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Prevalence, polyphasic identification, and molecular phylogeny of dagger and needle nematodes infesting vineyards in southern Spain

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Abstract The occurrence and geographic distribution of longidorid nematode species inhabiting the rhizosphere of grapevine plants in southern Spain were investigated. Nematode surveys were conducted on 77 vineyards during the spring seasons of 2006, 2007 and 2008 in the main Andalusian grapevine-growing areas, including the provinces of Cádiz, Córdoba, and Huelva. Morphological and morphometrical studies identified two *Longidorus* and nine *Xiphinema* species, *viz*.: *Longidorus alvegus*, *L. magnus, Xiphinema adenohystherum*, *X. hispidum*, *X. index*, *X. italiae*, *X. lupini*, *X. nuragicum*, *X. pachtaicum*, *X. rivesi*, and *X. turcicum*. Overall, frequencies of infestation were, in decreasing order: *X. pachtaicum* 90.8%, *X. index* 30.3%, *X. italiae* 13.2%, *L. magnus* 11.8%, *X. hispidum* 7.9%, *X. lupini* 3.9%, *L. alvegus* and *X. rivesi* 2.6%, and *X. adenohystherum, X. nuragicum* and *X. turcicum* 1.3%. *Xiphinema hispidum*, *X. lupini*, *L. alvegus* and *L. magnus* were compared with nematode type specimens and are reported for the first time in Spain. Furthermore, the male of *L. alvegus* is described for the first time in the literature. Molecular characterisation of these species using D2–D3 expansion regions of 28S rRNA, 18S rRNA and ITS1-rRNA was carried out and maximum likelihood and Bayesian inference analysis were used to reconstruct phylogenetic relationships among these species and with other longidorids. The monophily of the genera *Xiphinema* and *Longidorus* was accepted and the genera *Paralongidorus* and *Xiphidorus* were rejected by the Shimodaira-Hasegawa test based on tree topologies.

Keywords Bayesian inference · Grapevine · Longidorids · *Longidorus* · Maximum likelihood · rDNA · New geographic record · *Xiphinema*

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Introduction

Grapevine (*Vitis vinifera* L.) production for wine-making and table grapes is the second, after olive, most extensive agricultural system for commercial fruit crops in southern Spain (MARM 2008). Viticulture in southern Spain is concentrated mainly in Cádiz, Córdoba, and Huelva provinces. These provinces comprise three geographically-separated climatic zones that include the three major wine production areas corresponding to the officially recognized "wine denomination of origin (D.O.) zones": Condado de Huelva D.O. (Huelva province), Montilla-Moriles D.O. (Córdoba province), and Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda D.O. (Cádiz province). Plant-parasitic nematodes have often been found in soils where grapevines have shown reduced growth vigour in the majority of viticultural areas around the world (Brown et al. 1993). Among them, dagger

(*Xiphinema* spp.) and needle (*Longidorus* spp. and *Paralongidorus* spp.) nematodes cause damage to grapevines by their direct feeding on root cells and transmission of nepoviruses (Taylor and Brown 1997).

The accurate and timely identification of longidorid nematodes infecting vineyards is a prerequisite for designing effective management strategies. This is particularly relevant for grapevines because of the diversity of longidorid nematode species in several grapevine growing areas in the world (Taylor and Brown 1997). Reliable nematode identification allows distinction between virus vector and non-virus vector nematodes and assists in the exclusion of species under quarantine or regulatory strategies. The occurrence and geographical distribution of longidorids in the Iberian Peninsula was reviewed by Peña Santiago et al. (2003) who reported that 71 species (19 for *Longidorus*, three for *Paralongidorus* and 49 for *Xiphinema*) were present there. Recent preliminary studies on plant-parasitic nematodes infesting grapevines in southern Spain revealed the presence of *Longidorus macrosoma* Hooper, 1961, *Xiphinema index* Thorne & Allen, 1950, *X. italiae* Mey1, 1953, *X. pachtaicum* (Tulaganov 1938) Kirjanova, 1951, and *X. turcicum* Luc & Dalmasso, 1963 (Téliz et al. 2007). However, no detailed morphological, morphometrical or molecular characterizations were carried out in that study on those nematode species. Current availability of molecular techniques may help to provide tools for differentiating species and can significantly improve and facilitate the routine identification of those nematodes. Thus, use of ribosomal DNA (rDNA) sequences from partial 18S, ITS regions, and the D2 and D3 expansion segments of the 28S and mitochondrial DNA (mtDNA) such as protein-coding mitochondrial gene, cytochrome c oxidase 1 subunit (*COI*), have proved an useful diagnostic tool for the characterisation and establishment of phylogenetic relationships within plant-parasitic nematodes such as Longidoridae, especially in cases where morphological characters may lead to ambiguous interpretation (Wang et al. 2003; Ye et al. 2004; He et al. 2005; Barsi and De Luca 2008; Kumari et al. 2010). Polyphasic identification, based in an integrative strategy of combining molecular techniques with morphology and morphometry measurements for diagnosis of each species, are vital for a correct nematode identification. For this reason, in this research we have conducted an extensive nematode survey on the three main wine denomination of origin zones of Andalusian grapevine production, with the following objectives: *a*) to determine the prevalence of dagger and needle nematodes infesting vineyards in southern Spain; *b*) to characterise morphologically and morphometrically longidorid species and to compare them with previous records; *c*) to molecularly characterise the sampled longidorid populations using the D2–D3 expansion segments of 28S rRNA, ITS1 and partial 18S rRNA gene sequences; and *d*) to study the phylogenetic relationships of the identified longidorids with other longidorid species.

