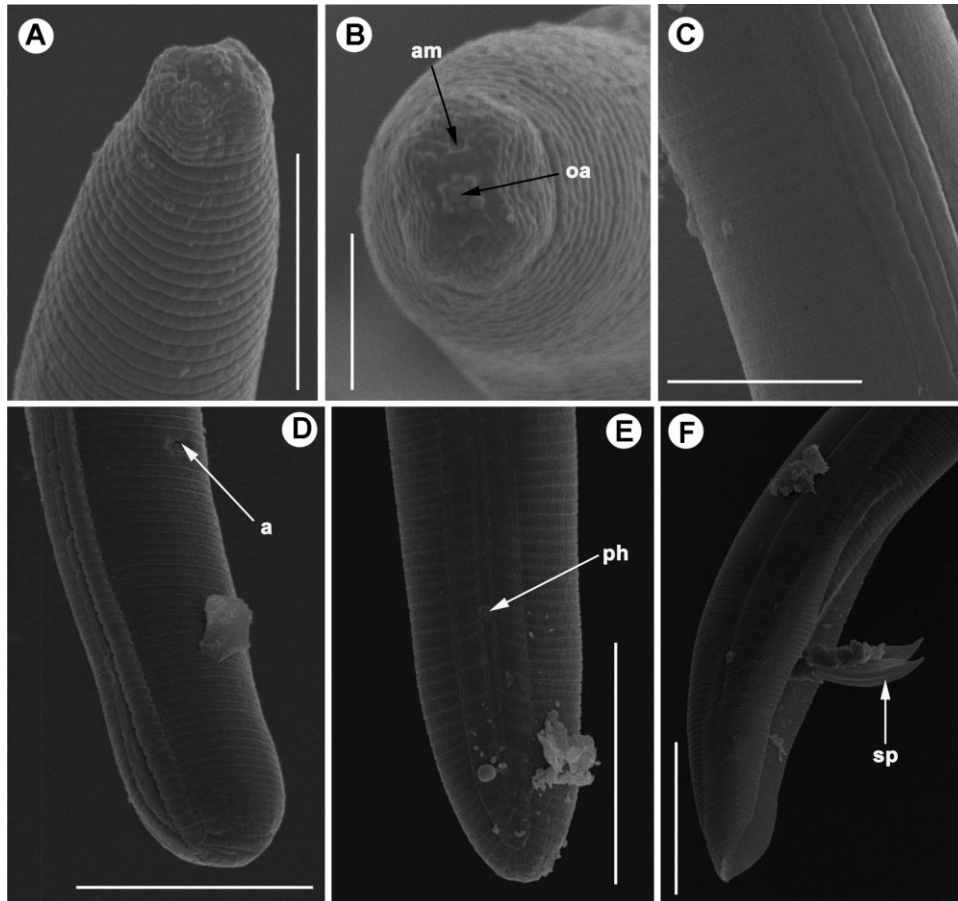


Figure 3. Scanning electron microscope photographs of *Bitylenchus hispaniensis* sp. nov. A, female lip region; B, *en face* view showing oral (oa) and amphidial (am) apertures; C, lateral fields at mid-body; D, E, female tails showing anus (a) and phasmid (ph); F, male tail showing spicules (sp). Scale bars: A, C = 10 μ m; B = 5 μ m; D–F = 20 μ m.

pattern, margins of labial disc interrupted laterally by amphidial openings (Fig. 6B, C). Oral aperture slit-like, orientated dorsoventrally with three papillae on each side parallel to long axis (Fig. 6B, C). Stylet moderately strong, shaft and conus equally long; knobs rounded and posteriorly directed. DGO about 2.5 to 3 μ m behind stylet base. Median pharyngeal bulb oblong to rounded, 16–18 μ m long, basal bulb 55–60 μ m long, pyriform or extending back over the intestine. Hemizonid usually just four to five annuli anterior to excretory pore, one to three annuli wide. Vulva a transverse slit slightly posterior to the middle of the body and distinctly protruding, most of the specimens had loose cuticle just above the vulval area. Epiptygma absent. Reproductive system amphidelphic, didelphic; anterior and posterior ovaries equally developed. Spermatheca rounded, filled with rounded spermatozoa. Tail conoid to cylindrical straight, with 42–81 annuli, tail terminus rounded, annulated. Phasmids located slightly anterior to middle of the tail. Postanal extension of intestine absent.

Description of the male: Abundant, morphologically similar to female except for sexual characters, and body in posterior region usually more curved than in female. Gubernaculum well developed, half of the spicule length. Tail terminus pointed.

Diagnosis: *Tylenchorhynchus mediterraneus* sp. nov. is characterized by a long body (850–1040 μ m), a lip region hemispherical, offset by a constriction, with five to seven annuli, stylet moderately strong, 19.0–22.0 μ m long with laterally to posteriorly directed rounded knobs, lateral fields with four incisures, areolated throughout the body, most of the specimens had loose cuticle just above the vulval area, tail conoid to cylindrical, straight, with 42–81 annuli, tail terminus rounded, annulated, phasmids located slightly anterior to middle of the tail, and postanal extension of intestine absent. Specific D2–D3, *ITS*, and *18S rRNA* sequences were deposited in GenBank with accession numbers KJ461553–KJ461558, KJ461584–KJ461587, and KJ461613–KJ461616, respectively.

Table 2. Morphometrics of paratypes and several populations of *Bitylenchus hispaniensis* sp. nov. from southern Spain*

Locality host-plant	Córdoba, Córdoba, Spain, wild olive			Castro del Río, Córdoba, Spain, olive			Albánchez, Jaén, Spain, olive			Bonares, Huelva, Spain olive,		
	Holotype	Paratype females	Paratype males	Females	Males	Males	Females	Males	Males	Females	Males	
<i>n</i>	7	10	10	5	5	5	10	6	6	3	3	
L	675.0	662 ± 38.4 (612–732)	640 ± 60.9 (548–760)	658 ± 65.8 (582–717)	682 ± 31.1 (645–727)	682 ± 31.1 (645–727)	711 ± 39.9 (657–765)	693 ± 67.7 (585–790)	714 ± 45.5 (660–765)	691 ± 57.8 (627–740)	691 ± 57.8 (627–740)	
a	30.6	30.4 ± 1.7 (27.8–32.5)	31.0 ± 3.9 (27.0–39.1)	31.3 ± 4.1 (27.6–35.9)	33.2 ± 2.1 (31.5–36.3)	33.2 ± 2.1 (31.5–36.3)	32.0 ± 1.0 (30.9–34.0)	34.0 ± 2.8 (30.2–38.5)	32.5 ± 1.1 (30.9–34.0)	30.2 ± 1.5 (28.5–31.3)	30.2 ± 1.5 (28.5–31.3)	
b	5.6	6.0 ± 0.4 (5.6–6.8)	6.1 ± 0.7 (5.4–7.8)	6.0 ± 0.8 (4.9–6.8)	6.0 ± 0.5 (5.6–6.9)	6.0 ± 0.5 (5.6–6.9)	6.2 ± 0.4 (5.7–6.9)	5.9 ± 0.7 (4.9–6.9)	6.3 ± 0.4 (5.7–6.8)	6.1 ± 0.4 (5.8–6.6)	6.1 ± 0.4 (5.8–6.6)	
c	15.0	14.2 ± 1.7 (11.8–16.8)	17.7 ± 1.4 (15.2–20.7)	14.8 ± 0.8 (13.8–15.8)	17.3 ± 2.1 (15.6–20.2)	17.3 ± 2.1 (15.6–20.2)	15.0 ± 0.6 (14.2–15.9)	18.7 ± 1.4 (16.6–20.0)	15.2 ± 0.6 (14.4–15.9)	14.5 ± 1.3 (13.1–15.7)	14.5 ± 1.3 (13.1–15.7)	
c'	2.7	2.9 ± 0.3 (2.5–3.3)	2.9 ± 0.3 (2.4–3.3)	2.7 ± 0.2 (2.5–3.0)	3.1 ± 0.3 (2.7–3.4)	3.1 ± 0.3 (2.7–3.4)	2.9 ± 0.2 (2.5–3.3)	2.9 ± 0.2 (2.7–3.2)	2.9 ± 0.2 (2.7–3.2)	2.8 ± 0.1 (2.7–2.8)	2.8 ± 0.1 (2.7–2.8)	
V or T %	54.0	54.6 ± 1.5 (52.5–57.0)	–	56.0 ± 1.0 (55.0–57.0)	–	–	55.0 ± 1.8 (53.0–57.5)	–	55.4 ± 1.7 (53.0–57.5)	–	–	
Stylet length	17.5	16.1 ± 0.5 (15.5–17.0)	15.9 ± 0.8 (15.0–18.0)	18.0 ± 0.4 (17.5–18.5)	17.0 ± 1.0 (16.0–18.0)	17.0 ± 1.0 (16.0–18.0)	17.8 ± 0.3 (17.5–18.0)	17.6 ± 0.6 (17.0–18.5)	17.7 ± 0.3 (17.5–18.0)	17.5 ± 0.5 (17.0–18.0)	17.5 ± 0.5 (17.0–18.0)	
Maximum body width	22.0	21.8 ± 1.0 (20.5–23.5)	20.7 ± 1.3 (18.0–23.0)	21.2 ± 2.2 (20.0–25.0)	20.6 ± 1.5 (19.0–23.0)	20.6 ± 1.5 (19.0–23.0)	22.6 ± 1.2 (20.0–24.0)	20.4 ± 1.5 (18.0–22.0)	22.0 ± 1.2 (20.0–23.0)	22.8 ± 1.0 (22.0–24.0)	22.8 ± 1.0 (22.0–24.0)	
Pharynx length	120.0	110.0 ± 6.5	105.4 ± 8.2	110.2 ± 9.0	114.0 ± 5.5	114.0 ± 5.5	114.6 ± 6.5	117.0 ± 4.5	114.0 ± 6.4	113.0 ± 8.5	113.0 ± 8.5	
Anterior end to excretory pore	88.0	(100.0–120.0)	(90.0–120.0)	(103.0–120.0)	(105.0–120.0)	(105.0–120.0)	(105.0–122.0)	(110.0–122.0)	(105.0–122.0)	(105.0–122.0)	(105.0–122.0)	
Anal body width	17.0	16.1 ± 1.4 (13.5–17.5)	12.8 ± 0.8 (12.0–15.0)	16.5 ± 2.8 (13.5–20.0)	12.9 ± 0.2 (12.6–13.0)	12.9 ± 0.2 (12.6–13.0)	16.6 ± 1.8 (14.0–20.0)	12.8 ± 1.0 (12.0–14.5)	16.3 ± 1.5 (14.0–18.0)	17.3 ± 1.2 (16.0–18.0)	17.3 ± 1.2 (16.0–18.0)	
Tail length	45.0	47.4 ± 6.3 (37.5–57.0)	36.6 ± 5.6 (30.0–50.0)	44.6 ± 5.5 (38.0–50.0)	39.8 ± 4.1 (35.0–44.0)	39.8 ± 4.1 (35.0–44.0)	47.4 ± 3.2 (43.0–52.0)	37.1 ± 3.3 (32.5–40.0)	47.0 ± 2.8 (44.0–51.0)	47.7 ± 2.5 (45.0–50.0)	47.7 ± 2.5 (45.0–50.0)	
Tail annuli	54	55.1 ± 8.1 (40–66)	–	48.8 ± 5.9 (40–56)	–	–	52.1 ± 6.6 (45–63)	–	51.5 ± 5.0 (46–58)	52.3 ± 5.1 (48–58)	52.3 ± 5.1 (48–58)	
Spicule	–	–	25.3 ± 1.8 (23.0–30.0)	–	25.2 ± 1.5 (23.0–27.0)	25.2 ± 1.5 (23.0–27.0)	–	25.3 ± 0.8 (24.0–26.0)	–	25.0 ± 1.0 (24.0–26.0)	25.0 ± 1.0 (24.0–26.0)	
Gubernaculum	–	–	11.4 ± 1.6 (10.0–15.0)	–	11.3 ± 0.6 (11.0–12.0)	11.3 ± 0.6 (11.0–12.0)	–	12.5 ± 0.9 (11.0–13.5)	–	12.3 ± 0.6 (12.0–13.0)	12.3 ± 0.6 (12.0–13.0)	

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

L, body length; a, body length/maximum body width; b, body length/pharyngeal length; c, body length/body width at anus; V, (distance from anterior end to vulva/body length) × 100; T (distance from cloacal aperture to anterior end of testis/body length) × 100.

Table 3. Morphometrics of several populations of *Bitylenchus hispaniensis* sp. nov. from southern Spain*

Locality host-plant	El Saucejo, Sevilla, Spain, olive		Montilla, Córdoba, Spain, grapevine		Génave, Jaén, Spain, olive	
	Females	Males	Females	Males	Females	Males
<i>n</i>	6	2	2	2	2	2
L	716 ± 44.4 (633–760)	683 ± 31.8 (660–705)	676 ± 26.9 (657–695)	683 ± 75.7 (630–737)	687 ± 24.7 (670–705)	701 ± 50.9 (585–790)
a	31.6 ± 0.2 (31.3–32.0)	32.2 ± 1.2 (31.3–33.0)	31.9 ± 1.4 (30.9–32.9)	31.4 ± 0.1 (31.4–31.5)	32.4 ± 1.5 (31.3–33.5)	30.5 ± 1.3 (29.6–31.4)
b	6.4 ± 0.4 (5.8–6.9)	6.0 ± 0.4 (5.8–6.3)	5.7 ± 0.0 (5.7–5.7)	6.1 ± 0.1 (6.0–6.1)	6.4 ± 0.3 (6.2–6.6)	6.0 ± 0.4 (5.8–6.3)
c	14.7 ± 0.6 (14.2–15.7)	15.2 ± 0.7 (14.7–15.7)	15.5 ± 0.4 (15.3–15.8)	14.1 ± 0.1 (14.0–14.2)	14.9 ± 0.2 (14.7–15.0)	14.0 ± 0.2 (13.9–14.2)
c'	2.8 ± 0.2 (2.5–3.0)	2.9 ± 0.1 (2.8–3.0)	2.8 ± 0.1 (2.8–2.9)	3.0 ± 0.0 (3.0–3.0)	3.1 ± 0.2 (2.9–3.3)	2.9 ± 0.1 (2.8–3.0)
V or T %	54.9 ± 1.5 (53.0–57.5)	–	55.5 ± 2.1 (54.0–57.0)	–	56.5 ± 0.7 (56.0–57.0)	–
Stylet length	17.8 ± 0.3 (17.5–18.0)	17.0 ± 1.4 (16.0–18.0)	17.8 ± 0.4 (17.5–18.0)	17.0 ± 1.4 (16.0–18.0)	17.0 ± 0.7 (16.5–17.5)	17.3 ± 0.4 (17.0–17.5)
Maximum body width	22.7 ± 1.4 (20.0–24.0)	21.3 ± 1.8 (20.0–22.5)	21.3 ± 1.8 (20.0–22.5)	21.8 ± 2.5 (20.0–23.5)	21.3 ± 1.8 (20.0–22.5)	23.0 ± 0.7 (22.5–23.5)
Pharynx length	112.8 ± 9.7 (98.0–122.0)	113.5 ± 12.0 (105.0–122.0)	118.5 ± 4.9 (115.0–122.0)	112.5 ± 10.6 (105.0–120.0)	108.0 ± 8.5 (102.0–114.0)	116.0 ± 1.4 (115.0–117.0)
Anterior end to excretory pore	99.5 ± 8.1 (87.0–107.0)	94.0 ± 2.8 (92.0–96.0)	100.0 ± 7.1 (95.0–105.0)	102.5 ± 3.5 (100.0–105.0)	90.5 ± 2.1 (89.0–92.0)	101.5 ± 2.1 (100.0–103.0)
Anal body width	17.3 ± 1.7 (15.0–20.0)	15.5 ± 0.7 (15.0–16.0)	15.5 ± 0.7 (15.0–16.0)	16.3 ± 1.8 (15.0–17.5)	15.0 ± 1.4 (14.0–16.0)	17.3 ± 0.4 (17.0–17.5)
Tail length	48.7 ± 3.3 (44.0–52.0)	46.0 ± 1.4 (45.0–47.0)	43.5 ± 0.7 (43.0–44.0)	48.5 ± 4.9 (45.0–52.0)	46.3 ± 1.1 (45.5–47.0)	50.0 ± 2.8 (48.0–52.0)
Tail annuli	54.8 ± 6.0 (48–63)	–	46.0 ± 1.4 (45–47)	–	51.5 ± 6.4 (47–56)	–
Spicule	–	25.0 ± 1.4 (24.0–26.0)	–	24.5 ± 0.7 (24.0–25.0)	–	25.5 ± 0.7 (25.0–26.0)
Gubernaculum	–	13.0 ± 1.4 (12.0–14.0)	–	12.5 ± 0.7 (12.0–13.0)	–	13.5 ± 0.7 (13.0–14.0)

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

L, body length; a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V; (distance from anterior end to vulva/body length) × 100; T (distance from cloacal aperture to anterior end of testis/body length) × 100.

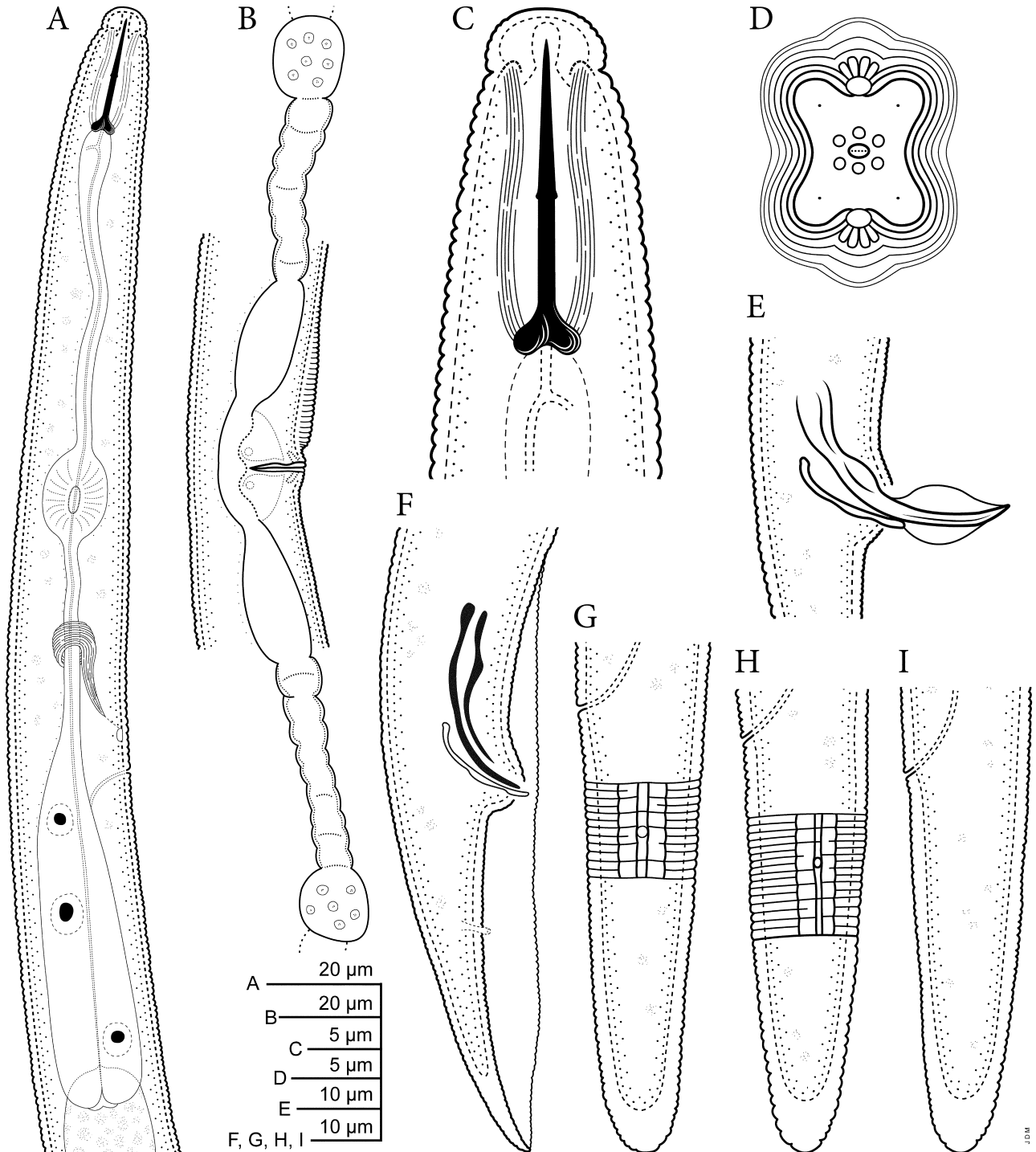


Figure 4. Line drawings of *Tylenchorhynchus mediterraneus* sp. nov. A, female pharyngeal region; B, vulval region showing part of gonads and spermatheca; C, female lip region; D, details of lip region showing oral disc (*en face* view); E, F, male tails showing spicules and gubernaculum; G–I, female tails, showing areolated lateral fields and phasmid in the middle.

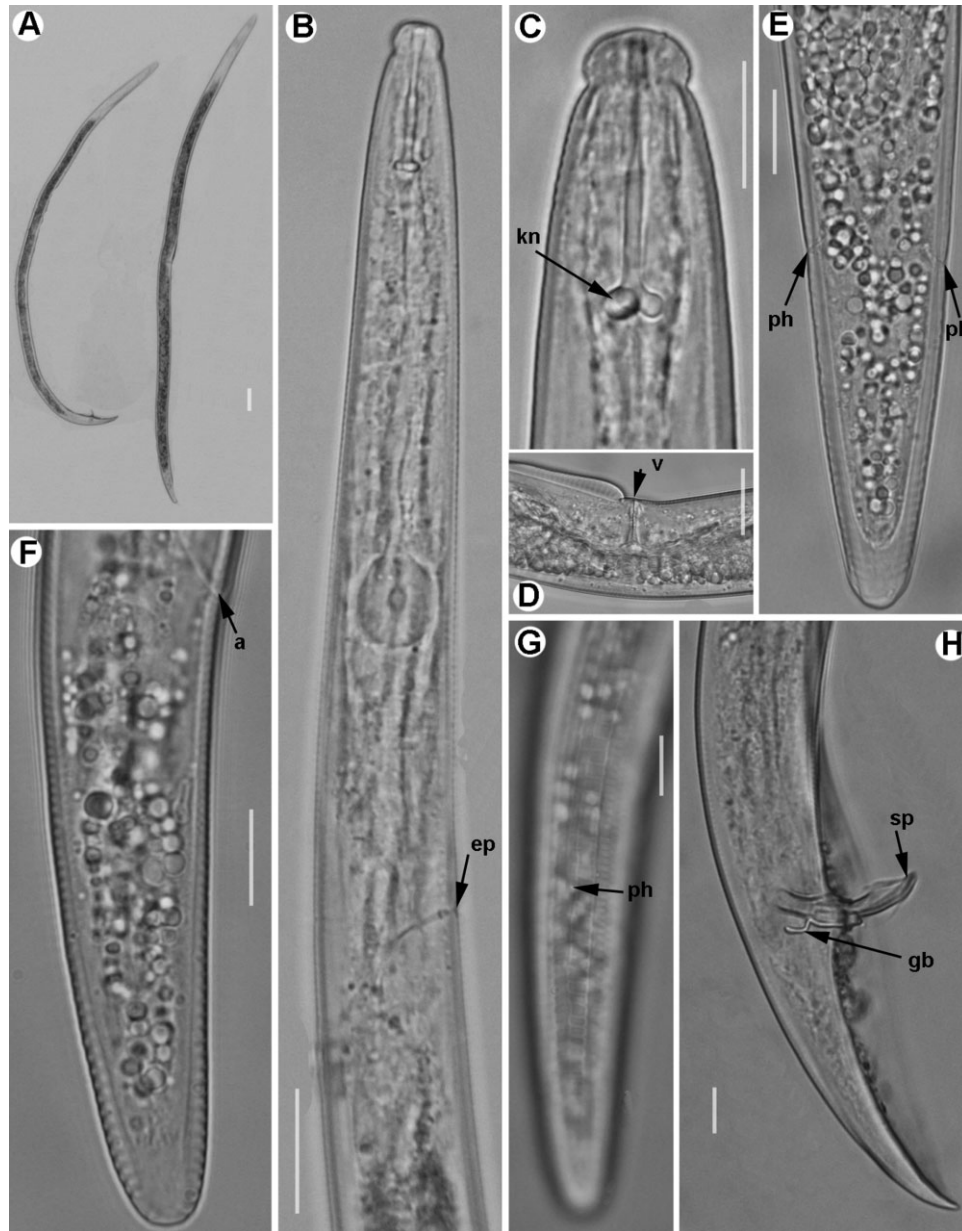


Figure 5. Photomicrographs of *Tylenchorhynchus mediterraneus* sp. nov. A, whole body of female and male; B, female pharyngeal region showing excretory pore (ep); C, female lip region showing stylet knobs (kn); D, vulval region; E–G, female tails showing anus (a) and phasmid (ph); H, male tail showing spicules (sp) and gubernaculum (gb). Scale bars: A = 50 μ m, B, D = 20 μ m; C, E–H = 10 μ m.

MORPHOLOGY AND MORPHOMETRICS OF
TELOTYLENCHIDAE AND OTHER KNOWN SPECIES
(FIGS S1–S19, TABLES S1–S8)

Dolichodorus mediterraneus Jiménez-Guirado,
Murillo Navarro, Liébanas, Landa & Castillo, 2007
(Table S1)

As the morphology of the new Spanish population of *D. mediterraneus* from Tarifa, Cádiz, found on the

rhizosphere of wild olive is almost identical to that published for this species in the original description, no morphological pictures of this new population are provided here. Discovery of this new population on wild olive constitutes a new record of this species for Spain and a new host-plant record. Minor morphometric differences of this population from the original description include stylet length (83.0–99.0 vs. 78.0–106.5 μ m) and gubernaculum

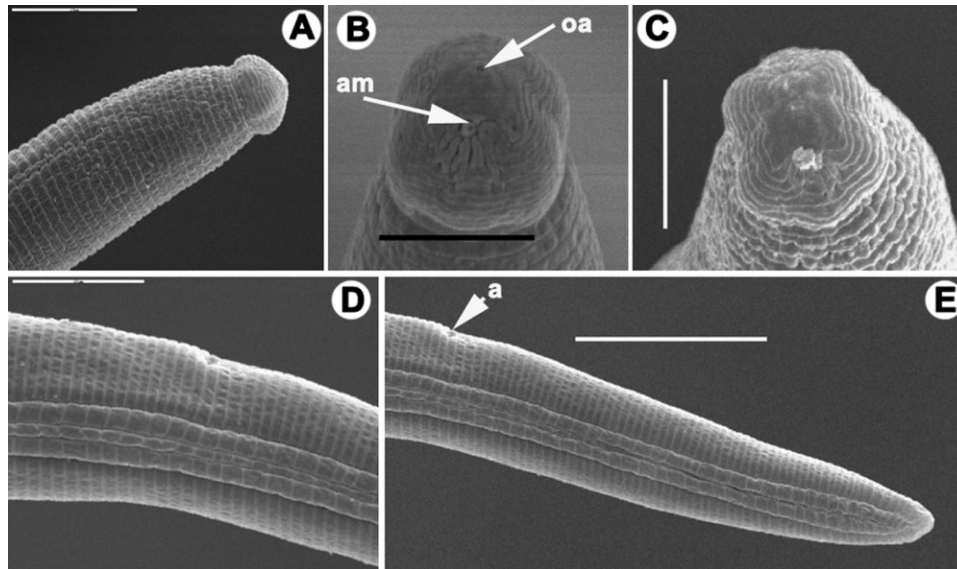


Figure 6. Scanning electron microscope photographs of *Tylenchorhynchus mediterraneus* sp. nov. A, female anterior region; B, C, *en face* view showing oral (oa) and amphidial (am) apertures; D, lateral fields at mid-body; E, female tail showing anus (a). Scale bars: A = 20 µm; B, C, D = 10 µm; E = 20 µm.

(17.0–22.0 vs. 14.0–22.0 µm) (Jiménez-Guirado *et al.*, 2007).

Paratrophurus bhutanensis (Ganguly *et al.*, 2004)
Andrássy, 2007 (Figs S1, S2; Table S1)

The Spanish population from Niebla, Huelva, found on the rhizosphere of a fig tree was characterized by having a straight to C-shaped body with distinct annuli, lip region continuous with a shallow depression, conoid to rounded with four to five annuli, and stylet delicate with rounded to slightly anteriorly directed knobs. The SEM *en face* view revealed a squarish labial disc bordering the amphidial apertures, with an oval oral opening surrounded by six raised projections, prongs, or labial papillae and that the anterior-most cephalic annulus is divided into six labial sectors (Fig. S2A–D). Lateral fields with four incisures with the outer ones areolated; spermatheca round with rounded sperms. Postrectal intestinal sac absent. Tail 2.3–3.3 anal body diameters long, cylindrical, clavate with broadly rounded annulated terminus bearing 16–25 annuli. Males morphologically similar to female except for sexual characters, and body in posterior region usually more curved than in female, bursa encircling the entire tail. Spicules and gubernaculum well developed, measuring 20–22.5 and 9–11 µm, respectively.

The morphology and morphometrics of the Spanish population are coincident with the original species description by (Ganguly *et al.*, 2004), except for minor intraspecific differences in the shape of lip region (conoid to rounded with a shallow depression vs. continuous, conoid-rounded), slightly longer stylet [20.1 (20.0–

21.0) vs. (18.0–20.0) µm], lower *c* ratio [body length/tail length; 17.1 (15.5–19.4) vs. (16.0–27.0)], higher *c'* ratio [tail length/body width at anus; 2.4 (2.2–2.7) vs. (1.5–2.4)], slightly shorter spicules (20.0–22.5 vs. 20–24 µm), and longer gubernaculum (9.0–11.0 vs. 6–9 µm) (Ganguly *et al.*, 2004).

This species was described from East Bhutan and this finding represents the second world record after the original description and a new country record for Spain, as well as a new host record for fig tree.

Paratrophurus loofi Arias, 1970 (Table S1)

The Spanish population from Coto Ríos, Jaén, on the rhizosphere of an ash tree was characterized by a cylindrical body, narrowing at anterior end. Lip region conoid-rounded without distinct annuli. Stylet conus longer than shaft, knobs 2.5–3.5 µm. Pharynx with median bulb oval, 10.1 µm long and isthmus about three times as long as median bulb. Vulva transverse, without epiptygma, ovaries outstretched with rounded spermatheca. Tail cylindrical, terminus striated with hyaline region 9–11 µm long. Male morphologically similar to female, apart from sexual characters.

As the morphology of this population is almost identical to that published for this species in the original description, no morphological pictures of these new population are provided here. Measurements of the studied population were similar to those given by Arias (1970) and Castillo *et al.* (1989), except for shorter spicules and gubernaculum (20–24 vs. 26–27 µm and 10–12 vs. 12–15 µm, respectively). This species has also been

Table 4. Morphometrics of paratypes and several populations of *Tylenchorhynchus mediterraneus* sp. nov. from southern Spain*

Locality host-plant	Jerez de la Frontera, Cádiz, Spain, grapevine			Marchena, Sevilla, Spain, olive			Huevar, Huelva, Spain, olive			Almonte, Huelva, Spain, stone pine		
	Holotype	Paratype females	Paratype males	Females	Males		Females	Males		Females	Males	
<i>n</i>	1	9	3	6	3		12	6		2	2	
L	995.0	1064 ± 109.2 (910–1220)	1005 ± 37.7 (965–1040)	852 ± 53.5 (815–960)	906 ± 94.0 (800–980)		897 ± 30.0 (842–942)	833 ± 59.3 (745–900)		953 ± 68.6 (905–1002)	913 ± 40.3 (885–942)	
a	27.5	38.4 ± 3.7 (34.3–45.3)	39.2 ± 1.8 (37.1–40.4)	37.5 ± 0.8 (36.3–38.4)	40.3 ± 3.2 (38.1–42.6)		42.9 ± 1.5 (40.8–46.6)	41.0 ± 2.8 (37.3–44.5)		42.4 ± 1.7 (41.1–43.6)	42.5 ± 0.5 (42.1–42.8)	
b	5.6	5.8 ± 0.4 (5.1–6.3)	5.1 ± 0.2 (4.9–5.3)	5.8 ± 0.3 (5.4–6.2)	6.0 ± 0.4 (5.8–6.3)		5.7 ± 0.3 (5.2–6.2)	5.5 ± 0.6 (4.9–6.3)		5.8 ± 0.1 (5.7–5.8)	5.6 ± 0.3 (5.4–5.8)	
c	16.0	17.2 ± 1.2 (16.3–20.0)	18.9 ± 1.7 (17.5–20.8)	18.1 ± 1.9 (15.3–20.6)	18.9 ± 0.9 (18.2–19.5)		15.9 ± 1.2 (14.6–18.1)	17.1 ± 2.0 (13.5–19.5)		15.6 ± 1.5 (14.6–16.7)	17.1 ± 2.4 (15.4–18.8)	
c'	3.6	3.3 ± 0.3 (3.0–3.7)	–	2.8 ± 0.1 (2.7–3.0)	3.2 ± 0.04 (3.1–3.2)		3.9 ± 0.3 (3.5–4.6)	3.7 ± 0.4 (3.4–4.0)		3.9 ± 0.1 (3.9–4.0)	4.1 ± 0.8 (3.5–4.6)	
V or T %	51.0	52.4 ± 1.6 (48.0–54.0)	–	54.4 ± 1.4 (53.0–56.0)	–		52.3 ± 1.5 (50.0–55.0)	–		52.5 ± 0.7 (52.0–53.0)	–	
Stylet length	20.5	21.1 ± 0.5 (21.0–22.0)	21.3 ± 0.6 (21.0–22.0)	20.2 ± 0.4 (20.0–21.0)	20.9 ± 1.2 (20.0–22.0)		19.1 ± 0.8 (18.0–20.0)	18.4 ± 0.9 (17.5–20.0)		20.5 ± 0.7 (20.0–21.0)	19.5 ± 0.7 (19.0–20.0)	
Maximum body width	27.5	28.3 ± 1.6 (26.0–30.0)	25.7 ± 0.6 (25.0–26.0)	22.9 ± 1.3 (21.5–25.0)	21.5 ± 0.7 (21.0–22.0)		20.9 ± 0.9 (19.0–22.0)	20.3 ± 0.5 (20.0–21.0)		22.5 ± 0.7 (22.0–23.0)	21.5 ± 0.7 (21.0–22.0)	
Pharynx length	177.0	187.3 ± 9.0 (170.0–195.0)	193.5 ± 2.1 (192.0–195.0)	149.3 ± 6.8 (142.0–155.0)	143.5 ± 6.4 (139.0–148.0)		157.7 ± 5.3 (150.0–170.0)	153.4 ± 11.4 (135.0–165.0)		165.0 ± 9.9 (158.0–172.0)	163.5 ± 2.1 (162.0–165.0)	
Anterior end to excretory pore	135.0	142.5 ± 8.7 (135.0–150.0)	–	–	–		118.2 ± 2.0 (115.0–120.0)	–		121.0 ± 4.2 (118.0–124.0)	116.5 ± 2.1 (115.0–118.0)	
Anal body width	17.5	18.9 ± 1.1 (17.0–20.0)	–	15.5 ± 0.8 (15.0–17.0)	14.5 ± 0.7 (14.0–15.0)		15 ± 1.0 (13.0–16.0)	13.5 ± 0.7 (13.0–14.0)		15.5 ± 0.7 (15.0–16.0)	13.3 ± 1.1 (12.5–14.0)	
Tail length	62.5	61.8 ± 6.1 (52.0–70.0)	53.3 ± 2.9 (50.0–55.0)	47.5 ± 4.6 (42.5–55.0)	46.9 ± 2.8 (44.0–48.0)		56.8 ± 3.8 (50.0–62.0)	49.4 ± 0.7 (41.0–60.0)		61.0 ± 1.4 (60.0–62.0)	53.8 ± 5.3 (50.0–57.5)	
Tail annuli	65	69.0 ± 6.4 (61.0–81.0)	–	53.2 ± 4.8 (46.0–59.0)	–		56.0 ± 8.8 (45–77)	–		62.0 ± 8.5 (56.0–68.0)	51.0 ± 8.5 (45.0–57.0)	
Spicule	–	–	27.7 ± 0.8 (27.0–28.5)	–	22.5 ± 3.5 (20.0–25.0)		–	24.3 ± 0.5 (24.0–25.0)		–	27.5 ± 0.7 (27.0–28.0)	
Gubernaculum	–	–	15.0 ± 0.0 (15.0–15.0)	–	13.0 ± 2.8 (11.0–15.0)		–	12.4 ± 0.2 (12.0–12.5)		–	15.5 ± 0.7 (15.0–16.0)	

*Measurements are in µm and in the form: mean ± SD (range).
 L, body length; a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100; T (distance from cloacal aperture to anterior end of testis/body length) × 100.

Table 5. Morphometrics of several populations of *Tylenchorhynchus mediterraneus* sp. nov. from southern Spain*

Locality host-plant	Benalup-Casas Viejas, Cádiz, Spain, pomelo		Montemayor, Córdoba, Spain, grapevine		Rota, Cádiz, Spain, sunflower		La Rambla, Córdoba, Spain, grapevine	
	Females	Males	Females	Males	Females	Males	Females	Males
<i>n</i>	2	2	5	3	10	5	2	2
L	886 ± 19.8 (872–900)	883 ± 2.1 (882–885)	849 ± 75.6 (791–980)	868 ± 17.6 (850–885)	949 ± 54.2 (867–1040)	944 ± 37.7 (900–1040)	873 ± 44.5 (842–905)	864 ± 30.4 (842–885)
a	43.2 ± 0.5 (42.9–43.6)	44.3 ± 3.2 (42.0–46.6)	46.5 ± 3.0 (42.5–49.4)	43.4 ± 0.9 (42.5–44.3)	30.9 ± 3.3 (25.8–37.8)	31.3 ± 2.3 (28.9–34.1)	42.6 ± 0.7 (42.1–43.1)	44.3 ± 3.2 (42.1–46.6)
b	5.5 ± 0.3 (5.3–5.7)	5.4 ± 0.2 (5.2–5.5)	5.4 ± 0.3 (5.2–5.9)	5.6 ± 0.1 (5.5–5.7)	5.6 ± 0.3 (5.1–6.1)	6.0 ± 0.7 (5.3–7.2)	5.5 ± 0.3 (5.3–5.7)	5.6 ± 0.1 (5.5–5.7)
c	15.1 ± 0.1 (15.0–15.2)	15.0 ± 0.5 (14.7–15.4)	16.9 ± 1.1 (15.8–18.7)	18.0 ± 0.8 (17.4–18.8)	17.6 ± 1.6 (15.1–19.1)	18.2 ± 1.8 (15.0–19.3)	15.7 ± 1.6 (14.6–16.8)	16.1 ± 1.0 (15.4–16.8)
c'	3.9 ± 0.1 (3.8–4.0)	4.3 ± 0.4 (4.0–4.6)	3.8 ± 0.3 (3.4–4.1)	–	2.4 ± 0.2 (2.0–2.7)	2.7 ± 0.0 (2.7–2.7)	3.7 ± 0.3 (3.5–3.9)	4.1 ± 0.8 (3.5–4.6)
V or T %	51.5 ± 0.7 (51.0–52.0)	–	52.7 ± 1.6 (51.0–55.0)	–	53.9 ± 1.0 (52.5–55.0)	–	52.0 ± 1.4 (51.0–53.0)	–
Stylet length	18.3 ± 0.4 (18.0–18.5)	18.8 ± 0.4 (18.5–19.0)	18.7 ± 0.8 (18.0–20.0)	19.7 ± 0.6 (19.0–20.0)	22.5 ± 0.3 (22.0–23.0)	22.1 ± 0.4 (21.5–22.5)	19.5 ± 0.7 (19.0–20.0)	19.0 ± 0.0 (19.0–19.0)
Maximum body width	20.5 ± 0.7 (20.0–21.0)	20.0 ± 1.4 (19.0–21.0)	18.3 ± 1.6 (16.0–20.0)	20.0 ± 0.0 (20.0–20.0)	31.0 ± 3.2 (26.0–37.0)	30.4 ± 1.7 (28.0–32.0)	20.5 ± 0.7 (20.0–21.0)	19.5 ± 0.7 (19.0–20.0)
Pharynx length	161.0 ± 12.7 (152.0–170.0)	165.0 ± 7.1 (160.0–170.0)	156.0 ± 6.5 (150.0–165.0)	155.0 ± 7.1 (150.0–160.0)	171.0 ± 8.8 (160.0–190.0)	159.0 ± 9.6 (145.0–170.0)	160.0 ± 0.0 (160.0–160.0)	154.5 ± 9.2 (148.0–161.0)
Anterior end to excretory pore	126.5 ± 7.8 (121.0–132.0)	119.0 ± 1.4 (118.0–120.0)	–	–	138.0 ± 4.5 (135.0–145.0)	134.2 ± 8.3 (120.0–140.0)	117.5 ± 3.5 (115.0–120.0)	116.5 ± 2.1 (115.0–118.0)
Anal body width	15.0 ± 0.0 (15.0–15.0)	13.8 ± 1.8 (12.5–15.0)	13.1 ± 1.7 (12.0–16.0)	–	22.1 ± 1.7 (19.0–25.0)	17.5 ± 0.0 (17.5–17.5)	15.0 ± 1.4 (14.0–16.0)	13.3 ± 1.1 (12.5–14.0)
Tail length	58.8 ± 1.8 (57.5–60.0)	58.8 ± 1.8 (57.5–60.0)	50.4 ± 6.7 (45.0–62.0)	48.3 ± 1.5 (47.0–50.0)	53.7 ± 5.6 (46.0–60.0)	52.2 ± 5.2 (48.0–60.0)	56.0 ± 8.5 (50.0–62.0)	53.8 ± 5.3 (50.0–57.5)
Tail annuli	61.5 ± 9.2 (55–68)	–	54.4 ± 6.1 (50.0–65.0)	–	49.8 ± 6.3 (42.0–60.0)	–	50.5 ± 7.8 (45.0–56.0)	–
Spicule	–	26.5 ± 0.7 (26.0–27.0)	–	24.2 ± 1.6 (23.0–26.0)	–	27.6 ± 1.0 (26.0–28.5)	–	25.5 ± 0.7 (25.0–26.0)
Gubernaculum	–	14.5 ± 0.7 (14.0–15.0)	–	11.8 ± 0.8 (11.0–12.5)	–	15.1 ± 0.7 (14.0–16.0)	–	15.5 ± 0.7 (15.0–16.0)

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

L, body length; a, body length/maximum body width; b, body length/pharyngeal length; c, body length/body width at anus; V, (distance from anterior end to vulva/body length) × 100; T (distance from cloacal aperture to anterior end of testis/body length) × 100.

Table 6. Morphometrics of several populations of *Tylenchorhynchus mediterraneus* sp. nov. from southern Spain*

Locality host-plant	Tabernas, Almería, Spain, olive		Chucena, Huelva, Spain, olive	
	Females	Males	Females	Males
<i>n</i>	2	2	2	2
L	905 ± 14.1 (895–915)	897 ± 21.2 (882–912)	908 ± 47.4 (875–942)	910 ± 51.6 (874–947)
a	44.2 ± 0.8 (43.6–44.8)	40.8 ± 1.7 (39.7–42.0)	42.2 ± 0.8 (41.7–42.8)	42.3 ± 1.0 (41.6–43.0)
b	5.6 ± 0.1 (5.5–5.7)	5.6 ± 0.1 (5.6–5.7)	5.7 ± 0.1 (5.7–5.8)	5.7 ± 0.0 (5.7–5.8)
c	15.2 ± 0.3 (15.0–15.4)	15.6 ± 0.9 (14.9–16.3)	17.2 ± 2.3 (15.6–18.8)	15.0 ± 0.3 (14.8–15.3)
c'	4.0 ± 0.2 (3.8–4.1)	3.8 ± 0.2 (3.7–4.0)	3.8 ± 0.3 (3.6–4.0)	3.9 ± 0.0 (3.9–3.9)
V %	52.0 ± 1.4 (51.0–53.0)	–	51.5 ± 0.7 (51.0–52.0)	–
Stylet length	19.5 ± 0.7 (19.0–20.0)	18.3 ± 0.4 (18.0–18.5)	18.5 ± 0.7 (18.0–19.0)	19.3 ± 1.1 (18.5–20.0)
Maximum body width	20.5 ± 0.7 (20.0–21.0)	22.0 ± 1.4 (21.0–23.0)	21.5 ± 0.7 (21.0–22.0)	21.5 ± 0.7 (21.0–22.0)
Pharynx length	161.0 ± 6.4 (157.0–166.0)	159.5 ± 2.1 (158.0–161.0)	158.0 ± 5.7 (154.0–162.0)	158.5 ± 9.2 (152.0–165.0)
Anterior end to excretory pore	117.5 ± 0.7 (117.0–118.0)	116.0 ± 2.8 (114.0–118.0)	118.0 ± 5.7 (114.0–122.0)	120.5 ± 4.9 (117.0–124.0)
Anal body width	15.0 ± 1.4 (14.0–16.0)	15.0 ± 1.4 (14.0–16.0)	14.0 ± 0.0 (14.0–14.0)	15.5 ± 0.7 (15.0–16.0)
Tail length	59.5 ± 2.1 (58.0–61.0)	57.5 ± 2.1 (56.0–59.0)	53.0 ± 4.2 (50.0–56.0)	60.5 ± 2.1 (59.0–62.0)
Tail annuli	56.5 ± 0.7 (56–57)	–	52.0 ± 5.7 (48.0–56.0)	–
Spicule	–	24.5 ± 0.7 (24.0–25.0)	–	24.0 ± 1.4 (23.0–25.0)
Gubernaculum	–	14.5 ± 0.7 (14.0–15.0)	–	12.5 ± 0.7 (12.0–13.0)

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

L, body length; a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100.

reported from Turkey (Saltukoglu, Geraert & Coomans, 1976) and Bulgaria (Katalan-Gateva & Tsoneva, 1982).

Paratrophurus striatus Castillo, Siddiqi & Gómez-Barcina, 1989 (Table S1)

Topotype specimens (females and males) of *P. striatus* collected at the type locality, in Arroyo Frío, Jaén, on black poplar studied under LM were identical to the type population (Table S1) described by Castillo *et al.* (1989). As this species has only been reported from the type locality, it should be considered an endemic of that area.

Trophurus imperialis Loof, 1956 (Table S2)

The Spanish population from Lebrija, Seville, on the rhizosphere of a grapevine was characterized by a body almost straight when relaxed, cylindrical, tapering anteriorly to a narrow, smooth lip region. Lateral fields 10–11 µm wide. Stylet with attenuated conus, shorter than shaft. DGO 2.0–3.5 µm from stylet base. Median bulb ovate, muscular. Cardia rounded, 4–7 µm long. Gonad monodelphic-prodelphic with a functional anterior branch and a postuterine sac. Tail cylindrical, with conoid smooth terminus having cuticle abnormally thickened, 10–15 µm long. Male morphologically similar to female, apart from sexual characters.

The morphological data for this population correspond well with previous descriptions of the species

(Loof, 1956; Castillo *et al.*, 1991). This species has been reported from several European countries including the Netherlands (Loof, 1956), Poland (Brzeski, 1968), England (Siddiqi, 1973), Turkey (Saltukoglu *et al.*, 1976), and Spain (Castillo *et al.*, 1991).

Bitylenchus brevilineatus (Williams, 1960)

Jairajpuri, 1982 (Figs S3, S4; Table S2)

The Spanish population of *B. brevilineatus* from Villalba del Alcor found on eucalyptus was characterized by having a slightly ventrally arcuate to C-shaped body, and a set-off lip region that is broadly rounded with five to seven annuli. Transverse striae and additional longitudinal lines marking the cuticle were noted at the anterior end of the body. The SEM *en face* view revealed a squarish labial disc with an oval oral opening surrounded by six raised labial projections/papillae and that the anterior-most labial annulus is divided into six labial sectors (Fig. S4A–C). Labial disc slightly raised above medial lips, dumbbell-shaped in *en face* view. Conspicuous round amphidial apertures (Fig. S4C). Stylet delicate, measuring 15.0–17.0 µm long with rounded, posteriorly directed knobs. Lateral fields with four incisures regularly areolated (Fig. S4). Spermatheca rounded with round sperms. Phasmids located in the middle or posterior half of tail. Tail conoid about 2.6–3.5 times anal body diameter in length with bluntly rounded, smooth terminus bearing 30–48 annuli.

Males common, bursa encircling entire tail, spicules 22.5–27.5 µm long, gubernaculum 11.0–12.5 µm long.

The morphology and morphometrics of this new Spanish population are coincident with the previous species description of *B. brevilineatus* by Williams (1960), except for a slightly higher number of tail annuli.

According to Geraert (2011) this species was described from Mauritius and Uttar Pradesh, India (= *Tylenchorhynchus indicus*) (Siddiqi, 1961) and later also reported from South Africa (Kleynhans & Heyns, 1984), India (Gupta & Uma, 1985), Pakistan (Maqbool & Shahina, 1987; Rashid & Heyns, 1990), and Sudan (Zeidan & Geraert, 1990).

Bitylenchus iphilus Minagawa, 1995 (Figs S5, S6; Table S5)

The Spanish population of *B. iphilus* from Villamanrique de la Condesa, Seville, on the rhizosphere of a cork oak was characterized by having a straight to slightly C-shaped body with a hemispherical, continuous to slightly offset lip region bearing five to six annuli. The lateral fields have four incisures and are areolated throughout the body. SEM revealed a lip region divided by shallow, hexaradial grooves, with the *en face* view revealing a squarish labial disc with an oval oral opening surrounded by six raised labial projections, prongs, or papillae and that the anterior-most labial annulus is divided into six labial sectors (Fig. S6A–D). The amphidial apertures are conspicuous (Fig. S6D). Stylet is thin with rounded, posteriorly directed knobs. Spermatheca round to oval with rounded sperms. Tail straight to slightly curved, 2.5–3.2 times anal body diameter in length, hemispherical to subhemispherical with a clavate to rounded terminus, bearing 35–43 annuli; tail terminus irregularly striated, usually rounded or truncate. Males common, morphologically similar to female except for sexual characters, and body in posterior region usually more curved than in female, bursa encircling the entire tail. Spicules and gubernaculum well developed, measuring 25 and 15 µm, respectively. Tail terminus pointed.

The morphology and morphometrics of this Spanish population were coincident with the original species description from Hiroshima, Japan (Minagawa, 1995), and those given in the compendium of species of *Tylenchorhynchus* by Brzeski & Dolinski (1998) and Handoo (2000), except for minor intraspecific differences in the number of tail annuli of females (35–43 vs. 24–41) and the female tail more broadly hemispherical to clavate with a truncate terminus. It is also close to *Tylenchorhynchus ibericus* and *Tylenchorhynchus huesingi* but differs from *Ty. huesingi* in tail shape and number of tail annuli and from *Ty. ibericus* in having a shorter stylet and body length, and in tail shape.

This record represents a new country record for Spain and a new host record for cork oak.

Bitylenchus maximus (Allen, 1955) Siddiqi, 1986 (Figs S7, S8, Table S6)

The Spanish populations of *B. maximus*, one from Andújar, Jaén, and the other from Niebla, Huelva, on the rhizospheres of olive and wild olive, respectively, were characterized by having a strong arcuate body that is often spiral-shaped. Lip region high to rounded, slightly offset by a constriction with six to seven annuli. SEM observations revealed a labial disc and an anterior lip annulus divided into six sectors, and longitudinal grooves on lip annuli behind amphids (Fig. S8A–C). Delicate stylet with small knobs, sloping posteriorly. Tail cylindrical to subcylindrical, occasionally clavate, with 32–41 annuli, tail terminus annulated. Males present, spicules 33–37 µm, gubernaculum 15 µm in length.

The morphology and morphometrics of these Spanish populations are in close conformity with those given for this species by Allen (1955), Maqbool & Shahina (1987), Handoo (2000), Yildiz *et al.* (2012), and Gómez Barcina *et al.* (1992).

This species was described from New York, USA, and later reported from various locations in North America, Europe, Pakistan (Maqbool & Shahina, 1987), Spain (Gómez Barcina *et al.*, 1992), north-western Turkey (Saltukoglu, 1974), and eastern Anatolian temperate pastures of Bingol, Turkey (Yildiz *et al.*, 2012).

Bitylenchus ventrosignatus (Tobar-Jiménez, 1969) Jairajpuri, 1982 (Figs S9, S10, Table S6)

The Spanish population of *B. ventrosignatus* from a sandy soil at Bollullos par del Condado, Huelva, on the rhizosphere of grapevine was characterized by having wave-like structures present close to the vulva on the ventral side of the body (Figs S9, S10) and a slightly ventrally curved body without any longitudinal striations. Lip region spherical, well offset with three to five annuli and weakly sclerotized labial framework. In *en face* view, SEM observations revealed a labial disc fused with labial sectors, resulting in a squarish pattern with slight indentations on dorsal and ventral sides, and longitudinal grooves on lip annuli behind amphids, margins of labial disc interrupted laterally by amphidial openings (Fig. S10A–C). Posterior to labial disc, a continuous lip annulus, broader on lateral sides (Fig. S10C). Oral aperture slit-like, orientated dorsoventrally, with three papillae on each side parallel to long axis. Lateral fields with four incisures, areolated throughout the body. Stylet slender, with backward-directed knobs. Tail subcylindrical with 32–42 annuli, tail terminus conical with a smooth tip. Phasmid located in the anterior half of tail. Male morphologically similar to female except for sexual

characters, and body in posterior region usually more curved than in female, bursa encircling the entire tail. Spicules and gubernaculum well developed, measuring 20.0–22.5 and 10–12 μm , respectively. Tail terminus pointed.

The morphology and morphometrics of this population are coincident with the original description (Tobar-Jiménez, 1969), except for some minor variations in the number of annuli in the lip region and on the tail (three to five vs. four and 32–42 vs. 28–32, respectively). This species was described from Algarrobo, Malaga, Spain, and later reported from Turkey (Geraert, Zepp & Borazanci, 1975). This is the second record of this species in Spain after the original description, also in southern Spain.

Tylenchorhynchus aduncus de Guiran, 1967
(Fig. S11, Table S1)

The Spanish population of *Ty. aduncus* from a sandy soil with *Juncus acutus* was characterized by an elongate-conoid to conical female tail with bluntly pointed to subdigitate, smoothly rounded terminus bearing 20–27 annuli (Fig. S11), lip region hemispherical, about 8.5 μm wide, body annuli without longitudinal lines outside the lateral fields, outer incisures of lateral fields areolated, lateral fields with four incisures. Males present, bursa covering the entire tail, spicules 25–27 μm long, gubernaculum 9–10 μm long with a distinct proximal end.

The morphology and morphometrics of this Spanish population are coincident with the original species descriptions by de Guiran (1967) from Montpellier, France, and Vovlas & Cham (1981) from Torre Cane, Italy (Table S1). This species has been widely reported in Europe including in France, Italy, and Spain, and in Africa, in Algeria (de Guiran, 1967; Vovlas & Cham, 1981; Ouanouki & Mitiche, 1991; Peña-Santiago *et al.*, 2003).

Tylenchorhynchus agri Ferris, 1963 (Fig. S12, Table S8)

The population of *Ty. agri* collected from dwarf date palm (*Phoenix roebelenii*) in Lake Worth, Florida, USA, was characterized by having a ventrally arcuate body with a coarsely striated cuticle. Lip region continuous with body contour or separated by slight depression bearing three to four annuli. Labial framework moderately sclerotized with outer margins extending two to three annuli into body. Stylet almost 21 μm long, with well-developed, anteriorly directed concave to straight knobs. Spermatheca not seen. Tail elongate, subcylindrical, 3.1 times anal body width long, with 25 annuli, terminus broadly rounded without annuli. Phasmid prominent in anterior part of tail.

The morphology and morphometrics of this Florida population are coincident with the original species de-

scription of *Ty. agri* by Ferris (1963). This species was first described from a corn field cropped continuously for 85 years at the University of Illinois, Urbana, Illinois, USA, and later reported from several places in the USA including Iowa, Michigan, and Tennessee (Ferris, 1963; Bernard, 1980; Knobloch & Bird, 1981; Norton *et al.*, 1984; Anderson & Potter, 1991).

In this work we considered the *Ty. annulatus* (EF030983) identified by Chen *et al.* (2006) as *Ty. agri* based on its similarity in morphology, morphometrics, and *ITS rRNA* gene sequences.

Tylenchorhynchus annulatus (Cassidy, 1930) Golden, 1971 (Fig. S13, Table S1)

The US populations of *Ty. annulatus* from Florida found on *Ficus* sp. and from California found on grasses were characterized by having a rounded to truncate lip region about 7.0–8.5 μm wide, continuous or slightly offset from the body with three to four annuli, stylet 16.0–21.0 μm long with distinct basal knobs and also flat to convex and posteriorly directed knobs. Phasmids prominent, located in the anterior half of tail. Tail elongate, subcylindrical, 2.7–3.5 times anal body width long, with 18–26 annuli and a broadly rounded terminus. Occasionally a clavate smooth tail terminus was noted in the Napa County, California, population and some specimens were parasitized by *Pasteuria* sp. spores (Fig. S13).

The morphology and morphometrics of these populations are coincident with previous species descriptions of *Ty. annulatus* (Cassidy, 1930; Hollis, 1962; Timm, 1963; Golden, 1971; Siddiqi, 1971, 1976). This species was described from Hawaii, USA, and reported from subtropical and tropical areas in all continents all over the world except for Europe and has been extensively reported in North America (Anderson & Potter, 1991).

Tylenchorhynchus clarus Allen, 1955 (Figs S14, S15; Tables S3, S4)

The Spanish and US populations of *Ty. clarus* collected from several hosts (alfalfa, cork oak, fig tree, grapevine, sunflower, tamarisk, and wheat) and localities (Chucena, Jerez de la Frontera, Niebla, Sanlúcar de Barrameda, and Merced and Calusa Counties, California) were similar to each other and characterized by having a straight, cylindrical body with a high, truncate lip region that is continuous with the body and has four to five lip annuli. In *en face* view (SEM) labial disc is flattened laterally, dorsally, and ventrally, resulting in a squarish pattern with slight indentations on the dorsal and ventral sides (Fig. S15B); margins of labial disc interrupted laterally by amphidial openings (Fig. S15A, B). Posterior to labial disc, a continuous lip annulus, broader on lateral sides. Oral aperture slit-like, orientated dorsoventrally, with three papillae on each side parallel to long axis. Stylet

15–17.5 µm long with anterior margins of knobs directed forward. Tail conoid-obtuse with smooth terminus; ten to 16 annuli on tail. Lateral fields with four incisures, outer ones crenate. Males present, morphologically similar to females except for sexual characters, and body in posterior region usually more curved than in females. Spicules and gubernaculum well developed, 16–20 and 9–11 µm long, respectively. Tail terminus pointed.

The morphology and morphometrics of the Spanish and US populations are coincident with previous species descriptions by Allen (1955) and Castillo *et al.* (1991). According to Geraert (2011), this species has been described from California, USA. It has also been reported from six other states of the USA (Norton *et al.*, 1984), the Netherlands (Loof, 1959; Dao, 1970), Egypt (Elmiligy, 1969), Mexico (Knobloch, 1975), Greece (Koliopanos & Vovlas, 1977), India (Ray & Das, 1983), Jordan (Hashim, 1983), South Africa (Kleynhans & Heyns, 1984), Canada and Morocco (Anderson & Potter, 1991), Spain (Castillo *et al.*, 1991), and Poland (Brzeski, 1998).

Tylenchorhynchus claytoni Steiner, 1937
(Fig. S16; Table S5)

The US population of *Ty. claytoni* has a rounded or button-shaped lip region, offset by a slight constriction; the tail tapered regularly to a rounded, smooth to bluntly rounded terminus, sometimes with annuli extending further back on the terminus, almost being an annulated terminus; tail with 17–20 annuli; phasmids located either in the centre or mostly off-centre of the lateral field.

The morphology and morphometrics of this US population are coincident with the original description from soil around the roots of tobacco in South Carolina by Steiner (1937) and other descriptions of this species provided by Golden, Maqbool & Handoo (1987) and Zeng *et al.* (2012).

Tylenchorhynchus leviterminalis Siddiqi, Mukherjee & Dasgupta, 1982 (Fig. S17, Table S5)

The US population from Wilton, Connecticut, on avocado was characterized by a ventrally arcuate to C-shaped body with a hemispherical to rounded smooth, continuous lip region. Stylet 19–20 µm long with posteriorly directed, rounded knobs. Tail subcylindrical to clavate, about 3.1 times anal body diameter long with a smooth, hyaline tail terminus. Phasmid located in anterior half of tail. Male morphologically similar to female except for sexual characters, and body in posterior region usually more curved than in female, bursa encircling the entire tail. Spicules and gubernaculum well developed, measuring 20 and 12 µm in length, respectively. Tail terminus pointed.

The morphology and morphometrics of the Wilton, Connecticut population are coincident with previous

species descriptions (Siddiqi *et al.*, 1982; Mizukubo, Toida & Keereewan, 1993; Talavera, Watanabe & Mizukubo, 2002; Chen *et al.*, 2006). This species has been reported from West Bengal, India (Siddiqi *et al.*, 1982), China (Vovlas & Cheng, 1988), Thailand (Mizukubo *et al.*, 1993), Japan (Talavera *et al.*, 2002), Assam, India (= *Tylenchorhynchus paranudus*) (Phukan & Sanwal, 1982; Pathak & Siddiqi, 1997), Papua New Guinea (Bridge & Page, 1984; Troccoli & Geraert, 1995), Vietnam (Nguyen *et al.*, 1997), and Taiwan (Chen *et al.*, 2006).

Our molecular study suggests that *Ty. leviterminalis* may represent a complex of sibling species.

Tylenchorhynchus thermophilus Golden, Baldwin & Mundo-Ocampo, 1995 (Fig. S18, Table S8)

The Indiana population of *Ty. thermophilus* from an unknown host in Indiana, USA, was characterized by having a cylindrical body with a coarsely striated cuticle. Lip region continuous with body contour, bearing four annuli. Labial framework moderately sclerotized. Stylet 22 µm long with well-developed, anteriorly directed concave knobs. Lateral fields with four incisures. Post-anal intestinal sac absent. Tail tapering, conoid, bearing 28 annuli without striations. Phasmids slightly off-centre, on anterior part of tail. Male similar to female. Spicules arcuate, about 24.5 µm long. Gubernaculum 12.5 µm long with proximal end slightly curved and bursa encircling entire tail.

The morphology and morphometrics of the Indiana population are coincident with the original species description of *Ty. thermophilus* (Golden *et al.*, 1995), except for the slightly longer stylet of about 22 vs. 19–20.2 µm and higher V ratio [(distance from anterior end to vulva/body length)/100; 59 vs. 53–56%]. This species was originally described from soil around the roots of saltgrass [*Distichlis spicata* (L.) Green] in Death Valley National Monument, California, USA, and this finding represents a new record of this species for Indiana.

Tylenchorhynchus zae Sethi & Swarup, 1968
(Fig. S19, Table S7)

The Spanish populations of *Ty. zae* from Manzanilla, Huelva, and Montilla, Córdoba, and from Santaella, also in Córdoba, on the rhizospheres of grapevine and olive, respectively, were characterized by having a cylindrical, slightly arcuate body with a continuous to slightly offset lip region with four to five annuli. In *en face* view (SEM) a depressed labial disc fused with labial sectors is seen, resulting in a squarish pattern with slight indentations on the dorsal and ventral sides, margins of labial disc interrupted laterally by amphidial openings (Fig. S19E, F). Oral aperture slit-like, orientated dorsoventrally, surrounded by four rounded, almost identical confluent lips/papillae. Stylet with anteriorly flattened knobs measuring 16.0–17.5 µm, with

anteriorly to laterally directed flattened knobs. Phasmids located in anterior half of tail. Tail conoid to subhemispherical, about 2.1–2.5 times anal body diameter in length, with obtuse smooth terminus bearing 13–20 annuli. Males common, bursa encircling entire tail, spicules 17.0–21.0 µm long; gubernaculum simple, rod-shaped, 10.0–11.0 µm long.

The morphology and morphometrics of these Spanish populations are coincident with the previous species descriptions of *Ty. zae* (Sethi & Swarup, 1968; Chen, Ni & Tsay, 2007). This species was described from Punjab, India, and reported from Taiwan (Chen *et al.*, 2007) and northern Spain (Arias & Romero, 1979).

Telotylenchus sp. (Table S8)

The Californian population of *Telotylenchus* sp. collected from June Lake from the rhizosphere of a grass species was characterized by having a straight body with a conoid, continuous lip region bearing four annuli. Stylet 24 µm long with posteriorly directed knobs. Lateral fields with four incisures, areolated throughout body. Tail subcylindrical to clavate, about 3.6 times anal body diameter long with a rounded, smooth, hyaline tail terminus. Phasmid located in anterior half of tail, about 22.5 µm from anus.

Telotylenchus sp. is close to *Telotylenchus verutus* Kleynhans, 1975, but differs from it in having a conoid lip region with four annuli vs. offset lip region with seven to eight annuli, clavate vs. conoid tail, and shape of stylet knobs. It is also close to *Telotylenchus laevis* Ivanova & Shagalina, 1988, but differs from it in having a shorter body length, in shape of tail and number of tail annuli, and in the absence of males; body length 0.79 vs. 1.03–1.4 mm, tail clavate vs. subcylindrical and number of tail annuli 39 vs. 17–32 in *T. laevis*. This *Telotylenchus* sp. population possibly represents a new species. However, further evaluation of this species is needed after the recovery of more specimens.

MOLECULAR CHARACTERIZATION OF *BITYLENCHUS HISPANIENSIS* SP. NOV., *TYLENCHORHYNCHUS MEDITERRANEUS* SP. NOV., AND OTHER KNOWN SPECIES STUDIED

Our newly obtained sequences of D2–D3 of the 28S *rRNA*, *ITS*, and partial 18S *rRNA* genes of *B. hispaniensis* sp. nov., *Ty. mediterraneus* sp. nov., and other studied species matched well with a few *Tylenchorhynchus* sequences deposited in GenBank in a Blast search (Table 7). These sequences were related to *B. dubius*, *B. maximus*, *Ty. annulatus*, *Ty. claytoni*, *Ty. leviterminalis*, *Ty. zae*, and *Telotylenchus ventralis* (Table 7). The intraspecific variation detected amongst the D2–D3 of the 28S *rRNA* gene sequences for the seven studied populations of *B. hispaniensis* sp. nov.

(five from olive, one from wild olive, and one from grapevine) ranged from one to two nucleotides (1%) and no indels. The intraspecific variation detected amongst the D2–D3 of the 28S *rRNA* gene sequences for the ten studied populations of *Ty. mediterraneus* sp. nov. (four from olive, three from grapevine, one from stone pine, one from grapefruit, and one from sunflower) ranged from two to four nucleotides (1%) and no indels. No intraspecific variability in *ITS* sequences for *Ty. zae* and *B. hispaniensis* sp. nov. was detected amongst individuals from the studied populations. Intraspecific sequence diversity for *Ty. mediterraneus* sp. nov. and *Ty. agri* varied from 0 to 1.6% (0–11 nucleotides) and from 0 to 1.3% (0–10 nucleotides), respectively. The sequence difference between type A and type B of *Ty. leviterminalis* was 12.3% (99 nucleotides). Similarly, the intraspecific variation detected amongst the partial 18S *rRNA* of the studied populations of *B. hispaniensis* sp. nov. was only one nucleotide (1%) and one indel, whereas for *Ty. mediterraneus* sp. nov. it ranged from two to three nucleotides (1%) and one indel. The partial 18S *rRNA* gene sequences of the other *Tylenchorhynchus* samples studied also matched well with the small number of *Tylenchorhynchus* spp. deposited in GenBank. Our Spanish population of *B. maximus* (KJ461611–KJ461612) from olive was 99% similar to a Belgian *B. maximus* population (AY993979), differing by just three nucleotides and no indels.

The D2–D3 of the 28S *rRNA* gene sequences of the other *Tylenchorhynchus* samples studied also matched well with the small number of *Tylenchorhynchus* spp. deposited in GenBank. Particularly, the sequence of the *Ty. claytoni* population from Mississippi, USA, was 99% similar to that of *Ty. claytoni* (EU368589) from South Carolina, USA. The sequence of the *Ty. leviterminalis* population from Wilton, Connecticut, USA, was 98% similar to that of *Ty. leviterminalis* (EU368591) from Vietnam. The sequence of *Dolichodoros mediterraneus* from Tarifa, Cádiz, Spain, was 98% similar to that of the type population of this species (DQ838803). The sequence of *Trophurus imperialis* from Lebrija, Sevilla, Spain was 86% similar to that of *Trophurus sculptus* (DQ328709), the only species of this genus deposited in GenBank so far.

PHYLOGENETIC RELATIONSHIPS WITHIN TELOTYLENCHINAE

The phylogenetic relationships amongst subfamily Telotylenchinae species inferred from the analyses of D2–D3 expansion segments of 28S, *ITS*, and the partial 18S *rRNA* gene sequences using BI and ML are given in Figures 7, 8, and 9, respectively. No significant differences in topology were obtained between the BI and ML approaches although there were several exceptions in the positions of clades with low statistical

Table 7. Similarity values (%) of rRNA sequences amongst *Bitylenchus* (*B.*) and *Tylenchorhynchus* (*Ty.*) species. Above diagonal ITS region and below diagonal D2–D3 expansion segments of 28S rRNA, based on multiple sequence alignment of 620 and 648 bp in length, respectively*

	<i>Ty. dubius</i> (DQ328707)	<i>Ty. aduncus</i> (KJ461531)	<i>Ty. annulatus</i> (EF030983)	<i>Ty. annulatus</i> (KJ461571–KJ461572)	<i>B. brevilineatus</i> (KJ461533)	<i>Ty. clarus</i> (KJ461534)	<i>Ty. clarus</i> (KJ461573–KJ461575)	<i>Ty. clarus</i> (KJ461576–KJ461578)	<i>B. iphilus</i> (KJ461579) (EF030983)	<i>Ty. agri</i> (KJ461591–KJ461593)	<i>Ty. thermophilus</i> (KJ461594–KJ461600)	<i>Ty. zeae</i> (KJ461596)
<i>Ty. dubius</i> (DQ328707)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Ty. aduncus</i> (KJ461531)	77	-	-	-	-	-	-	-	-	-	-	-
<i>Ty. annulatus</i> (EF030983)	78	80	-	-	-	-	-	-	-	-	-	-
<i>B. brevilineatus</i> (KJ461533)	82	88	84	-	-	-	-	-	-	-	-	-
<i>Ty. clarus</i> (KJ461534)	76	88	88	85	-	-	-	-	-	-	-	-
<i>Ty. claytoni</i> (KJ461542–KJ461543)	77	91	95	87	88	-	-	-	-	-	-	-
<i>B. hispaniensis</i> sp. nov. (KJ461544–KJ461548)	90	85	86	89	83	84	63	65	63	64	63	65
<i>B. iphilus</i> (KJ461549)	79	87	88	91	85	87	65	85	66	66	66	74
<i>Ty. leviterminalis</i> (EU368591)	79	92	95	87	89	93	87	87	88	88	88	72
<i>B. maximus</i> (KJ461551–KJ461552)	81	87	88	94	86	87	88	92	88	68	68	72
<i>Ty. mediterraneus</i> sp. nov. (KJ461553–KJ461558)	79	89	88	89	86	88	87	88	87	70	71	72
<i>Ty. agri</i> (KJ461549, KJ461559–KJ461560)	77	91	94	87	89	93	87	87	96	85	85	80
<i>Ty. thermophilus</i> (KJ461561–KJ461562)	78	91	95	88	89	97	84	88	95	94	71	83
<i>B. ventrosignatus</i> (KJ461567)	77	84	83	85	82	81	84	83	83	83	82	72
<i>Ty. zeae</i> (KJ461563–KJ461566)	78	92	93	88	91	92	85	89	93	93	92	85

*Sequences of new species are in bold letters. -, Not available.

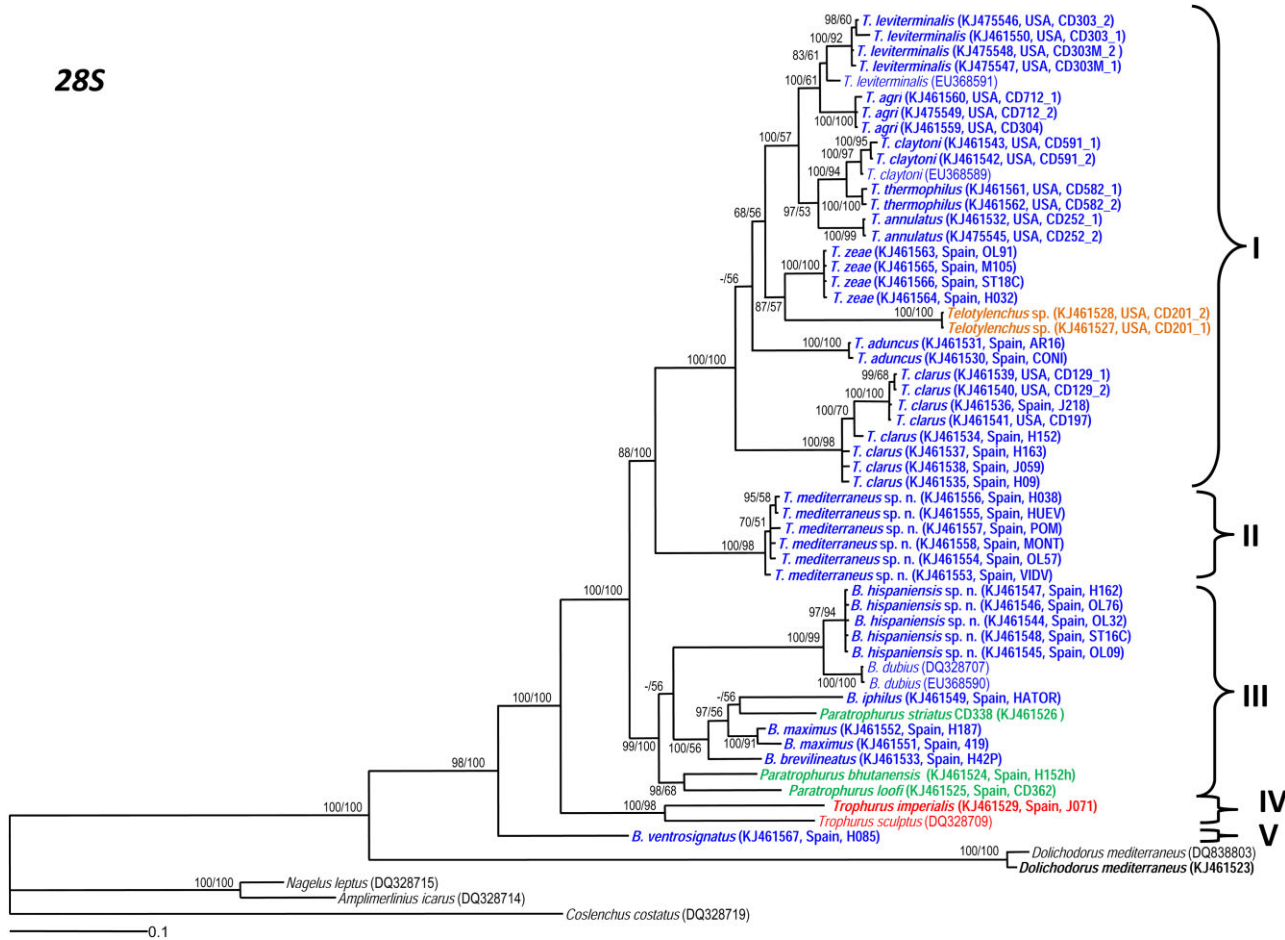


Figure 7. The 50% majority rule consensus trees from Bayesian analysis generated from the D2–D3 of 28S rRNA gene data set with a general time reversible of invariable sites and a gamma-shaped distribution model. Posterior probabilities more than 65% are given for appropriate clades; bootstrap values greater than 50% are given on appropriate clades in the maximum likelihood analysis. Newly obtained sequences are in bold letters.

support. Clade numbering was assigned based on common species groupings observed in all studied trees.

The D2–D3 of the 28S rRNA gene sequence alignment contained 59 sequences, five of which were outgroups. The alignment was 705 bp in length. The 50% majority rule consensus BI and ML trees showed five major highly or moderately supported clades (Fig. 7). Clade I (PP and BS = 100%) comprised eight *Tylenchorhynchus* species (*Ty. aduncus*, *Ty. annulatus*, *Ty. clarus*, *Ty. claytoni*, *Ty. leviterminalis*, *Ty. zaeae*, *Ty. agri*, *Ty. thermophilus*) and one *Telotylenchus* species. Clade II (PP = 100; BS = 98) included *Ty. mediterraneus* sp. nov. only. Clade III (PP = 99; BS = 100) contained five *Bitylenchus* species (*B. hispaniensis* sp. nov., *B. brevilineatus*, *B. dubius*, *B. iphilus*, *B. maximus*), and three species of *Paratrophurus* (*P. bhutanensis*, *P. loofi*, and *P. striatus*). Clade IV (PP = 100; BS = 98) contained two species of *Trophurus* (*Tr. imperialis* and *Tr. sculptus*). Clade V included only *B. ventrosignatus*,

which occupied a basal position in the tree and was clearly separated from all other *Bitylenchus* spp.

The ITS rRNA gene sequence alignment consisted of 37 ITS sequences and was 804 bp in length. The 50% majority rule consensus BI and ML trees resolved four highly supported major clades (Fig. 8). Clade I (PP = 100; BS = 90) comprised seven *Tylenchorhynchus* species [*Ty. aduncus*, *Ty. annulatus*, *Ty. clarus*, *Ty. leviterminalis* (type A and type B), *Ty. zaeae*, *Ty. agri*, and *Ty. thermophilus*]. Clade II (PP and BS = 100) comprised seven sequences of *Ty. mediterraneus* sp. nov. Clade III (PP = 100; BS = 88) contained three *Bitylenchus* species (*B. hispaniensis* sp. nov., *B. iphilus*, and *B. maximus*) and *Paratrophurus bhutanensis*. The basal fourth clade named here as Clade IV included a single species, *B. ventrosignatus*.

The partial 18S rRNA gene sequence included 25 sequences and was 862 bp in length. The 50% majority rule consensus BI and ML trees resolved six highly

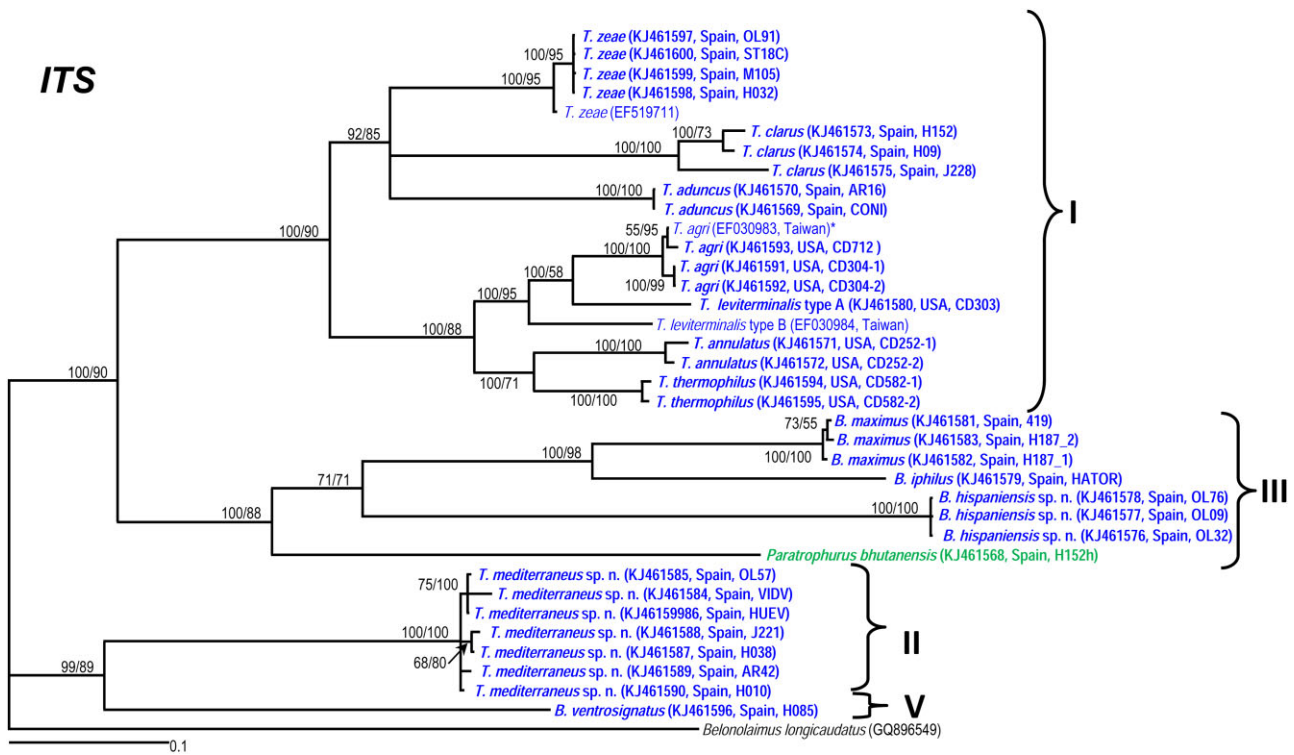


Figure 8. The 50% majority rule consensus trees from Bayesian analysis generated from the *ITS rRNA* gene data set with a transversional model of invariable sites and a gamma-shaped distribution model. Posterior probabilities more than 65% are given for appropriate clades; bootstrap values greater than 50% are given on appropriate clades in the maximum likelihood analysis. Newly obtained sequences are in bold letters.

supported major clades (Fig. 9). Clade I (PP = 100; BS = 97) comprised a group of four *Tylenchorhynchus* species; *Ty. aduncus*, *Ty. clarus*, *Ty. leviterminalis*, and *Ty. zaeae*. Clade II (PP = 100; BS = 77) contained sequences of *Ty. mediterraneus* sp. nov. populations and *Telotylenchus ventralis*. The third clade, named here as IIIa (PP and BS = 100), consisted of two *Bitylenchus* species: *B. hispaniensis* sp. nov. and *B. dubius*. Clade IV included only *Trophurus imperialis*. Clade V was in a basal position and contained a single species *B. ventrosignatus*. The sixth clade, named IIIb (PP = 96; BS = 98), consisted of *Bitylenchus brevilineatus*, *B. iphilus*, *B. maximus*, and *Paratrophurus bhutanensis*.

The results of the SH tests for alternative topologies using the three *rRNA* gene fragment alignments with a reduced number of taxa are shown in Table 8. Analyses of all data sets accepted the hypothesis of monophyly for the genus *Tylenchorhynchus sensu* Siddiqi (2000) when all studied *Tylenchorhynchus* sequences were constrained into one group. The SH test for the D2–D3 of *28S rRNA* data set also accepted the hypothesis of monophyly for the genus *Paratrophurus*. The hypothesis of monophyly for the genus *Bitylenchus sensu* Gómez Barcina *et al.* (1992) and Siddiqi (2000) was accepted for all three data sets only after the exclusion of

B. ventrosignatus from this genus. Monophyly of the genus *Tylenchorhynchus sensu* Fortuner & Luc (1987) was rejected for the D2–D3 of *28S rRNA* data set.

MORPHOLOGICAL MATRIX AND MAPPING OF MORPHOLOGICAL CHARACTERS

Several characters used in the species identification key were used to reconstruct the ancestry of some characters by parsimony (Figs S20, S21). Shapes of tail, tail terminus, and lip region showed mosaic evolution patterns. Shape of tail ranged from cylindrical, subcylindrical, and conoid for *Tylenchorhynchus*, whereas tails for the other genera were more variable. Annulated tail tip terminus is likely to be the ancestral character state for Telotylenchidae, which then evolved independently to a smooth state three times. Posterior stylet knob inclination seems to be the ancestral character state that then changed to lateral or anterior in several groups during nematode evolution.

DISCUSSION

The present morphological and morphometrics studies confirmed that diagnosis and identification of *Bitylenchus*

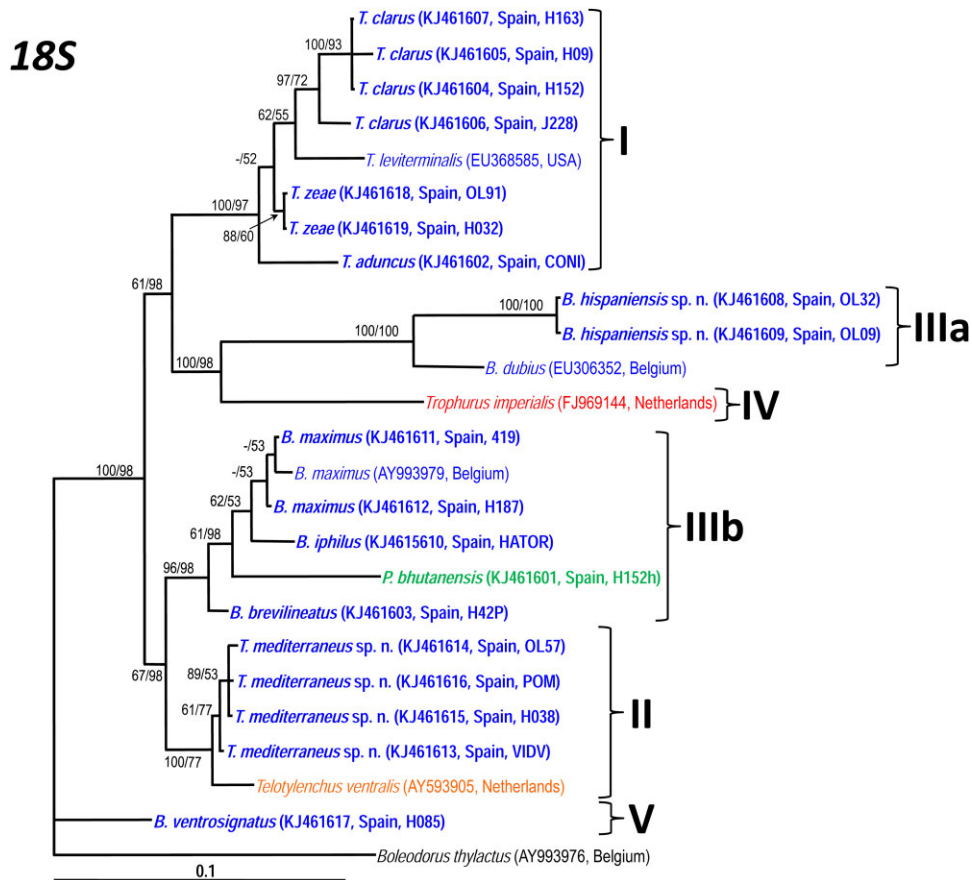


Figure 9. The 50% majority rule consensus trees from Bayesian analysis generated from the 18S *rRNA* gene data set with a transitional model of invariable sites and a gamma-shaped distribution model. Posterior probabilities more than 65% are given on appropriate clades; bootstrap values greater than 50% are given on appropriate clades in the maximum likelihood analysis. Newly obtained sequences are in bold letters.

and *Tylenchorhynchus* species based solely on morphometric features are quite problematic because there is an almost continuous range of these within populations of the same species as well as amongst species (Tables 2–6, S1–S7).

MORPHOLOGICAL COMPARISON OF *BITYLENCHUS* *HISPANIENSIS* SP. NOV. AND *TYLENCHORHYNCHUS* *MEDITERRANEUS* SP. NOV. WITH RELATED TAXA

Bitylenchus hispaniensis sp. nov. from the type and other localities were identical morphologically, but some minor morphometric differences amongst them were detected and considered as geographical intraspecific variability (Tables 2, 3). *Bitylenchus hispaniensis* sp. nov. is very close to *Bitylenchus iphilus* Minagawa, 1995, *Bitylenchus teeni* Hashim, 1983, and *Bitylenchus parvus* Allen, 1955. It differs from *B. iphilus* in having on average a shorter stylet (15.5–18.5 vs. 17–20 μm); a truncate, continuous lip region vs. hemispherical, offset

lip region; non-areolated lateral fields vs. areolated throughout; the shape of female tail being bluntly rounded with a hemispherical to clavate terminus bearing 52 (40–66) annuli vs. straight to curved, narrowly clavate tail with 31 (24–41) annuli; absence of postrectal intestinal sac vs. present, 8.1–32.3 μm long; and spicules being longer 25.3 (23–30) μm , with a smooth terminus vs. shorter 23.8 (20–25.7) μm with a minutely bifurcate terminus. From *B. teeni* it differs by the shape of female tail being bluntly rounded with a hemispherical to clavate terminus bearing 52 (40–66) annuli vs. cylindrical tail with a hemispherical to subhemispherical terminus bearing 44–57 annuli; absence of postanal intestinal sac vs. postanal intestinal sac present and occupying the entire tail cavity; and vulval flap and epiptygma absent vs. present in the form of a double epiptygma. From *B. parvus* it differs in the shape of female tail being bluntly rounded with a hemispherical to clavate terminus bearing 40–66 annuli vs. cylindrical tail with hemispherical

Table 8. Results of the Shimodaira–Hasegawa tests for alternative hypotheses using maximum likelihood (ML) trees reconstructed from *rRNA* gene sequence alignments

Hypothesis	D2–D3 of 28S <i>rRNA</i>		ITS <i>rRNA</i>		18S <i>rRNA</i>	
	–LnL	Difference in –LnL	–LnL	Difference in –LnL	–LnL	Difference in –LnL
ML tree	5927.76651	Best	7418.7943	Best	2919.55647	Best
Species of <i>Tylenchorhynchus sensu</i> Siddiqi (2000) constrained into a monophyletic group	5954.44202	26.67550	7430.8519	12.05754	2931.61844	12.06196
Species of <i>Bitylenchus sensu</i> Siddiqi (2000) constrained into a monophyletic group	5975.07088	47.30437	7474.81084	56.01649	2939.07295	19.51647
Species of <i>Bitylenchus sensu</i> Siddiqi (2000)† constrained into a monophyletic group without <i>B. ventrosignatus</i>	5939.14684	11.38032	7430.69237	11.89802	2925.21759	5.66112
Species of <i>Paratrophurus sensu</i> Siddiqi (2000) constrained into a monophyletic group	5952.30564	24.53913	–	–	–	–
Species of <i>Bitylenchus sensu</i> Gómez Barcina <i>et al.</i> (1992)‡ constrained into a monophyletic group without <i>B. ventrosignatus</i>	5939.95422	12.18771	7418.7943	Best	2919.84967	0.29319
Species of <i>Tylenchorhynchus sensu</i> Fortuner & Luc (1987)§ constrained into a monophyletic group	5975.08754	47.32102	7430.49454	11.70019	2935.36198	15.80551

**P* < 0.05 indicates the significant differences between the two inferred tree topologies.†*Bitylenchus maximus* is considered as a representative of *Saueritylenchus*.‡Systematics of the genus *Bitylenchus* accepted in the present study.§*Bitylenchus* and *Telotylenchus* are synonyms of *Tylenchorhynchus*.

terminus bearing 35–43 annuli; and in the absence of postanal intestinal sac vs. postanal intestinal sac present.

Tylenchorhynchus mediterraneus sp. nov. from the type and other localities were identical morphologically, but some minor morphometric differences amongst them were detected and considered as geographical intraspecific variability (Tables 4–6). *Tylenchorhynchus mediterraneus* sp. nov. is very close to *Tylenchorhynchus dewaelei* Kleyhans, 1992, that was originally described from Namibia. However, it differs from *Ty. dewaelei* in having a shorter tail [55.0 (42.5–70.0) vs. 84 (71–92) μm]; slightly longer stylet [20.4 (18–23) vs. 19.4 (17–21.3) μm]; vulva without recessed, double epiptygma vs. epiptygma present; higher number of tail annuli [57 (42–81) vs. (41–65)], shorter anal body width [17.1 (12–25) vs. 27.6 (23.4–32.5) μm]; large phasmids vs. inconspicuous; and slightly shorter spicule length [25.5 (20.0–28.5) vs. 32 (28.8–35) μm]. It is also similar to *Tylenchorhynchus canalis* and other species described from Spain such as *Tylenchorhynchus serranus* and *Tylenchorhynchus pratensis*, but these all differ from *Ty. mediterraneus* sp. nov. in one or more other characters. For example, from *Ty. canalis* it differs in the shape of lip region, stylet knobs, tail terminus, and number of tail annuli [high, rounded to hemispherical offset lip region vs. truncate, flattened lip region; posteriorly directed knobs vs. strong anteriorly directed knobs; rounded, smooth terminus vs. annulated tail terminus; and higher number of tail annuli (42–81 vs. 66)].

Some specimens of this species have pharyngeal glands overlapping the intestine. Seinhorst (1971) and Fortuner & Luc (1987) discussed the taxonomic importance of this character and noted that intermediate forms existed between two morphologies described as typical for *Tylenchorhynchus* and *Telotylenchus*. *Tylenchorhynchus brassicae*, *Ty. clarus*, *Ty. indicus*, and *Tylenchorhynchus mashhoodi* have the pharyngeal glands slightly overlapping, and the dorsal gland nucleus in the posterior half of the gland. We also noticed a few specimens with these features in *Ty. dewaelei*.

PHYLOGENY OF THE SUBFAMILY TELOTYLENCHINAE

The genus *Bitylenchus* was not recognized by Fortuner & Luc (1987) and Geraert (2011), but it was accepted in Siddiqi's (2000) classification. In this study we distinguished ten *Tylenchorhynchus* species and five *Bitylenchus* species. The species of these genera clustered separately in most trees. Although in the D2–D3 of the 28S and 18S trees representatives of the genera *Telotylenchus* and *Paratrophurus* were nested within *Tylenchorhynchus* and *Bitylenchus*, respectively, and made these two genera paraphyletic, the ML tests accepted all hypotheses with monophyly

of *Tylenchorhynchus* and *Bitylenchus* without *B. ventrosignatus*. Fortuner & Luc (1987) noted that *B. ventrosignatus* lacks a postanal intestinal sac, which is a generic character. In all trees this species occupied a basal position within Telotylenchinae, suggesting that it might be considered under a separate genus. Analyses of all data sets accepted the hypothesis of monophyly for the genus *Tylenchorhynchus sensu* Siddiqi (2000) and rejected the 'large-genus idea' advocated by Fortuner & Luc (1987).

The genus *Sauertylenchus* was established by Sher (1974) with a single species *Sauertylenchus labiodiscus*, which was only distinguished from other related genera by a conspicuous labial disc and stylet over 30 μm long. Gómez Barcina *et al.* (1992) synonymized *Sauertylenchus* with *Bitylenchus* based on the results of SEM analysis of lip regions, which showed structural similarity for both taxa. However, Geraert (2011) and Siddiqi (2000) did not accept this synonymization. Siddiqi (2000) included five valid species in the genus *Sauertylenchus*, amongst them *Sauertylenchus maximus*, whereas Geraert (2011) considered *Sauertylenchus* as a monospecific genus. The present study revealed that *B. maximus* (= *S. maximus*) clustered within *Bitylenchus* species (i.e. *B. brevilineatus*, *B. hispaniensis* sp. nov., and *B. iphilus*), thus supporting the view of Gómez Barcina *et al.* (1992) on the relationships between *Sauertylenchus* and *Bitylenchus*. However, the position of the type species *S. labiodiscus* within Telotylenchidae still needs to be analysed in order to make a final conclusion with regard to maintaining the genus *Sauertylenchus*.

The close relationship of *Telotylenchus* with *Tylenchorhynchus* was pointed out by Siddiqi (1960). *Telotylenchus* shares most morphological characters with *Tylenchorhynchus* and differs from this genus only in having the dorsal pharyngeal gland extending over the intestine and in having a different *en face* view and structure of the gubernaculum (Siddiqi, 2000). In our study the genus *Telotylenchus* was always nested within *Tylenchorhynchus* species in the phylogenetic trees. However, constrained trees, in which representatives of *Telotylenchus* were outside *Tylenchorhynchus*, were not significantly worse than the best tree, and thus do not give a sufficient reason for synonymization of it with *Tylenchorhynchus*. Other genera (*Histotylenchus*, *Telotylenchoides*, and *Trichotylenchus*) that have pharyngeal glands extending over the intestine should be included in future phylogenetic analyses in order to better understand the evolution of this character within nematodes.

The genus *Paratrophurus* is morphologically similar to *Tylenchorhynchus*, from which it differs only in the abnormally thickened terminal cuticle of the tail (Castillo *et al.*, 1989; Siddiqi, 2000). Castillo *et al.* (1989) noted that both genera have similar SEM *en face* views with

labial disc and labial sectors fused into a quadrangular structure, which provides strong evidence for the close relationship between these genera. Our current analysis confirmed close relationships of *Paratrophurus* with *Bitylenchus*. In the phylogenetic trees, *Paratrophurus* species clustered with *Bitylenchus* and the SH test did not reject the constrained tree when *Paratrophurus* formed a monophyletic group.

Trophurus has a similar lip region, pharynx, and tail to those of *Paratrophurus*, this genus is characterized by a posterior genital branch reduced to a uterine sac. Bert *et al.* (2008) noticed that there were relatively few switches in the number of gonad arms in the order Tylenchida, possibly indicating that the monodelphic vs. didelphic state has relatively stronger historical and genetic determinants than suggested from traditional classifications. Analysis by Bert *et al.* (2008) also indicated that monodelphy is ancestral for tylenchid nematodes. In our D2–D3 tree, *Trophurus* species clustered in the basal position of Telotylenchinae, thus suggesting that the monodelphic genital branch is the ancestral morphological character for this subfamily. However, further testing and more detailed phylogenies need to be used in order to completely assess this hypothesis.

Some characters used in the species identification key (Handoo, 2000) were selected here to reconstruct the ancestry of some characters by parsimony. The shapes of the tail, terminus and lip region did not show a clear evolution pattern, as has also been suggested for the shape of the tail in a broader phylogenetic analysis by Carta *et al.* (2010). The position of the stylet knobs appears to have evolved from a posterior position to an anterior position; however, the intermediate character is shared amongst several species lineages and anterior knobs are shared in deeper branches in our tree. Anteriorly directed stylet knobs are associated with the protractor muscles linked to the stylet and may relate to the enforcement of the perforation function (Ryss, 2002). Specifically, in our data set some branches (*Ty. thermophilus* clade) of the tree show the logical transition of posterior–lateral–anterior, whereas in other anterior clades the transition character species are not yet studied molecularly. Tail tip annulations look like an ancestral character that has evolved to the smooth state; however, smooth tail tip has multiple independent origins and appears in two lineages of *Bitylenchus* (*B. ventrosignatus* and *B. brevilineatus*).

CONCLUSIONS

The present research establishes the importance of using integrative taxonomic identification by highlighting the time-consuming aspects and difficulty of correct species-level identification within the genera *Tylenchorhynchus* and *Bitylenchus*. Overall, the data reported here

strengthen the idea that *Tylenchorhynchus* and *Bitylenchus* species delimitation can be refined and improved based on studies that integrate morphology, morphometry, and molecular taxonomic identification and phylogeny using the D2–D3 region, *ITS* of rRNA, and partial *18S rRNA* sequences. These molecular markers provide precise and unequivocal diagnosis of some of the stunt nematode species in cultivated and native vegetation. This point is particularly important because nematode morphology is quite similar and mixed populations of several species and genera within Telotylenchidae may occur in the same soil sample. Additionally, the discovery of several new records of species from the family Telotylenchidae present in Spain and the USA suggests that the biodiversity of these nematodes is still not fully clarified, and requires further study. Phylogenetic analyses based on the three molecular markers used here resulted in a general consensus of species groupings because lineages were maintained for the majority of species. Consequently, the genera *Tylenchorhynchus* and *Bitylenchus* can be clearly separated on the basis of integrative taxonomy that includes both morphological and molecular studies, as well as SH testing for alternative topologies obtained from the D2–D3 of *28S*, *ITS*, and partial *18S rRNA* gene sequences.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Photomicrographs of *Paratrophurus bhutanensis* (Ganguly *et al.*, 2004) Andrassy, 2007. A, whole body of female; B, female pharyngeal region; C, female lip region; D, lateral fields at mid-body; E, vulval region; F, G, female tails. Scale bars: A = 50 µm, B = 20 µm; C–G = 10 µm.

Figure S2. Scanning electron microscope photographs of *Paratrophurus bhutanensis* (Ganguly *et al.*, 2004) Andrassy, 2007. A, D, female anterior region; B, C, *en face* view showing oral (oa) and amphidial (am) apertures; E, vulval region; F, G, female tail; H, male tail. Scale bars: A, D, H = 20 µm; B, C, E–G = 10 µm.

Figure S3. Photomicrographs of *Bitylenchus brevilineatus* (Williams, 1960) Jairajpuri, 1982. A, female pharyngeal region; B, C, female lip region; D, female tail; E, male tail. Scale bars: A = 20 µm, B–E = 10 µm.

Figure S4. Scanning electron microscope photographs of *Bitylenchus brevilineatus* (Williams, 1960) Jairajpuri, 1982. A, female anterior region; B, C, *en face* view showing oral (oa) and amphidial (am) apertures; D, lateral fields at mid-body; E–G, female tails; H, male tail. Scale bars: A–D, F, = 10 µm; E, G, H = 20 µm.

Figure S5. Photomicrographs of *Bitylenchus iphilus* Minagawa, 1995. A, whole body of female; B, female pharyngeal region showing excretory pore (arrowed); C, female lip region; D, vulval region; E–J, female tails showing anus and phasmid (arrowed); K, male tail. Scale bars: A = 50 µm, B = 20 µm; C–K = 10 µm.

Figure S6. Scanning electron microscope photographs of *Bitylenchus iphilus* Minagawa, 1995. A, female anterior region showing start of lateral fields (arrowed); B, C, female lip region; D, *en face* view showing oral (oa) and amphidial (am) apertures; E, lateral fields at mid-body showing areolated bands (arrowed); F, G, female tails showing anus (arrowed); H, male tail. Scale bars: A, B, E = 10 μ m; C, D = 5 μ m; F–H = 20 μ m.

Figure S7. Photomicrographs of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1976. A, female pharyngeal region; B, C, female lip region; D–F, female tails showing anus and phasmid (arrowed). Scale bars: A = 20 μ m, B–F = 10 μ m.

Figure S8. Scanning electron microscope photographs of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1976. A, female lip region; B, C, *en face* view showing oral (oa) and amphidial (am) apertures; D–F, female tails showing anus (a) and phasmid (p). Scale bars: A = 2 μ m; B, C = 1 μ m; D = 2.5 μ m; E, F = 5 μ m.

Figure S9. Photomicrographs of *Bitylenchus ventrosignatus* (Tobar-Jiménez, 1969) Jairajpuri, 1982. A, whole body of female and male; B, female pharyngeal region; C, D, female lip region; E, F, vulval region showing wave-like structures present close to the vulva (arrowed); G–I, female tails; J, male tail. Scale bars: A = 50 μ m, B = 20 μ m; C–J = 10 μ m.

Figure S10. Scanning electron microscope photographs of *Bitylenchus ventrosignatus* (Tobar-Jiménez, 1969) Jairajpuri, 1982. A, B, female anterior region showing excretory pore (arrowed); C, *en face* view; D, E, ventral view of vulval region showing wave-like structures present (arrowed); F, lateral fields at mid-body; G, female tail showing anus (a); H, male tail. Scale bars: A, H = 20 μ m; B, C, F = 5 μ m; D, E, G = 100 μ m.

Figure S11. Photomicrographs of *Tylenchorhynchus aduncus* de Guiran, 1967. A, whole body of female and male; B, female pharyngeal region; C, female lip region; D, vulval region; E, lateral fields at mid-body; F–I, female tails; J, male tail. Scale bars: A = 50 μ m, B = 20 μ m; C–J = 10 μ m.

Figure S12. Photomicrographs of *Tylenchorhynchus agri* Ferris, 1963. A, female lip region; B, detail of pharyngeal gland; C, lateral fields at mid-body; D, E, female tails. Scale bars: A–E = 10 μ m.

Figure S13. Photomicrographs of *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971. A, female pharyngeal region showing adhered *Pasteuria* sp. spores and excretory pore (arrowed); B, vulval region; C, lateral fields at mid-body; D, E, female tails, showing anus and phasmid (arrowed). Scale bars: A–E = 25 μ m.

Figure S14. Photomicrographs of *Tylenchorhynchus clarus* Allen, 1955. A, whole body of female and male; B, female pharyngeal region showing excretory pore (ep); C, D, female lip region; E, lateral fields at mid-body; F, vulval and posterior region showing vulva (v) and anus (a); G, H, female tails; I, vulval region. Scale bars: A = 50 μ m, B, E, F = 20 μ m; C, D, G–I = 10 μ m.

Figure S15. Scanning electron microscope photographs of *Tylenchorhynchus clarus* Allen, 1955. A, female anterior region showing start of lateral fields (arrowed); B, *en face* view showing oral (oa) and amphidial (am) apertures, and lateral sectors (ls) fused to oral disc; C, lateral fields at mid-body; D, E, female tails. Scale bars: A–D = 10 μ m; E = 20 μ m.

Figure S16. Photomicrographs of *Tylenchorhynchus claytoni* Steiner, 1937. A, whole body of female; B, female pharyngeal region; C–E, female lip region; F, lateral fields at mid-body; G, H, male tails; I, female tail. Scale bars: A = 50 μ m, B = 20 μ m; C–I = 10 μ m.

Figure S17. Photomicrographs of *Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982. A, whole body of female; B, C, female lip region; D, vulval region; E, lateral fields at mid-body; F, G, female tails. Scale bars: A = 50 μ m, B–G = 10 μ m.

Figure S18. Photomicrographs of *Tylenchorhynchus thermophilus* Golden, Baldwin & Mundo-Ocampo, 1995. A, female pharyngeal region; B, female lip region; C, whole body of female and male; D, male tail; E, F, female tails. Scale bars: A–F = 10 μ m.

Figure S19. Light and scanning electron microscope photographs of *Tylenchorhynchus zae* Siddiqi, 1961 (A–D and E–H, respectively). A, female lip region; B, vulval region showing spermatheca (arrowed); C, female tail; D, male tail; E, F, female lip region; G, lateral fields at mid-body; H, male tail. Scale bars: A–D 10 μ m; E, F = 10 μ m, G, H = 20 μ m.

Figure S20. Morphological character history reconstruction for tail tip annulations and inclination of stylet knobs using the phylogenetic tree obtained for the D2–D3 of the 28S rRNA gene sequences. A, tail tip annulation; B, inclination of stylet knobs. The most representative value for each character was considered for the morphological matrix. The criterion of parsimony was used to optimize character state evolution on the maximum likelihood tree using MESQUITE 2.73.

Figure S21. Morphological character history reconstruction for the shape of tail terminus, shape of tail, and shape of lip region using the phylogenetic tree obtained for the D2–D3 of the 28S rRNA gene sequences. A, shape of tail terminus; B, shape of tail; C, shape of lip region. The most representative value for each character was considered for the morphological matrix. The criterion of parsimony was used to optimize character state evolution on the maximum likelihood tree using MESQUITE 2.73.

Table S1. Morphometrics of *Dolichodorus mediterraneus* Jiménez-Guirado *et al.*, 2007, *Paratrophurus bhutanensis* (Ganguly *et al.*, 2004) Andrassy, 2007, *Paratrophurus loofi* Arias, 1970, and *Paratrophurus striatus* Castillo *et al.*, 1989, from southern Spain.

Table S2. Morphometrics of *Trophurus imperialis* Loof, 1956, *Tylenchorhynchus aduncus* de Guiran, 1967, and *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971, from southern Spain and California, USA.

Table S3. Morphometrics of *Bitylenchus brevilineatus* (Williams, 1960) Jairajpuri, 1982, and several populations of *Tylenchorhynchus clarus* Allen, 1955, from southern Spain.

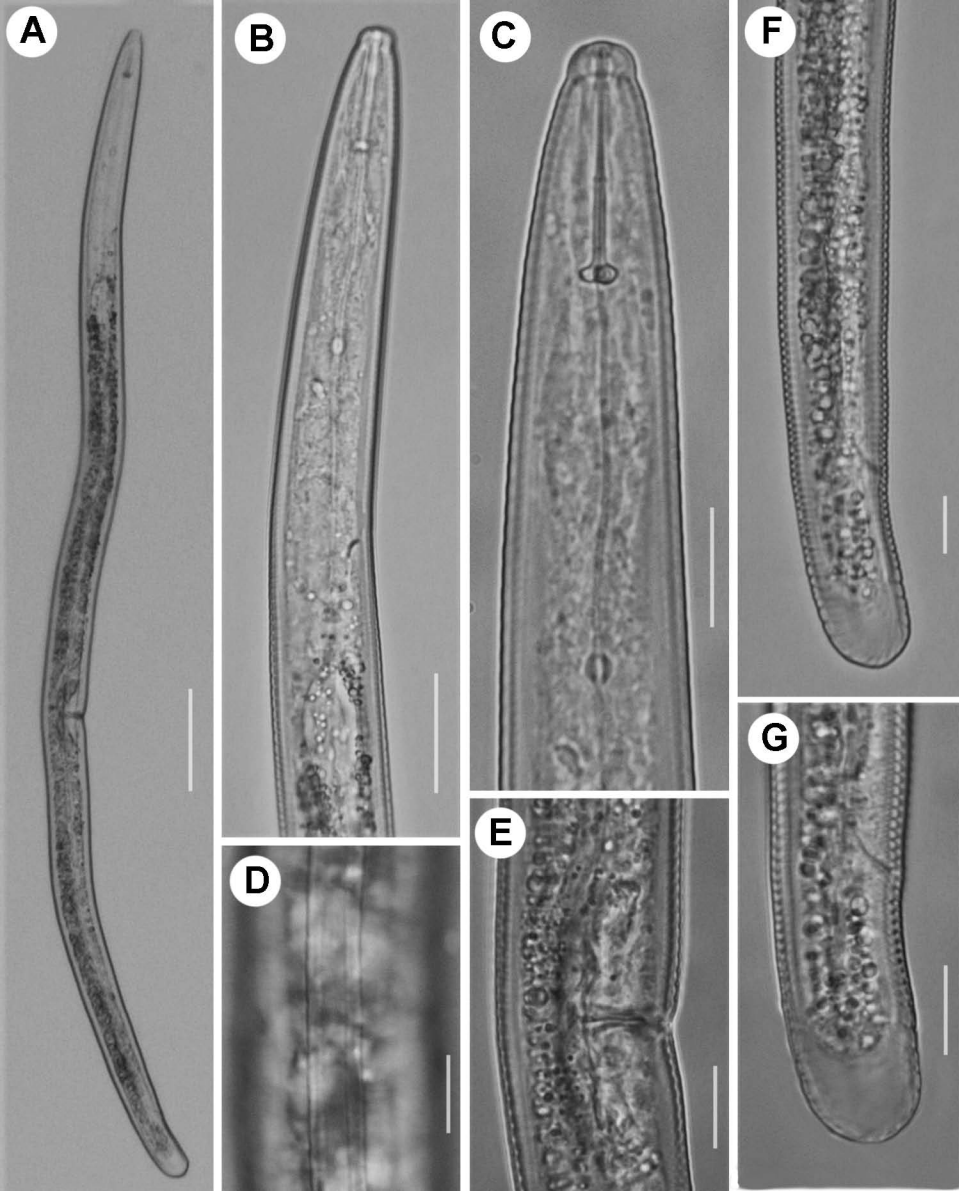
Table S4. Morphometrics of several populations of *Tylenchorhynchus clarus* Allen, 1955, from southern Spain and from California, USA.

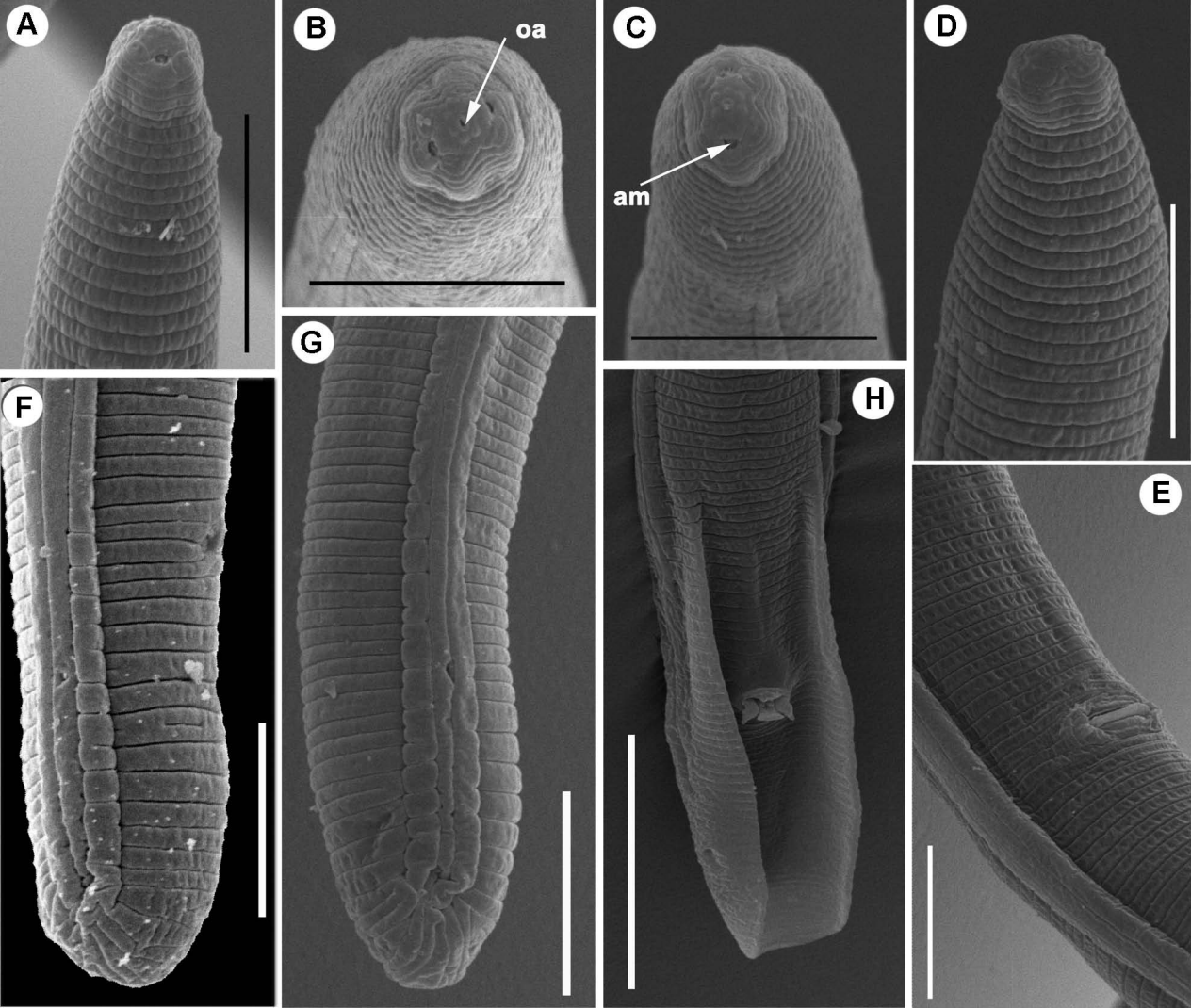
Table S5. Morphometrics of *Tylenchorhynchus claytoni* Steiner, 1937, *Bitylenchus iphilus* Minagawa, 1995, and *Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982, from California, USA, and southern Spain.

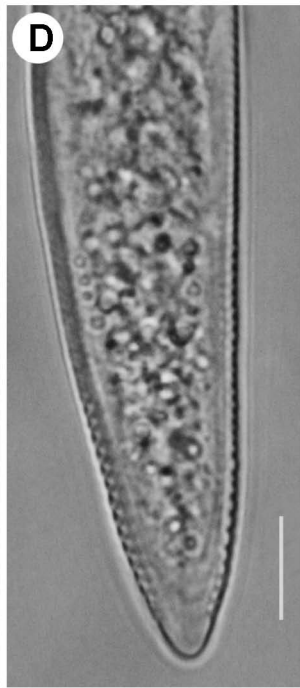
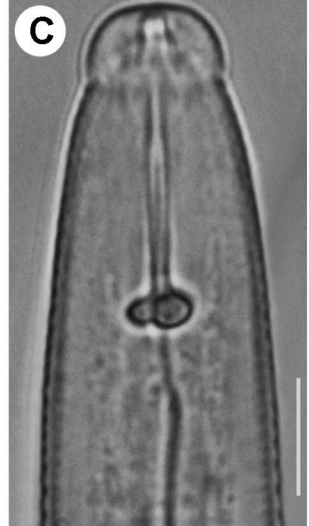
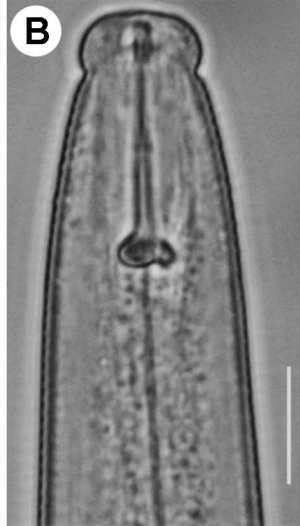
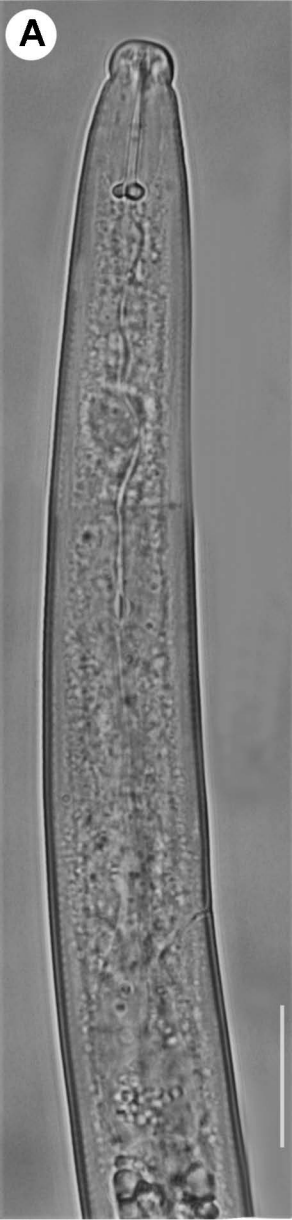
Table S6. Morphometrics of *Bitylenchus maximus* (Allen, 1955) Siddiqi, 1986, and *Bitylenchus ventrosignatus* (Tobar-Jiménez, 1969) Jairajpuri, 1982, from southern Spain.

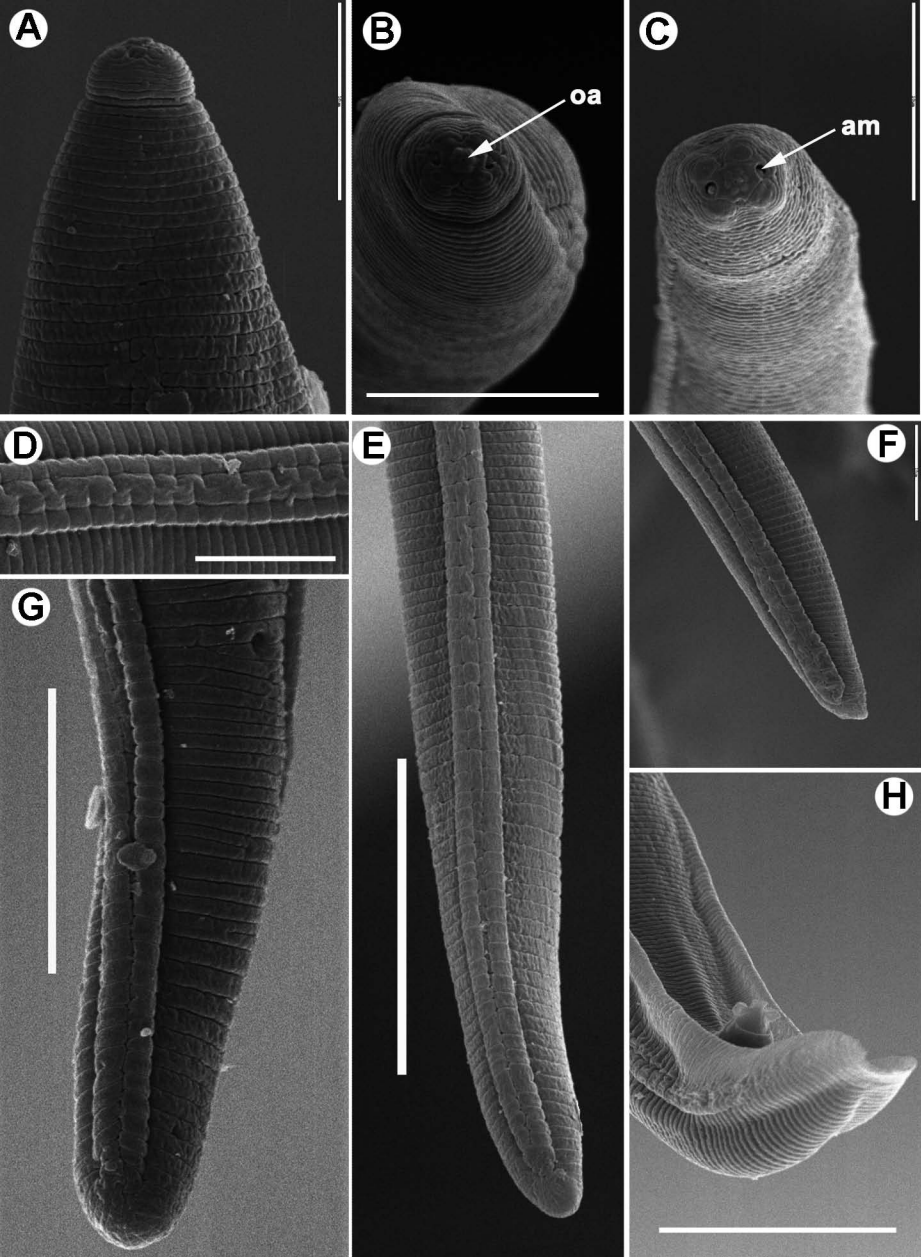
Table S7. Morphometrics of several populations of *Tylenchorhynchus zae* Sethi & Swarup, 1968, from southern Spain.

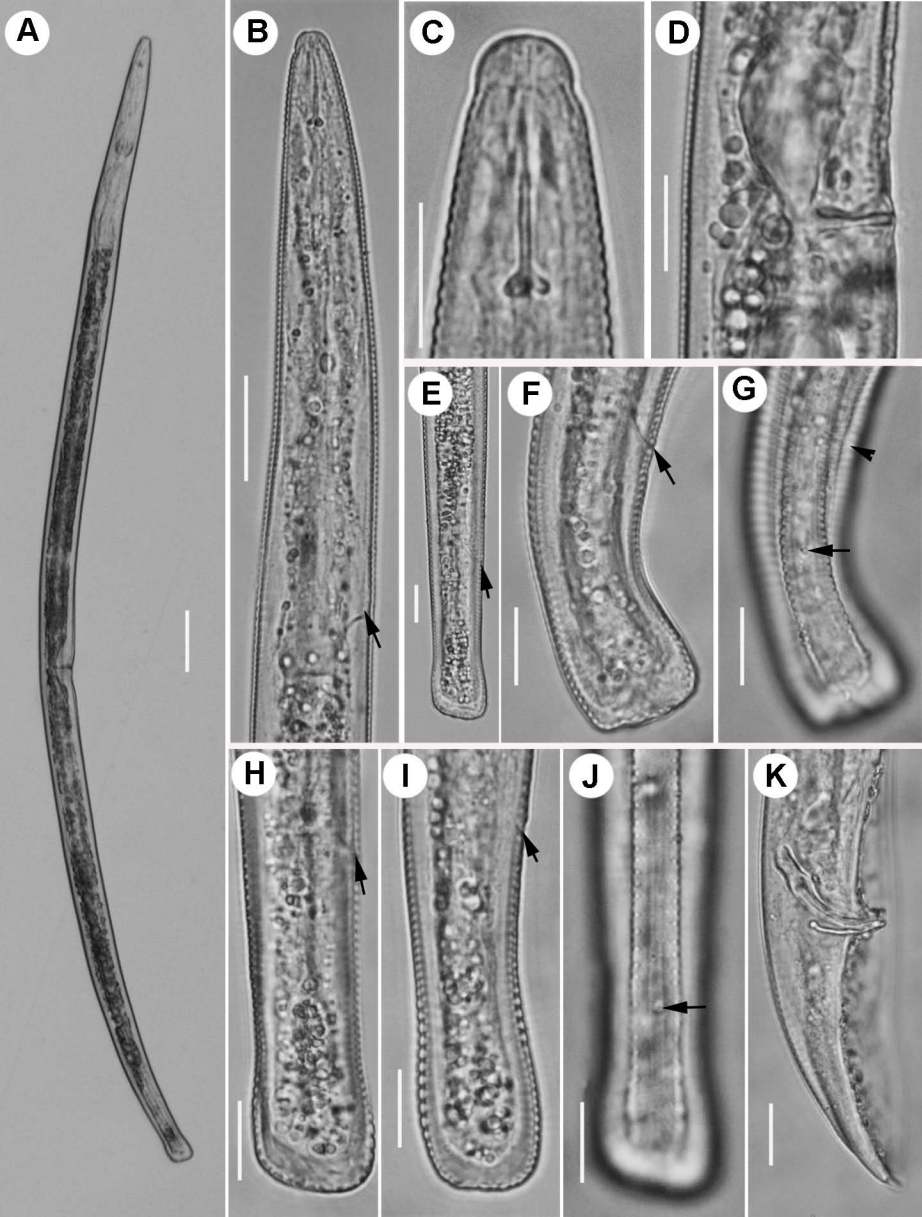
Table S8. Morphometrics of *Tylenchorhynchus agri* Ferris, 1963, *Tylenchorhynchus thermophilus* Golden, Baldwin & Mundo-Ocampo, 1995, and *Telotylenchus* sp. from the USA.

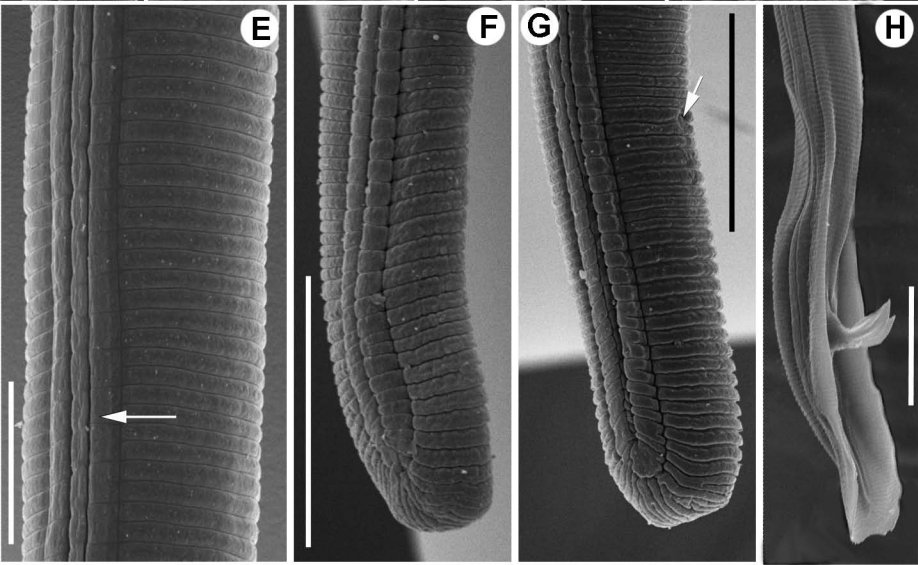
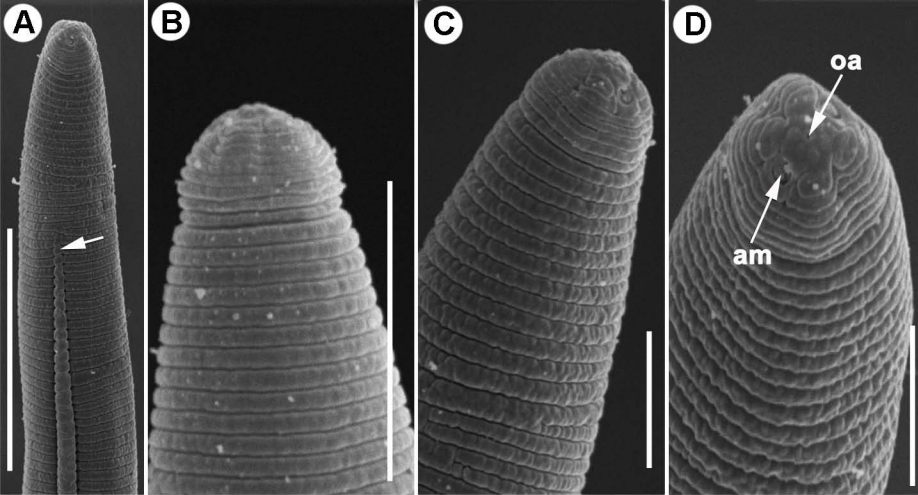


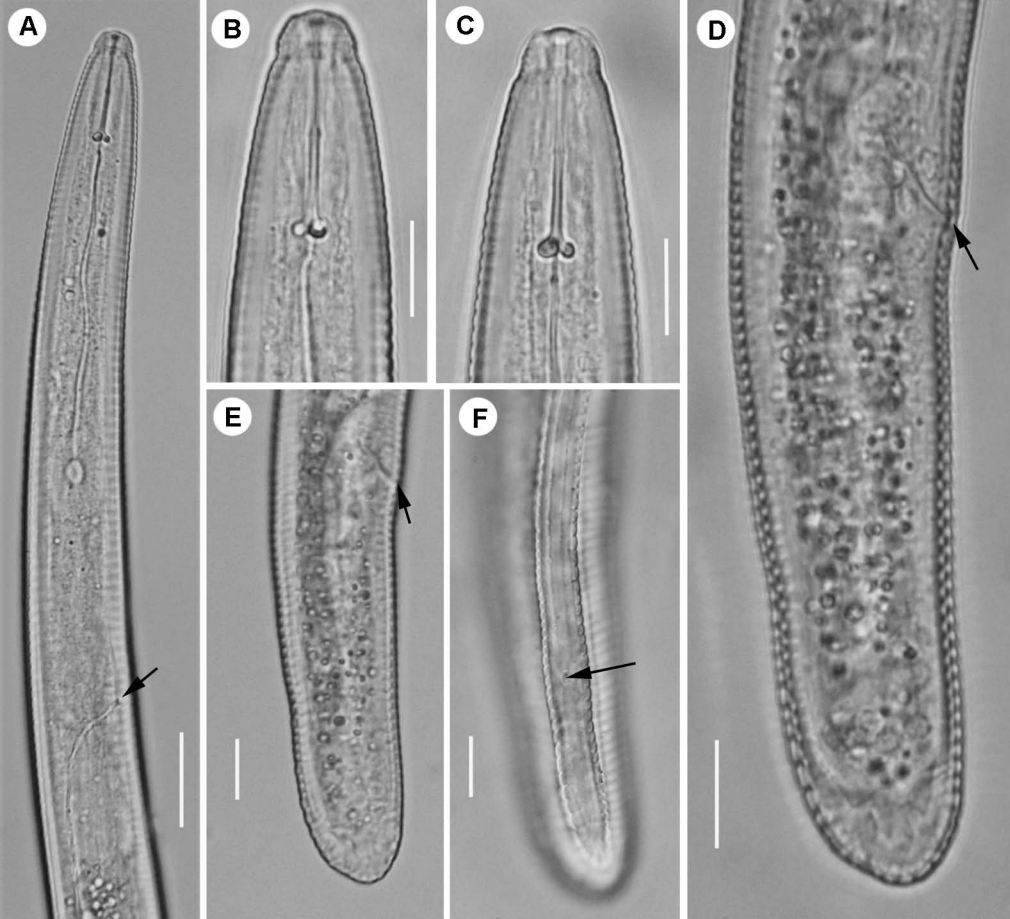


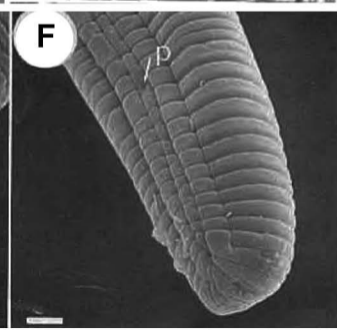
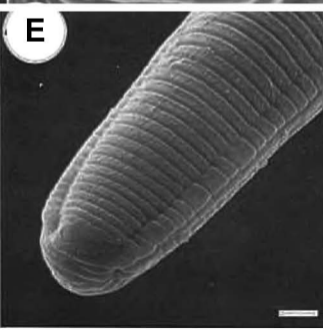
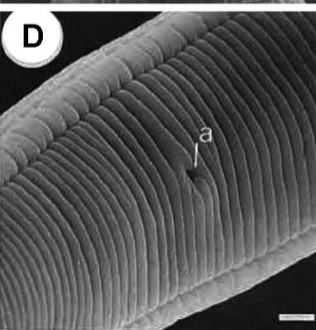
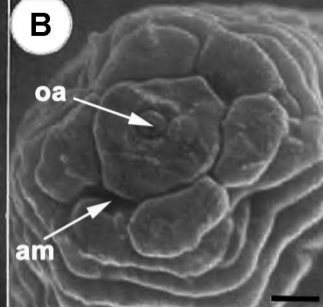
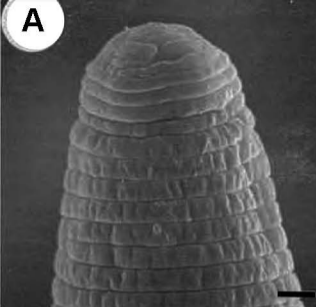


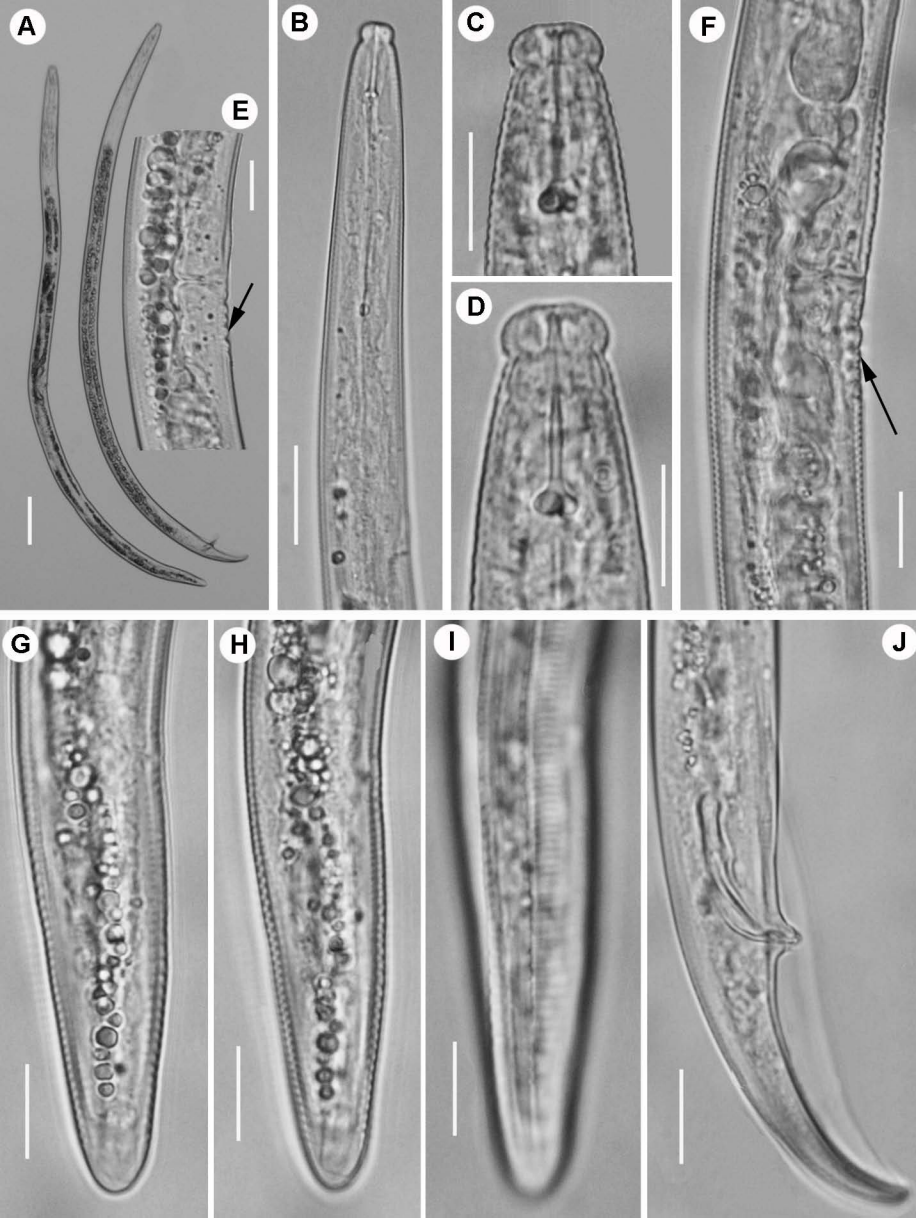


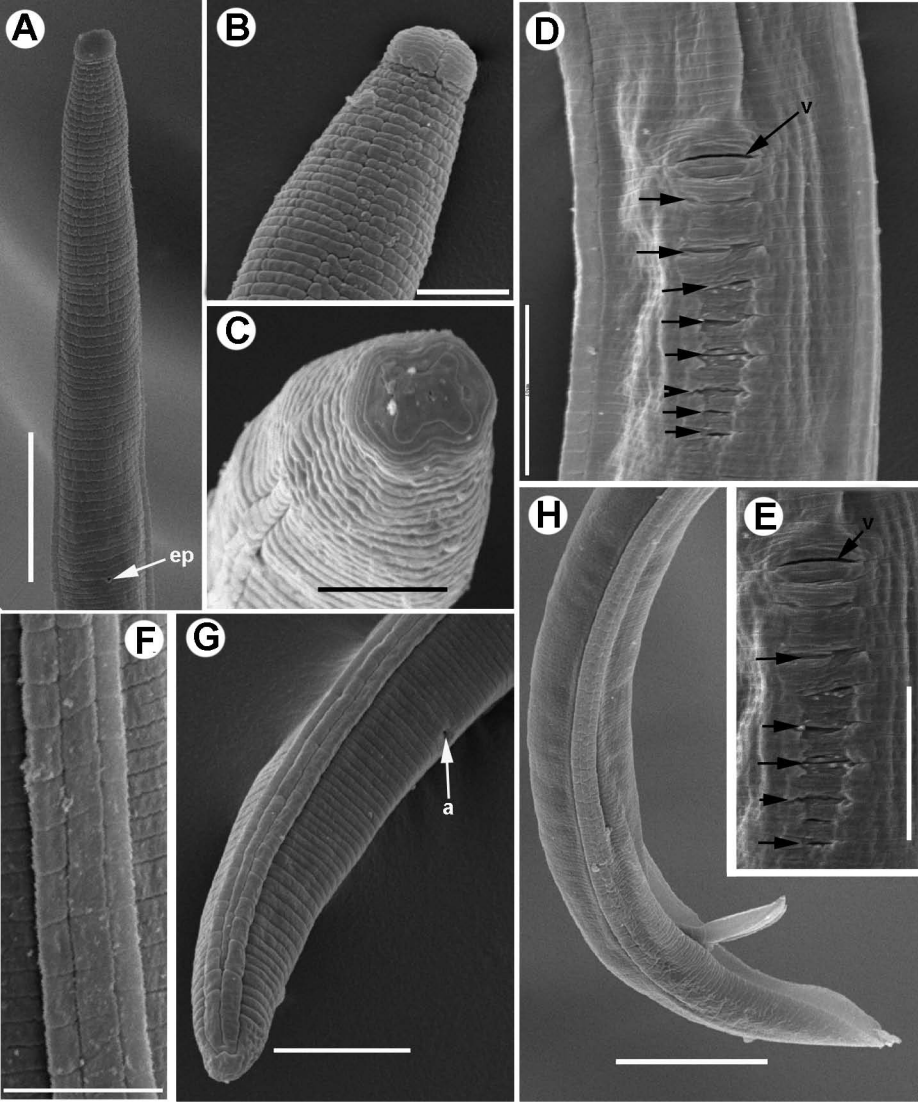


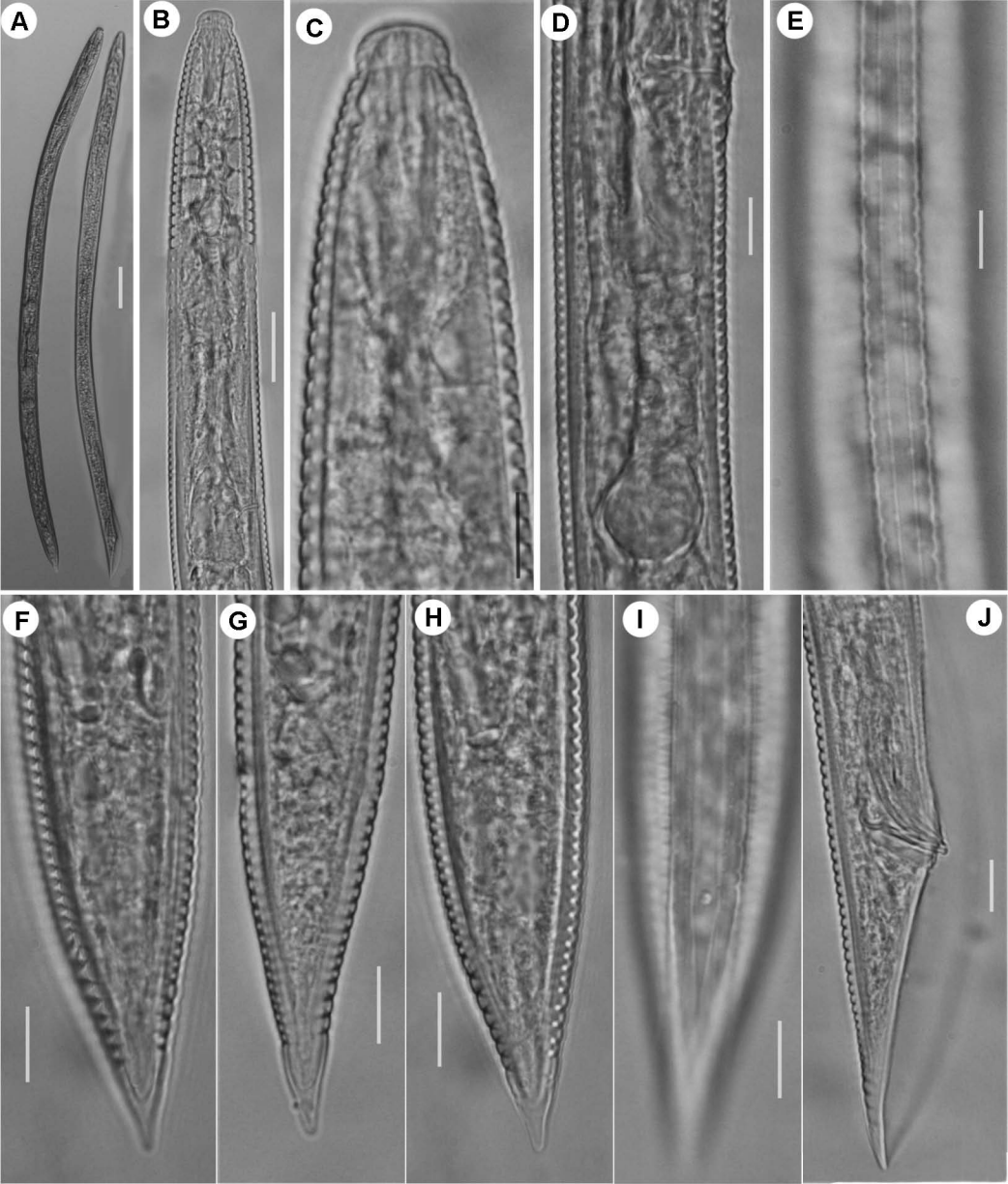


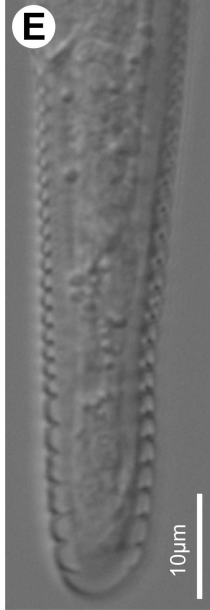
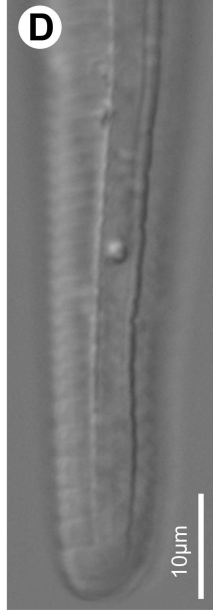
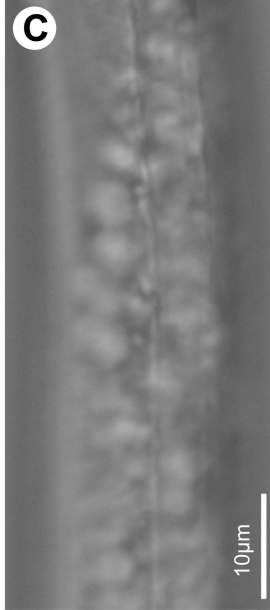
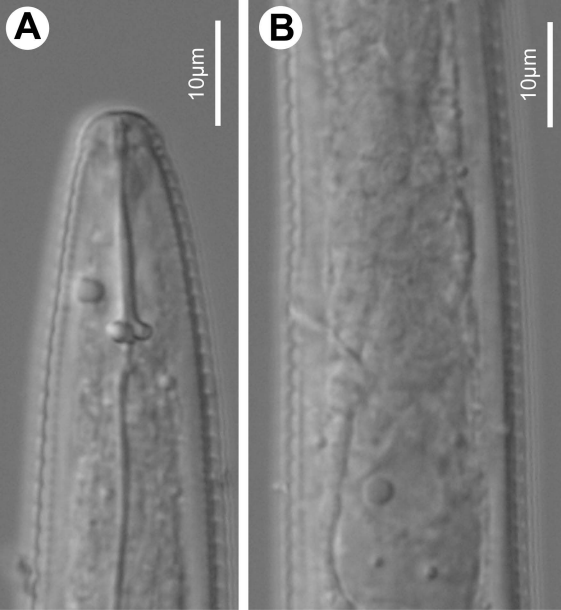


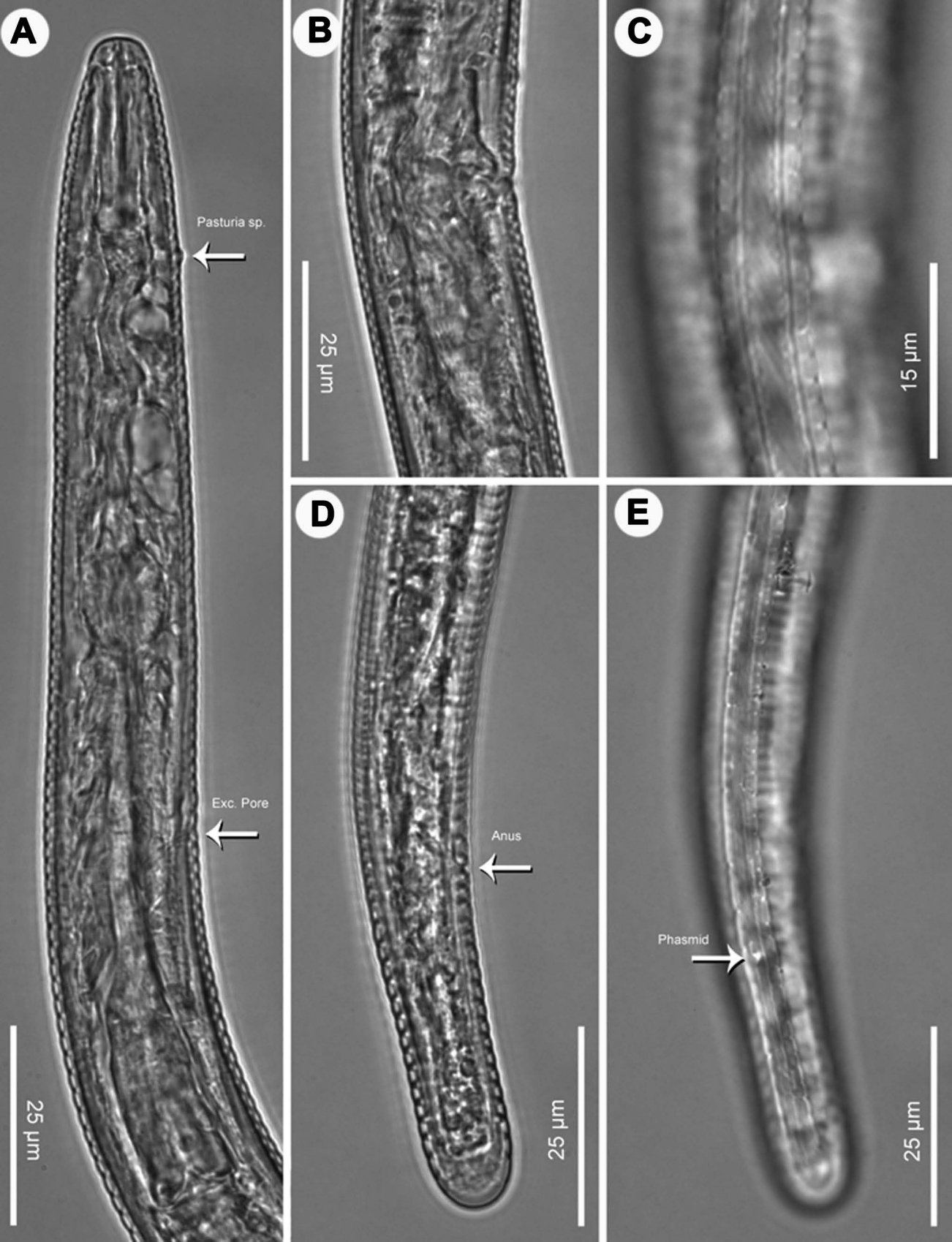


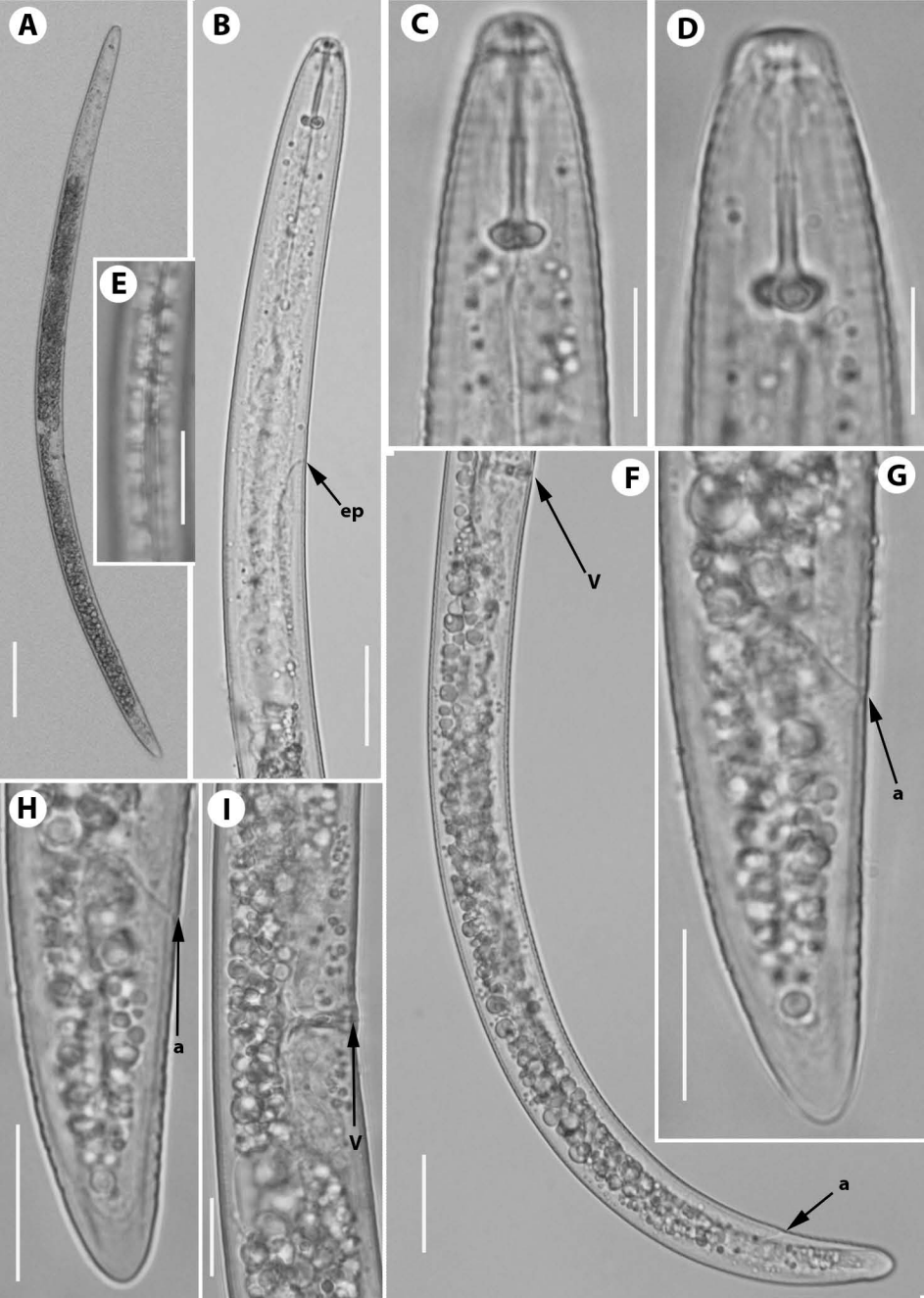


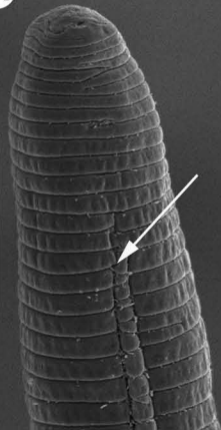
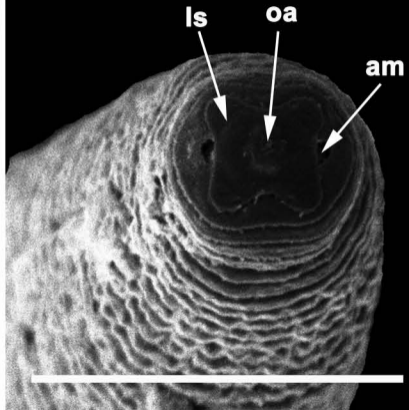
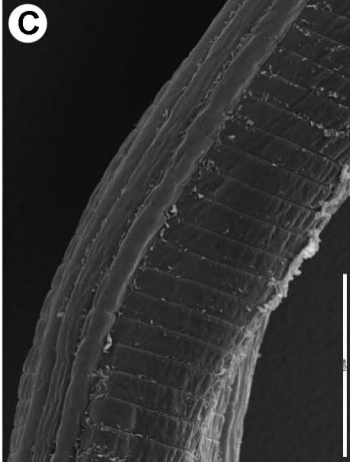
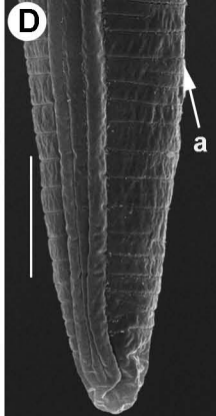


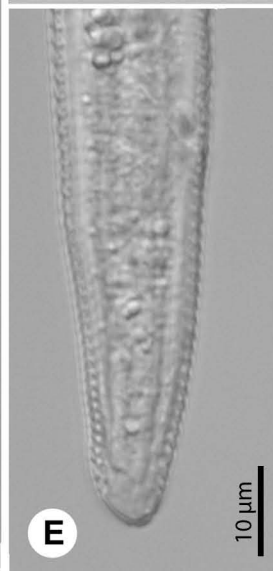
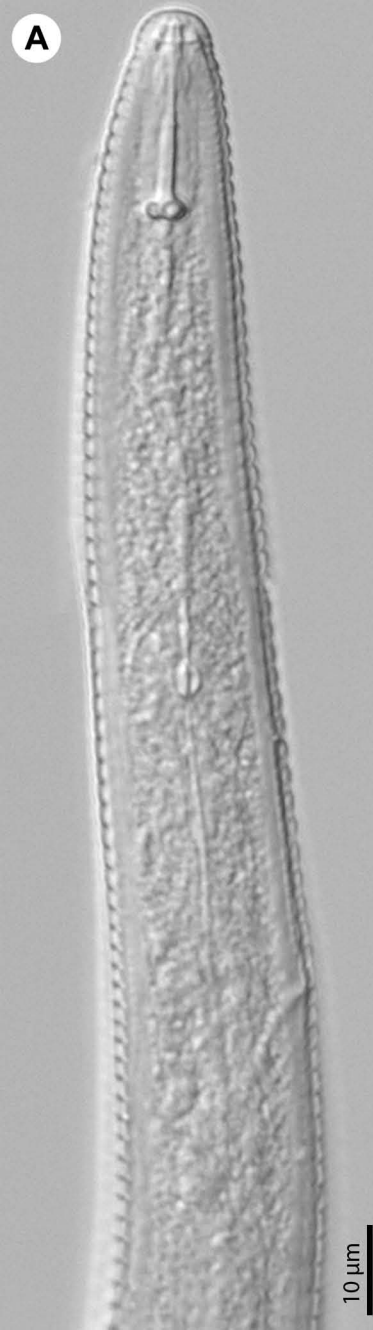


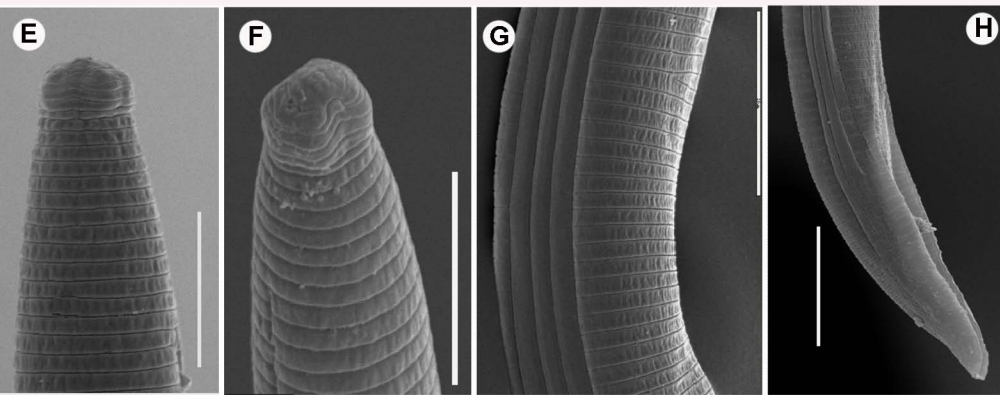
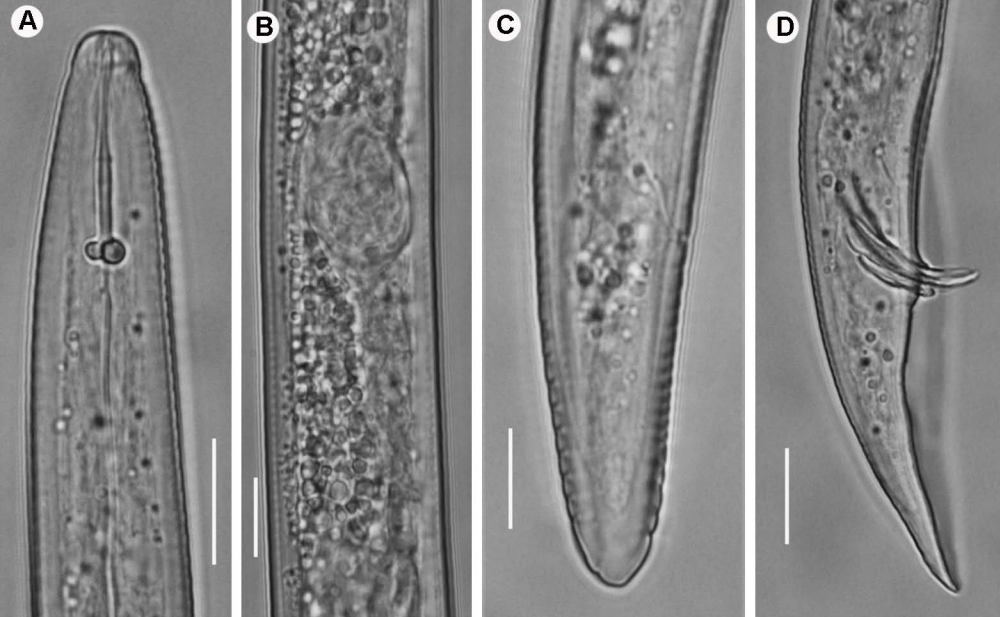


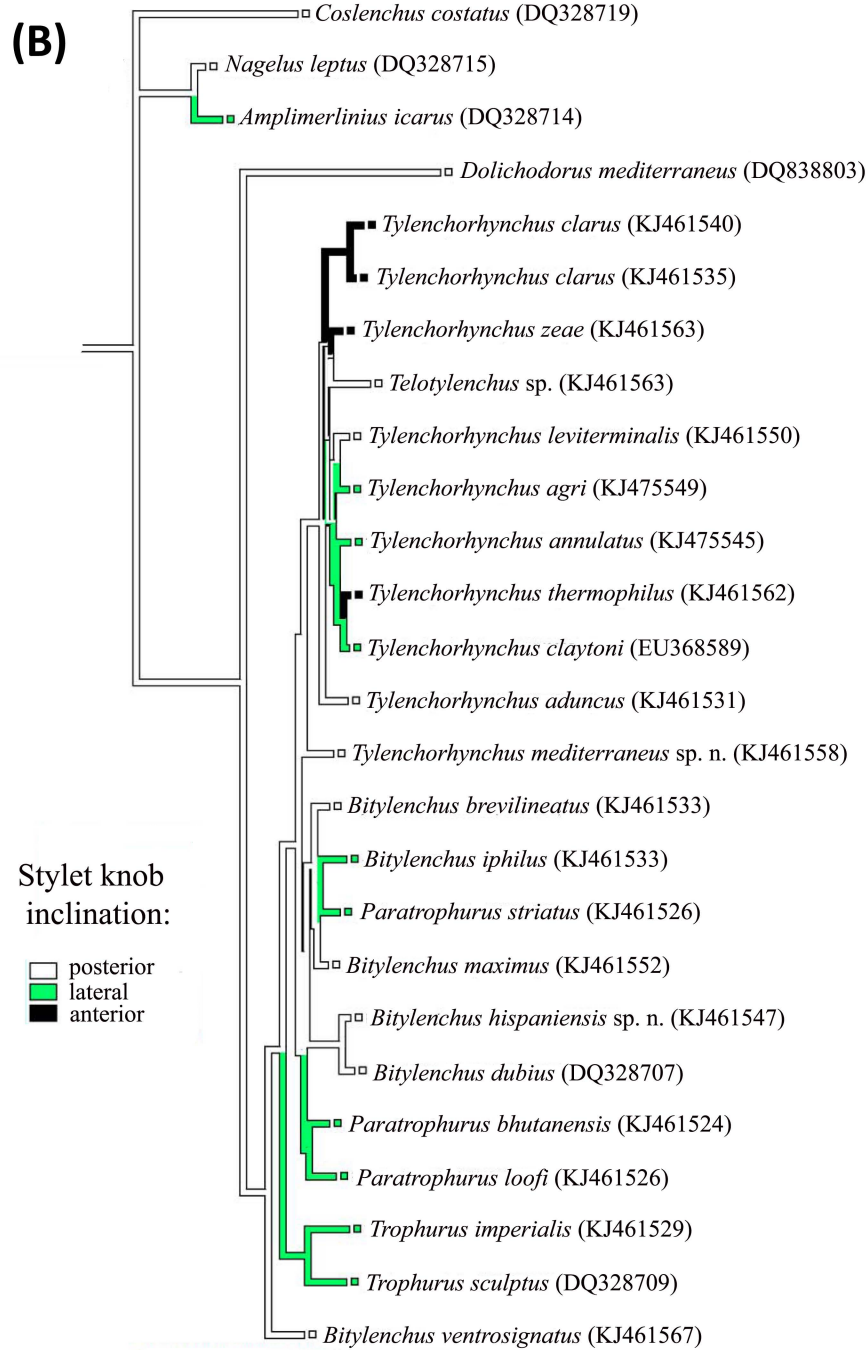
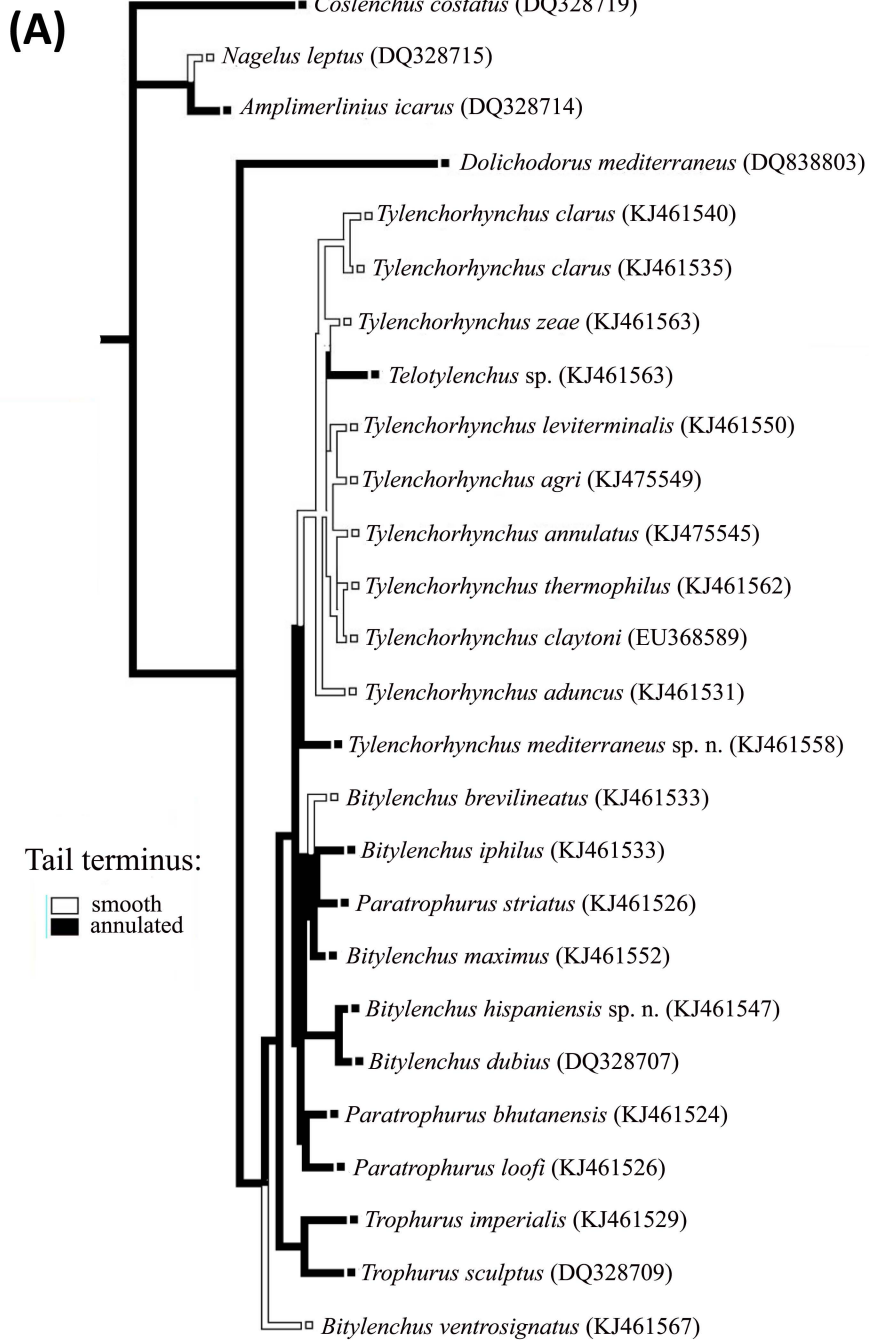




A**B****C****D****E**







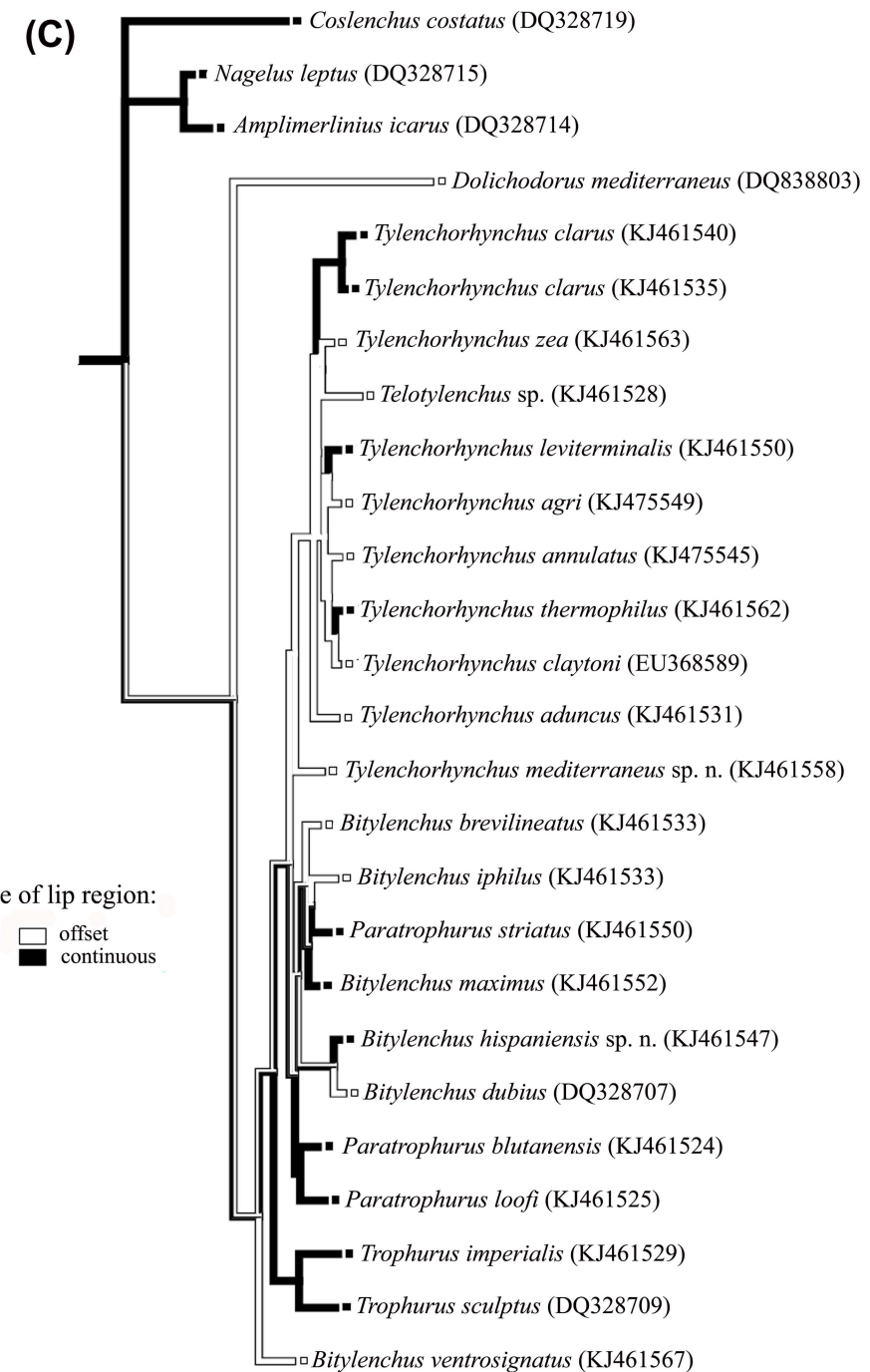
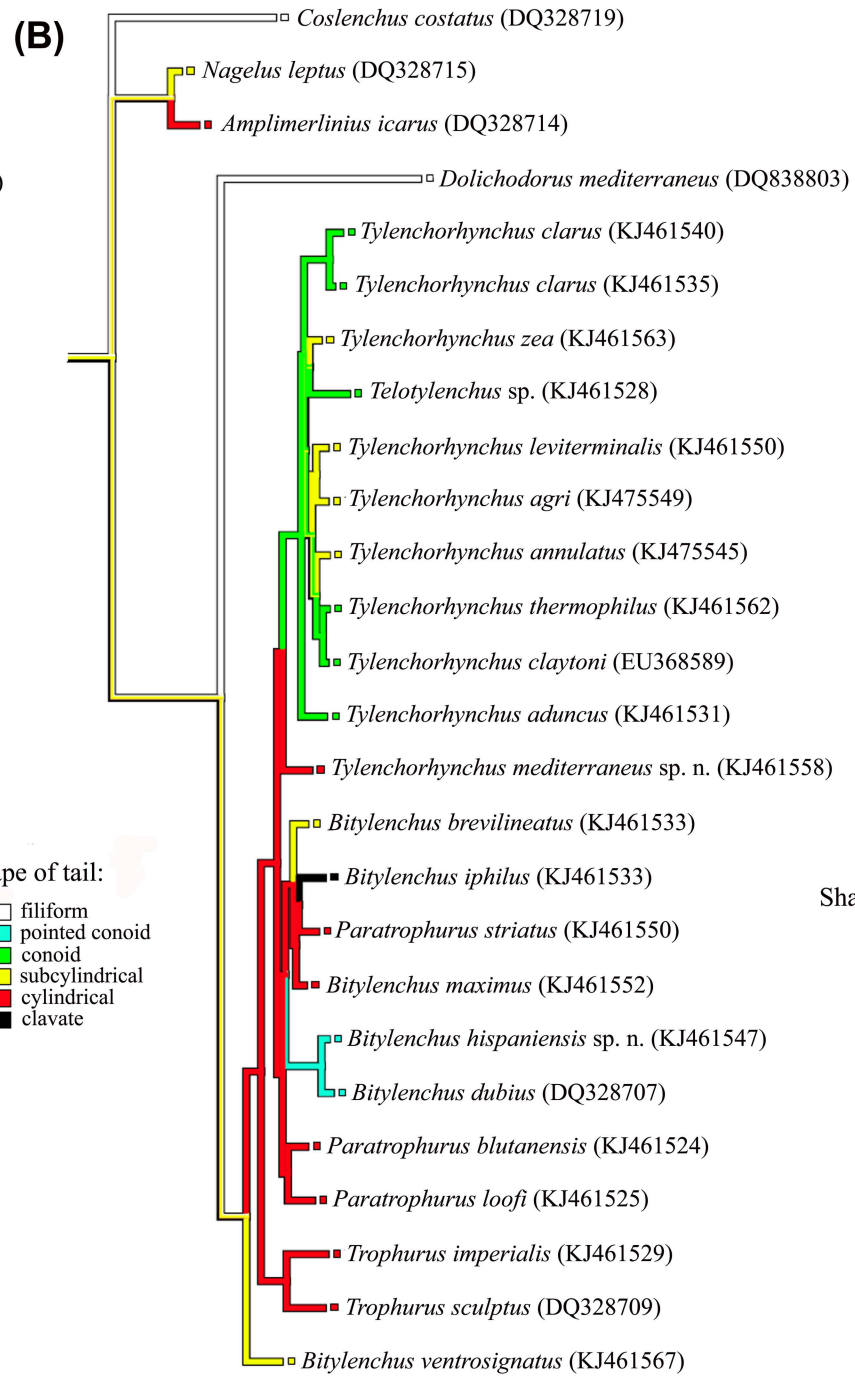
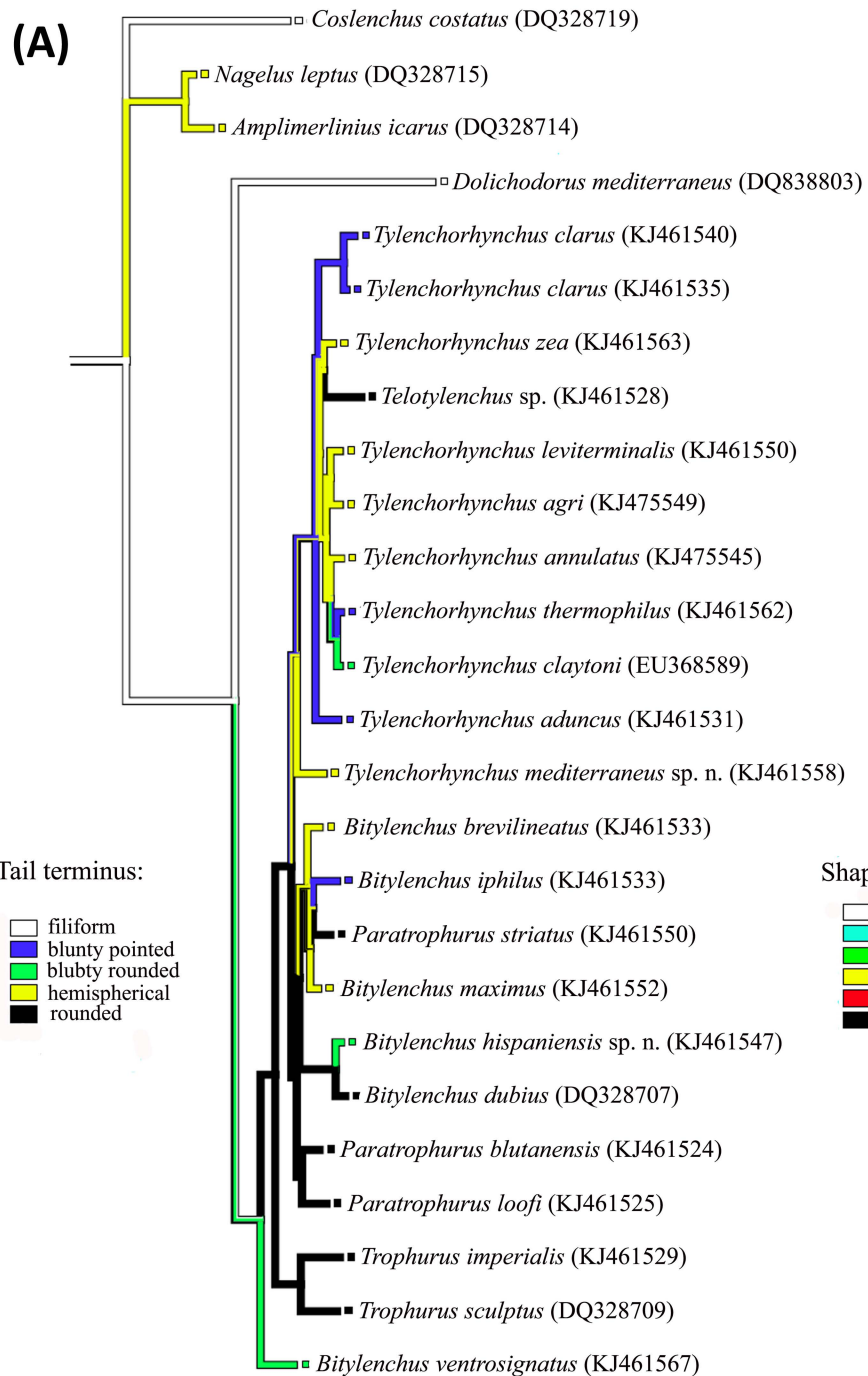


Table S1. Morphometrics of *Dolichodoros mediterraneus* Jiménez Guirado *et al.*, 2007, *Paratrophurus bhutanensis* (Ganguly, Lal & Procter, 2004) Andrásy, 2007, *Paratrophurus loofi* Arias, 1970, and *Paratrophurus striatus* Castillo *et al.* 1989 from southern Spain*.

Species	<i>Dolichodoros mediterraneus</i>		<i>Paratrophurus bhutanensis</i>		<i>Paratrophurus loofi</i>		<i>Paratrophurus striatus</i>	
	Tarifa, Cádiz, Spain wild olive		Niebla, Huelva, Spain fig tree		Coto Ríos, Jaén, Spain ash tree		Arroyo Frío, Jaén, Spain black poplar	
Locality host-plant	Females	Males	Females	Males	Females	Males	Females	Males
n	12	12	10	5	4	4	4	4
L ^a	1838 ± 210.1 (1483-2172)	1594 ± 157.4 (1428-1828)	523 ± 54.4 (465-595)	555 ± 56.3 (490-610)	668 ± 74.3 (569-749)	594 ± 44.2 (531-630)	777 ± 116.6 (623-878)	723 ± 62.3 (645-794)
a	60.7 ± 6.6 (48.3-68.0)	63.6 ± 8.9 (54.2-83.1)	28.8 ± 1.6 (26.4-31.0)	33.1 ± 1.7 (31.6-35.9)	32.7 ± 1.3 (30.9-33.7)	33.9 ± 1.8 (31.2-35.2)	30.7 ± 1.0 (29.7-31.7)	30.8 ± 0.9 (29.7-31.8)
b	7.8 ± 0.8 (6.4-9.4)	7.5 ± 0.7 (6.5-8.9)	4.9 ± 0.4 (4.2-5.4)	5.3 ± 0.4 (4.9-6.0)	5.4 ± 0.4 (4.9-5.8)	5.8 ± 0.2 (5.6-6.0)	5.7 ± 0.2 (5.5-5.9)	5.4 ± 0.1 (5.3-5.5)
c	20.6 ± 2.6 (16.6-25.9)	49.8 ± 7.9 (39.8-63.0)	17.1 ± 1.3 (15.5-19.4)	17.2 ± 1.8 (15.2-19.7)	17.2 ± 1.1 (15.8-18.4)	13.5 ± 0.6 (12.9-14.0)	20.8 ± 0.5 (20.4-21.5)	18.7 ± 0.3 (18.2-19.0)
c'	3.4 ± 0.6 (2.7-4.5)	1.5 ± 0.2 (1.1-1.9)	2.4 ± 0.1 (2.2-2.7)	2.9 ± 0.2 (2.7-3.3)	2.5 ± 0.1 (2.3-2.5)	2.9 ± 0.2 (2.7-3.2)	2.1 ± 0.1 (1.9-2.2)	2.1 ± 0.1 (2.0-2.2)
V or T %	52.1 ± 1.5 (50.0-54.0)	43.9 ± 5.9 (35.0-54.0)	59.0 ± 2.4 (55.0-62.0)	-	56.5 ± 1.3 (55.0-58.0)	-	54.0 ± 2.2 (51.0-56.0)	46.5 ± 11.6 (30.0-57.0)
Stylet length	94.6 ± 5.3 (83.0-99.0)	90.5 ± 4.4 (81.0-95.0)	20.1 ± 0.3 (20.0-21.0)	19.4 ± 0.9 (18.0-20.0)	20.8 ± 1.3 (19.0-22.0)	20.3 ± 1.0 (19.0-21.0)	21.0 ± 1.8 (19.0-23.0)	20.8 ± 1.5 (19.0-22.0)
Max. body width	39.9 ± 6.7 (25.0-45.0)	24.8 ± 3.8 (21.0-29.0)	18.3 ± 2.4 (15.0-22.5)	16.8 ± 1.8 (15.0-19.0)	20.5 ± 2.6 (17.0-23.)	17.5 ± 0.6 (17.0-18.0)	25.3 ± 3.1 (21.0-28.0)	23.5 ± 1.9 (21.0-25.0)
Pharynx length	237.8 ± 29.8 (198.0-298.0)	212.0 ± 17.7 (176.0-230.0)	106.2 ± 4.9 (95.0-110.0)	104.0 ± 4.3 (100.0-110.0)	124.3 ± 19.5 (100.0-141.0)	103.0 ± 10.2 (89.0-112.0)	135.8 ± 16.7 (112.0-148.0)	133.8 ± 11.0 (120.0-144.0)
Ant. end to Excretory pore	153.9 ± 27.3 (124.0-215.0)	136.1 ± 16.4 (104.0-146.0)	87.1 ± 5.8 (75.0-95.0)	91.8 ± 4.7 (85.0-97.0)	91.5 ± 11.9 (78.0-105.0)	88.3 ± 11.1 (78.0-101.0)	108.5 ± 15.8 (86.0-121.0)	102.8 ± 10.2 (89.0-111.0)
Anal body width	26.5 ± 3.5 (21.0-32.0)	21.5 ± 2.3 (18.0-24.0)	12.8 ± 1.3 (11.0-15.0)	11.2 ± 0.8 (10.0-12.0)	15.8 ± 2.2 (13.0-18.0)	15.0 ± 0.8 (14.0-16.0)	17.8 ± 2.2 (15.0-20.0)	18.3 ± 1.0 (17.0-19.0)
Tail length	89.9 ± 11.6 (77.0-122.0)	32.9 ± 2.4 (29.0-36.0)	30.6 ± 2.1 (27.0-34.0)	32.4 ± 1.9 (30.0-35.0)	39.0 ± 5.9 (31.0-44.0)	44.3 ± 4.8 (38.0-48.0)	37.5 ± 6.5 (29.0-43.0)	38.8 ± 3.4 (34.0-42.0)
Tail annuli	-	-	18.9 ± 2.8 (16-25)	-	30.3 ± 4.6 (24-35)	-	30.8 ± 5.0 (24-35)	-
Spicule	-	37.9 ± 2.4 (34.0-42.0)	-	21.5 ± 1.0 (20.0-22.5)	-	21.8 ± 1.7 (20.0-24.0)	-	31.5 ± 1.9 (29.0-33.0)
Gubernaculum	-	18.8 ± 1.6 (17.0-22.0)	-	10.2 ± 0.8 (9.0-11.0)	-	10.8 ± 1.0 (10.0-12.0)	-	15.0 ± 0.8 (14.0-16.0)

* Measurements are in µm and in the form: mean ± standard deviation (range)

^a L, body length, a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100

Table S2. Morphometrics of *Trophurus imperialis* Loof, 1956, *Tylenchorhynchus aduncus* de Guiran, 1967, and *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971 from southern Spain and California, USA*.

Species	<i>Trophurus imperialis</i>		<i>Tylenchorhynchus aduncus</i>		<i>Tylenchorhynchus aduncus</i>		<i>Tylenchorhynchus annulatus</i>
	Locality host-plant		Locality host-plant		Locality host-plant		Locality host-plant
	Lebrija, Sevilla, Spain grapevine		Conil de la Frontera, Cádiz, Spain sharp rush		Sanlúcar de Barrameda, Cádiz, Spain wild olive		Napa County, California, USA undetermined grasses
	Females	Males	Females	Males	Females	Males	Females
n	5	3	7	2	3	2	2
L ^a	1075 ± 91.9 (965-1198)	949 ± 61.7 (880-998)	720 ± 41.6 (690-810)	975 ± 14.1 (665-685)	789 ± 82.9 (695-850)	775 ± 41.1 (730-810)	640 ± 28.3 (620-660)
a	40.4 ± 2.4 (37.3-42.8)	36.0 ± 1.9 (33.8-37.0)	26.8 ± 1.7 (25.1-28.9)	28.5 ± 1.5 (27.4-29.6)	28.1 ± 2.3 (25.7-30.4)	28.7 ± 0.6 (28.1-28.9)	30.8 ± 0.2 (30.7-31.0)
b	9.7 ± 0.4 (9.4-10.3)	9.1 ± 0.2 (8.9-9.2)	5.6 ± 0.1 (5.4-5.8)	5.6 ± 0.1 (5.5-5.7)	6.0 ± 0.3 (5.8-6.0)	7.7 ± 1.5 (6.0-9.0)	4.5 ± 0.3 (4.3-4.7)
c	28.8 ± 2.3 (26.1-31.5)	19.8 ± 0.9 (18.7-20.4)	16.8 ± 1.0 (15.6-17.9)	13.6 ± 0.3 (13.4-13.9)	18.9 ± 1.4 (17.4-19.3)	15.4 ± 0.4 (14.9-15.6)	15.3 ± 0.3 (15.1-15.5)
c'	1.6 ± 0.04 (1.5-1.7)	2.1 ± 0.1 (2.0-2.1)	2.1 ± 0.2 (1.9-2.3)	-	2.1 ± 0.06 (2.1-2.2)	2.4 ± 0.2 (2.2-2.6)	3.2 ± 0.1 (3.1-3.3)
V or T %	55.4 ± 2.1 (53.0-58.0)	30.3 ± 8.0 (22.0-38.0)	58.6 ± 1.1 (57.0-59.5)	-	59.0 ± 1.0 (58.0-60.0)	29.0 ± 6.2 (22.0-34.0)	53.7 ± 1.8 (52.0-55.0)
Stylet length	18.9 ± 1.2 (17.0-20.0)	18.0 ± 1.0 (17.0-19.0)	19.9 ± 0.7 (19.0-21.0)	19.5 ± 0.7 (19.0-20.0)	18.5 ± 1.5 (17.0-20.0)	18.0 ± 1.0 (17.0-19.0)	20.5 ± 0.7 (20.0-21.0)
Max. body width	26.6 ± 1.1 (25.0-28.0)	26.3 ± 0.6 (26.0-27.0)	26.8 ± 1.4 (25.0-28.0)	23.8 ± 1.8 (22.5-25.0)	28.0 ± 1.0 (27.0-29.0)	27.0 ± 1.0 (26.0-28.0)	20.8 ± 1.1 (20.0-21.5)
Pharynx length	115.2 ± 8.6 (102.0-126.0)	112.3 ± 3.5 (109.0-116.0)	145.0 ± 8.7 (135.0-150.0)	120.0 ± 0.0 (120.0-120.0)	130.7 ± 11.0 (120.0-142.0)	135.7 ± 6.0 (130.0-142.0)	142.5 ± 3.5 (140.0-145.0)
Ant. end to Excretory pore	110.8 ± 6.1 (102.0-116.0)	104.0 ± 5.0 (99.0-109.0)	127.5 ± 6.9 (120.0-140.0)	-	126.3 ± 2.1 (124.0-128.0)	112.3 ± 3.5 (109.0-116.0)	99.0 ± 1.4 (98.0-100.0)
Anal body width	23.4 ± 0.5 (23.0-24.0)	22.7 ± 1.2 (22.0-24.0)	20.6 ± 0.8 (20.0-22.0)	-	19.5 ± 0.5 (19.0-20.0)	21.0 ± 1.0 (20.0-22.0)	13.0 ± 0.7 (12.5-13.5)
Tail length	37.3 ± 1.0 (36.0-38.0)	48.0 ± 1.0 (47.0-49.0)	43.1 ± 4.1 (40.0-51.0)	49.5 ± 2.1 (48.0-51.0)	41.7 ± 2.1 (40.0-44.0)	50.3 ± 1.5 (49.0-52.0)	17.5 ± 0.7 (17.0-18.0)
Tail annuli	-	-	23.7 ± 2.8 (20-27)	-	20.0 ± 2.0 (18-22)	-	-
Spicule	-	22.3 ± 2.5 (20.0-25.0)	-	26.3 ± 1.8 (25.0-27.5)	-	22.3 ± 2.5 (20.0-25.0)	-
Gubernaculum	-	7.7 ± 1.5 (6.0-9.0)	-	9.5 ± 0.7 (9.0-10.0)	-	7.7 ± 1.5 (6.0-9.0)	-

* Measurements are in µm and in the form: mean ± standard deviation (range)

^a L, body length, a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100

Table S3. Morphometrics of *Bitylenchus brevilineatus* (Williams, 1960) Jairajpuri, 1982, and several populations of *Tylenchorhynchus clarus* Allen, 1955 from southern Spain*.

Species	<i>Bitylenchus brevilineatus</i>		<i>Tylenchorhynchus clarus</i>		<i>Tylenchorhynchus clarus</i>		<i>Tylenchorhynchus clarus</i>	
	Villalba del Alcor, Huelva, Spain eucalyptus		Niebla, Huelva, Spain fig tree		Chucena, Huelva, Spain cork oak		Sanlúcar de Barrameda, Cádiz, Spain tamarisk	
Locality host-plant	Females	Male	Females	Males	Females	Male	Females	Males
n	6	1	10	5	6	1	4	3
L ^a	681 ± 105 (520-782)	720	517 ± 39.1 (467-578)	501 ± 24.8 (470-537)	530 ± 28.3 (480-548)	470	513 ± 31.2 (475-550)	500 ± 10.0 (490-510)
a	33.1 ± 2.7 (28.6-35.5)	35.4	32.7 ± 2.6 (28.2-36.7)	34.8 ± 1.0 (33.6-35.8)	32.0 ± 1.3 (30.7-34.1)	29.4	34.3 ± 2.7 (31.6-36.7)	34.9 ± 0.9 (34.0-35.7)
b	5.3 ± 0.3 (5.0-5.8)	5.4	4.6 ± 0.3 (4.2-5.2)	4.6 ± 0.3 (4.3-5.0)	4.9 ± 0.4 (4.5-5.4)	4.4	4.6 ± 0.4 (4.2-5.2)	4.6 ± 0.4 (4.3-5.0)
c	16.9 ± 2.2 (14.4-16.6)	23.2	18.7 ± 2.0 (16.7-22.2)	18.1 ± 1.9 (15.7-20.7)	19.7 ± 0.8 (18.9-21.0)	17.7	17.7 ± 0.7 (16.8-18.3)	18.1 ± 1.1 (17.0-19.0)
c'	3.1 ± 0.6 (2.6-3.9)	-	2.7 ± 0.2 (2.3-3.1)	3.1 ± 0.2 (2.9-3.3)	2.5 ± 0.1 (2.3-2.6)	1.9	2.9 ± 0.2 (2.7-3.1)	3.0 ± 0.1 (2.9-3.0)
V %	54.7 ± 2.2 (52.9-57.7)	-	58.5 ± 1.6 (56.4-61.0)	-	59.6 ± 1.6 (57.5-62.0)	-	58.2 ± 2.0 (56.4-61.0)	-
Stylet length	16.0 ± 1.4 (15.0-17.0)	15.5	16.7 ± 0.5 (16.0-17.0)	15.9 ± 0.7 (15.0-17.0)	16.7 ± 0.9 (15.0-17.5)	15	16.8 ± 0.5 (16.0-17.0)	15.8 ± 1.0 (15.0-17.0)
Max. body width	21.5 ± 1.0 (20.0-22.5)	17.5	15.9 ± 1.7 (13.0-19.0)	14.4 ± 0.5 (14.0-15.0)	16.6 ± 1.1 (15.0-17.5)	16	15.0 ± 1.4 (13.0-16.0)	14.3 ± 0.6 (14.0-15.0)
Pharynx length	125.4 ± 17.0 (100.0-145.0)	133.5	113.9 ± 7.6 (100.0-122.0)	108.4 ± 8.5 (100.0-120.0)	109.2 ± 9.0 (101.0-122.0)	107	112.5 ± 9.6 (100.0-120.0)	110.0 ± 10.0 (100.0-120.0)
Ant. end to Excretory pore	-	-	83.5 ± 4.1 (80.0-90.0)	79.0 ± 8.2 (70.0-90.0)	-	-	83.3 ± 2.9 (80.0-85.0)	80.0 ± 8.7 (75.0-90.0)
Anal body width	14.3 ± 1.5 (13.0-16.0)	-	10.4 ± 0.7 (10.0-12.0)	9.3 ± 0.5 (9.0-10.0)	10.9 ± 0.5 (10.0-11.5)	10	10.1 ± 0.3 (10.0-10.5)	9.3 ± 0.6 (10.0-9.0)
Tail length	44.7 ± 8.3 (38.0-54.0)	31.0	27.9 ± 2.3 (25.0-32.0)	27.8 ± 2.0 (26.0-30.0)	26.9 ± 1.5 (25.0-29.0)	26.5	29.0 ± 1.8 (27.0-31.0)	27.7 ± 2.1 (26.0-30.0)
Tail annuli	36.3 ± 5.9 (32-43)	-	13.7 ± 1.9 (10-16)	-	10.3 ± 0.5 (10-11)	-	13.8 ± 1.0 (13-15)	-
Spicule	-	25.0	-	18.0 ± 1.2 (17.0-20.0)	-	17.5	-	17.7 ± 0.6 (17.0-18.0)
Gubernaculum	-	12.5	-	9.8 ± 0.4 (9.0-10.0)	-	10	-	9.7 ± 0.6 (9.0-10.0)

* Measurements are in µm and in the form: mean ± standard deviation (range)

^a L, body length, a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100

Table S4. Morphometrics of several populations of *Tylenchorhynchus clarus* Allen, 1955 from southern Spain and California, USA*.

Species Locality host-plant	<i>Tylenchorhynchus clarus</i> Niebla, Huelva, Spain wheat		<i>Tylenchorhynchus clarus</i> Jerez de la Frontera, Cádiz, Spain sunflower		<i>Tylenchorhynchus clarus</i> Merced County, California, USA alfalfa	<i>Tylenchorhynchus clarus</i> Calusa County, California, USA grapevine
	Females	Male	Females	Males	Females	Female
n	2	1	3	3	5	1
L ^a	509 ± 5.3 (505-513)	489	503 ± 31.4 (468-528)	501 ± 12.5 (489-514)	610 ± 52.3 (570-687)	575,0
a	32.9 ± 1.9 (31.6-34.3)	32.6	32.9 ± 0.7 (32.1-33.4)	35.0 ± 0.7 (34.3-35.7)	29.5 ± 3.0 (25.0-32.7)	32,9
b	4.4 ± 0.3 (4.2-4.6)	4.1	4.7 ± 0.5 (4.3-5.2)	4.7 ± 0.3 (4.4-5.1)	4.4 ± 0.7 (3.8-5.0)	4,8
c	17.9 ± 0.2 (17.7-18.0)	16.3	17.6 ± 0.2 (17.3-17.7)	18.2 ± 1.6 (16.3-19.2)	13.3 ± 1.4 (12.1-15.7)	12,8
c'	2.8 ± 0.1 (2.8-2.9)	3.0	2.7 ± 0.1 (2.6-2.9)	3.0 ± 0.1 (2.9-3.0)	3.6 ± 0.6 (2.7-4.3)	3,8
V or T %	57.3 ± 1.3 (56.4-58.2)	34.0-	57.7 ± 1.5 (56.0-59.0)	-	54.4 ± 2.5 (50.0-56.0)	57.0
Stylet length	16.8 ± 0.4 (16.5-17.0)	17.0	16.8 ± 0.8 (16.0-17.5)	15.5 ± 0.5 (15.0-16.0)	13.5 ± 0.0 (13.5-13.5)	14,5
Max. body width	15.5 ± 0.7 (15.0-16.0)	15.0	15.3 ± 1.2 (14.0-16.0)	14.3 ± 0.6 (14.0-15.0)	19.8 ± 1.3 (18.0-21.0)	17,5
Pharynx length	116.3 ± 5.3 (112.5-120.0)	120.0	108.7 ± 9.5 (99.0-118.0)	107.0 ± 7.0 (99.0-112.0)	136.7 ± 16.5 (115.0-155.0)	119,0
Ant. end to Excretory pore	84.2 ± 1.2 (83.0-85.0)	94.0	83.0 ± 3.6 (79.0-86.0)	76.0 ± 1.7 (75.0-78.0)	-	85,0
Anal body width	10.3 ± 0.4 (10.0-10.5)	10.0	10.5 ± 0.9 (10.0-11.5)	9.3 ± 0.6 (9.0-10.0)	12.7 ± 1.2 (11.0-14.0)	12,0
Tail length	28.5 ± 0.7 (28.0-29.0)	30.0	28.7 ± 1.5 (27.0-30.0)	27.7 ± 2.1 (26.0-30.0)	49.0 ± 2.5 (47.0-52.5)	45,0
Tail annuli	13.5 ± 0.7 (13-14)	-	13.7 ± 0.6 (13-14)	-	-	52,0
Spicule	-	18.0	-	17.0 ± 1.0 (16.0-18.0)	-	-
Gubernaculum	-	10.0	-	10.0 ± 1.0 (9.0-11.0)	-	-

* Measurements are in µm and in the form: mean ± standard deviation (range)

^a L, body length, a, body length/maximum body width; b, body length/pharyngeal length; c, body length/tail length; c', tail length/body width at anus; V, (distance from anterior end to vulva/body length) × 100

Table S5. Morphometrics of *Tylenchorhynchus claytoni* Steiner, 1937, *Bitylenchus iphilus* Minagawa, 1995, and *Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982, from California, USA and southern Spain^a.

Species Locality host-plant	<i>Tylenchorhynchus claytoni</i>		<i>Bitylenchus iphilus</i>		<i>Tylenchorhynchus leviterminalis</i>
	Mississippi, USA unknown plant		Villamanrique de la Condesa, Huelva, Spain cork oak		Wilton, Connecticut, USA avocado
	Females	Male	Females	Males	Female
n	4	4	3	3	1
L ^b	613±49.2 (550-670)	620±29 (583-648)	778.3 ± 33.3 (740-800)	796.7 ± 7.6 (790-805)	470.0
a	25.9±1.2 (24.4-27.5)	28.7±1.3 (26.8-30.4)	26.5 ± 2.6 (24.7-28.4)	31.9 ± 1.2 (30.6-32.9)	27.6
b	4.6±0.1 (4.4-4.7)	4.9±0.1 (4.8-5.1)	5.2 ± 0.5 (4.6-5.7)	5.8 ± 0.1 (5.6-5.9)	4.1
c	18.6±1.4 (17.6-20.6)	14.7±0.8 (13.7-15.5)	14.0 ± 0.9 (13.0-14.5)	17.3 ± 0.5 (16.8-17.7)	12.5
c'	1.9±0.5 (1.6-2.5)	2.5±0.1 (2.4-2.7)	2.9 ± 0.3 (2.7-3.2)	-	3.1
V %	56.2±1.3 (55-58)	-	53.5 ± 1.1 (52.2-54.3)	-	51.4
Stylet length	23.1±0.8 (21.8-23.8)	21.6± 1.6 (20-23.8)	17.0 ± 0.9 (16.0-17.5)	16.3 ± 1.2 (15.0-17.0)	20.0
Max. body width	23.8± 3.1 (20.0-27.5)	21.8±0.8 (21.5-22.5)	29.0 ± 1.4 (28.0-30.0)	25.0 ± 1.0 (24.0-26.0)	17.0
Pharynx length	133± 8.5 (125-145)	125.8± 3.3 (123-130)	151.7 ± 10.4 (140.0-160.0)	138.3 ± 2.9 (135.0-140.0)	115.0
Ant. end to Excretory pore	103.3± 6.5 (97.5-112.5)	102 ±4.8 (95-105)	-	-	82.0
Anal body width	17.3± 1.1 (16.3-18.8)	16.4± 0.4 (16.3-16.9)	19.3 ± 2.1 (17.0-21.0)	-	12.0
Tail length	33.1±1.6 (31.3-35.0)	42.3±2.3 (40-45)	55.7 ± 1.2 (55.0-57.0)	46.0 ± 1.7 (45.0-48.0)	37.5
Tail annuli	14± 3.6 (10-17)	-	39.0 ± 4.0 (35-43)	-	-
Spicule	-	24.4±0.6 (23.8-25)	-	25.0 ± 0.0 (25.0-25.0)	-
Gubernaculum	-	11.1±0.8 (10-11.9)	-	15.0 ± 0.0 (15.0-15.0)	-

^a Measurements are in µm and in the form: mean ± standard deviation (range)

^b Abbreviations as defined in Siddiqi (2000)

Table S6. Morphometrics of *Bitylenchus maximus* (Allen, 1955) Siddiqi (1986), *Bitylenchus ventrosignatus* (Tobar Jiménez, 1969) Jairajpuri, 1982 from southern Spain^a.

Species Locality host-plant	<i>Bitylenchus maximus</i>		<i>Bitylenchus maximus</i>		<i>Bitylenchus ventrosignatus</i>	
	Andújar, Jaén, Spain wild olive		Niebla, Huelva, Spain wild olive		Bollullos par del Condado, Huelva, Spain grapevine	
	Females	Males	Females	Males	Females	Males
n	10	2	4	2	7	3
L ^b	1306 ± 69.9 (1210-1430)	1256 ± 19.8 (1242-1270)	1310 ± 208.9 (1031-1510)	979 ± 61.5 (935-1022)	650 ± 43.1 (610-722)	520 ± 37.5 (478-550)
a	44.6 ± 2.3 (40.8-49.0)	41.9 ± 2.5 (40.1-43.7)	45.8 ± 5.6 (39.7-50.7)	36.9 ± 1.3 (36.0-37.9)	31.3 ± 2.1 (28.9-35.1)	31.5 ± 1.4 (29.9-32.4)
b	8.1 ± 0.4 (7.4-8.8)	7.6 ± 0.7 (7.6-7.7)	8.5 ± 1.5 (7.3-10.6)	6.1 ± 0.3 (5.9-6.3)	5.6 ± 0.3 (5.3-6.0)	4.5 ± 0.3 (4.3-4.8)
c	22.4 ± 1.0 (21.4-23.9)	25.1 ± 1.0 (24.4-25.9)	23.5 ± 2.0 (21.5-25.4)	19.6 ± 0.1 (19.5-19.7)	14.2 ± 1.0 (12.6-15.0)	15.3 ± 0.8 (14.5-16.2)
c'	2.8 ± 0.2 (2.5-3.0)	3.7	2.4 ± 0.2 (2.2-2.5)	2.4 ± 0.0 (2.4-2.4)	3.0 ± 0.3 (2.6-3.3)	2.7 ± 0.1 (2.6-2.7)
V or T %	52.0 ± 1.1 (50.0-55.0)	-	53.3 ± 2.8 (50.0-56.0)	35.0 ± 5.7 (31.0-39.0)	54.6 ± 1.3 (52.6-55.7)	37.7 ± 6.1 (31.0-43.0)
Stylet length	21.5 ± 0.7 (20.5-22.5)	21.2 ± 1.8 (20.0-22.5)	21.8 ± 1.3 (20.0-23.0)	21.5 ± 0.7 (21.0-22.0)	13.6 ± 0.8 (13.0-15.0)	13.3 ± 0.6 (13.0-14.0)
Max. body width	29.3 ± 2.8 (26.0-35.0)	30.0 ± 1.4 (29.0-31.0)	28.5 ± 1.9 (26.0-30.0)	26.5 ± 0.7 (26.0-27.0)	20.8 ± 1.8 (18.0-22.5)	16.5 ± 0.5 (16.0-17.0)
Pharynx length	160.2 ± 4.6 (155.0-170.0)	163.5 ± 7.6 (162.0-165.0)	154.5 ± 19.9 (134.0-174.0)	160.5 ± 2.1 (159.0-162.0)	116.0 ± 6.0 (105.0-124.0)	115.7 ± 4.7 (112.0-121.0)
Ant. end to Excretory pore	136.1 ± 6.4 (125.0-145.0)	135 ± 7.0 (130.0-140.0)	134.5 ± 15.3 (116.0-148.0)	128.0 ± 5.7 (124.0-132.0)	99.0 ± 2.8 (97.0-101.0)	86.7 ± 4.0 (82.0-89.0)
Anal body width	20.7 ± 0.9 (19.0-22.0)	14	23.3 ± 3.1 (19.0-26.0)	21.0 ± 1.4 (20.0-22.0)	15.0 ± 1.4 (12.5-16.0)	12.7 ± 0.6 (12.0-13.0)
Tail length	58.3 ± 4.3 (55.0-67.0)	50.0 ± 2.8 (48.0-52.0)	55.5 ± 5.3 (48.0-60.0)	50.0 ± 2.8 (48.0-52.0)	46.3 ± 3.9 (41.0-50.0)	34.0 ± 1.0 (33.0-35.0)
Tail annuli	37.0 ± 3.0 (32-41)	-	32.5 ± 3.1 (28-35)	-	37.6 ± 4.7 (32-42)	-
Spicule	-	35.2 ± 3.1 (33.0-37.5)	-	32.0 ± 1.4 (31.0-33.0)	-	21.4 ± 1.1 (20.0-22.5)
Gubernaculum	-	15.0 ± 0.0 (15.0-15.0)	-	15.5 ± 0.7 (15.0-16.0)	-	11.5 ± 1.0 (10.0-12.0)

^a Measurements are in µm and in the form: mean ± standard deviation (range)

^b Abbreviations as defined in Siddiqi (2000)

Table S7. Morphometrics of several populations of *Tylenchorhynchus zeae* Sethi & Swarup, 1968 from southern Spain^a.

Species Locality host-plant	<i>Tylenchorhynchus zeae</i> Alcalá la Real, Jaén, Spain olive		<i>Tylenchorhynchus zeae</i> Manzanilla, Huelva, Spain grapevine		<i>Tylenchorhynchus zeae</i> Montilla, Córdoba, Spain olive		<i>Tylenchorhynchus zeae</i> Santaella, Córdoba, Spain olive	
	Females	Males	Females	Males	Females	Males	Female	Male
n	7	2	7	3	3	2	1	1
L ^b	638 ± 30.7 (595-677)	652 ± 24.7 (635-670)	593 ± 34.3 (540-617)	583 ± 29.4 (559-616)	530 ± 31.9 (509-530)	509 ± 9.2 (503-516)	559	506
a	28.1 ± 1.4 (27.0-29.8)	29.0 ± 0.2 (28.9-29.1)	26.6 ± 1.9 (23.5-28.0)	25.7 ± 1.3 (24.3-26.8)	24.5 ± 1.2 (23.4-24.5)	23.7 ± 0.4 (23.5-24.0)	24,3	22,0
b	5.4 ± 0.6 (4.8-5.7)	5.6 ± 0.2 (5.5-5.8)	5.1 ± 0.3 (4.8-5.2)	4.9 ± 0.4 (4.6-5.2)	4.8 ± 0.4 (4.5-4.8)	4.8 ± 0.2 (4.7-4.9)	5,1	4,6
c	21.2 ± 2.6 (16.7-24.0)	20.2 ± 3.0 (18.1-22.3)	19.9 ± 1.8 (17.6-22.0)	19.9 ± 1.3 (18.6-21.3)	18.9 ± 0.7 (18.2-19.1)	17.6 ± 0.5 (17.2-18.0)	18,6	16,9
c'	2.2 ± 0.1 (2.1-2.5)	2.4 ± 0.4 (2.1-2.7)	2.2 ± 0.2 (2.1-2.5)	2.2 ± 0.1 (2.1-2.3)	2.2 ± 0.1 (2.2-2.3)	2.2 ± 0.2 (2.1-2.3)	2,2	2,2
V %	57.1 ± 1.4 (54.0-58.1)	-	57.1 ± 1.4 (54.0-58.0)	-	57.3 ± 1.5 (57.0-59.0)	-	57,2	-
Stylet length	16.9 ± 0.5 (16.0-17.5)	16.0 ± 0.0 (16.0-16.0)	16.9 ± 0.5 (16.0-17.0)	16.5 ± 0.7 (16.0-17.0)	16.7 ± 0.8 (16.0-17.0)	16.3 ± 0.4 (16.0-16.5)	17	16
Max. body width	22.7 ± 0.4 (22.0-23.0)	22.5 ± 0.7 (22.0-23.0)	22.4 ± 0.7 (21.0-23.0)	22.7 ± 0.6 (22.0-23.0)	21.7 ± 0.6 (21.0-22.0)	21.5 ± 0.7 (21.0-22.0)	23	23
Pharynx length	120.3 ± 4.0 (118.0-125.0)	115.5 ± 0.7 (115.0-116.0)	114.1 ± 6.4 (107.0-125.0)	117.3 ± 8.0 (109.0-125.0)	112.0 ± 3.0 (109.0-115.0)	106.0 ± 5.7 (102.0-110.0)	109	109
Ant. end to Excretory pore	-	-	83.1 ± 4.1 (79.0-90.0)	80.7 ± 1.5 (79.0-82.0)	77.7 ± 2.5 (75.0-80.0)	78.0 ± 2.8 (76.0-80.0)	79	79
Anal body width	13.6 ± 1.5 (11.0-15.0)	13.5 ± 0.7 (13.0-14.0)	13.5 ± 1.0 (12.0-14.0)	13.5 ± 1.5 (12.0-15.0)	13.0 ± 1.0 (12.0-13.0)	13.3 ± 0.4 (13.0-13.5.0)	13,5	13,5
Tail length	30.6 ± 4.1 (25.0-37.0)	32.5 ± 3.5 (30.0-35.0)	30.1 ± 3.0 (27.0-35.0)	29.3 ± 2.1 (27.0-31.0)	28.0 ± 1.0 (27.0-29.0)	29.0 ± 1.4 (28.0-30.0)	30	30
Tail annuli	16.3 ± 2.3 (13-20)	-	17.0 ± 2.8 (13-21)	-	18.3 ± 1.5 (17-20)	17.5 ± 2.1 (16-19)	16	16
Spicule	-	19.0 ± 2.8 (17.0-21.0)	-	18.0 ± 1.0 (17.0-19.0)	-	16.8 ± 0.4 (16.5-17.0)	-	17
Gubernaculum	-	10.5 ± 0.7 (10.0-11.0)	-	11.0 ± 1.0 (10.0-12.0)	-	10.3 ± 0.4 (10.0-10.5)	-	10

^a Measurements are in µm and in the form: mean ± standard deviation (range)

^b Abbreviations as defined in Siddiqi (2000)

Table S8. Morphometrics of *Tylenchorhynchus agri* Ferris, 1963, *Tylenchorhynchus thermophilus* Golden, Baldwin & Mundo-Ocampo, 1995 and *Telotylenchus* sp. from USA^a.

Species Locality host-plant	<i>Tylenchorhynchus agri</i>	<i>Tylenchorhynchus thermophilus</i>		<i>Telotylenchus</i> sp.
	Lake Worth, FL, USA date palm Female	Indiana, USA unknown plant Female Male		Bank of June Lake, CA, USA grasses Female
n	1	7	3	1
L ^b	662	705	655	795
a	32.9	24.7	26.7	33.1
b	4.9	4.3	4.3	3.2
c	13.3	16.2	16.9	14.0
c'	3.1	2.6	2.1	3.6
V %	55.1	59.3	-	59.7
Stylet length	21.0	22.0	22.0	24.0
Max. body width	20.1	28.5	24.5	24.0
Pharynx length	135.0	163.0	152.0	248
Ant. end to Excretory pore	112.0	115.5	-	120
Anal body width	15.5	17.0	18.0	16.0
Tail length	50.0	43.5	39.0	57
Tail annuli	25	28	-	39
Spicule	-	-	24.5	-
Gubernaculum	-	-	12.5	-

^a Measurements are in μm and in the form: mean \pm standard deviation (range)

^b Abbreviations as defined in Siddiqi (2000)