Material and methods

Nematode population sampling

Nematode surveys were conducted from 2006 to 2008 during the spring season on 77 commercial vineyards which area representative of the main grapevine-growing regions in the three main D.O. zones of Andalusia, *viz.* Montilla-Moriles D.O. (Córdoba province), Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda D.O. (Cádiz province), and Condado de Huelva D.O. (Huelva province). Samples were collected with a shovel from the upper 50 cm of soil of four to five plants arbitrarily chosen in each vineyard. Nematodes were extracted from 500 cm^3 of soil by centrifugal flotation (Coolen 1979) and a modification of Cobb's decanting and sieving (Flegg 1967) methods. In some cases, additional soil samples were collected afterwards from the same vineyards for completing the necessary specimens for morphological and/or molecular identification.

Prevalence of infestation and population density of plant-parasitic nematodes were determined. Prevalence of infestation was calculated as the percentage of samples in which a nematode species was diagnosed with respect to total number of samples. Nematode population density in soil was assessed for each sample and calculated as the average of the soil count.

Nematode morphological identification

Specimens for light microscopy were killed by gentle heat, fixed in a solution of 4% formaldehyde +1% propionic acid and processed to pure glycerine using

Seinhorst's method (1966). Specimens were examined using a Zeiss III compound microscope with Nomarski differential interference contrast at powers up to 1,000× magnification. Measurements were done using a drawing tube attached to a light microscope and, unless otherwise indicated in text. All measurements were done in relation to the nematode body and expressed in micrometres (μm). All other abbreviations used are as defined in Jairajpuri and Ahmad (1992). Morphometric data were processed using Statistix 9.0 (NH Analytical Software, Roseville, MN, USA). Morphometric values and ratios of eight nematode populations of *X. pachtaicum* were subjected to analysis of variance (ANOVA) and means were compared using Tukey honestly significant difference test (HSD) at $P=0.05$. In addition, a comparative morphological and morphometrical study on type specimens of some species were conducted with specimens kindly provided by Mrs. A. Agostinelli, from the nematode collection at the Istituto per la Protezione delle Piante, Sede di Bari, Consiglio Nazionale delle Ricerche, (C.N.R.), Bari, Italy (*viz. Longidorus alvegus*, *L. magnus*, *Xiphinema lupini*, *X. rivesi*) and Dr Z.A. Handoo, from the USDA Nematode Collection, Beltsville, MD, USA (viz. *Xiphinema hispidum* slides T-4435p, T-4436p).

Nematode molecular identification

For molecular analyses, two live nematodes from each sample were temporary mounted in a drop of 1M NaCl containing glass beads and 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 rDNA was amplified using the D2A (5′-ACAAGTACCGTGAGG GAAAGTTG-3′) and D3B (5′-TCGGAAGGAAC CAGCTACTA-3′) primers (Castillo et al. 2003; He et al. 2005; Palomares-Rius et al. 2008). The ITS1 region was amplified using forward primer 18S (5′TTGAT TACGTCCCTGCCCTTT-3′) and reverse primer rDNA1 (5′-ACGAGCCGAGTGATCCACCG-3′) as described in Wang et al. (2003). Finally, the 18S rDNA gene was amplified using the SSU_F_07 (5′-AAAGATTAAGCCATGCATG-3′) and SSU_R_81 (5′-TGATCCWKCYGCAGGTTCAC-3′) primers (http://www.nematodes.org/barcoding/sourhope/nemoprimers.html).

All PCR assays were carried out with the following conditions: one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing temperature of 57°C for 45 s, 72°C for 3 min and finally one cycle of 72°C for 10 min. PCR products were purified after amplification with a gel extraction kit (Geneclean turbo; Q-BIOgene SA, Illkirch Cedex, France) or Exo-SAP-IT® (Affymetrix, Inc., Cleveland, Ohio, USA) for PCR product clean-up, PCR purified products were quantified using a Nanodrop spectrophotometer (Nano-drop Technologies, Wilmington, DE, USA) and used for direct DNA sequencing. For the 18S gene sequencing the internal primer SSU_R_13R (5′-GGGCATCA CAGACCTGTTA-3′) (http://www.nematodes.org/barcoding/sourhope/nemoprimers.html) was also used. DNA fragments from two independent PCR amplifications from two different nematodes were sequenced in both directions using the same primers with a terminator cycle sequencing ready reaction kit (BigDye; Perkin-Elmer Applied Biosystems, Warrington, UK) according to the manufacturer's instructions The resulting products were purified and run on a DNA multicapillary sequencer (Model 3130XL genetic analyzer; Applied Biosystems, Foster City, CA, USA) at the STABVIDA sequencing facilities (Monte da Caparica, Portugal). Sequences were deposited in the National Center of Biotechnology Information (GenBank) database under accession numbers listed in Table 1.

Phylogenetic analyses

D2–D3 expansion segments of 28S, ITS1 and partial 18S rDNA sequences of different genera from Longidoridae family from GenBank were used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen according to previous published data (He et al. 2005). The newly obtained and published sequences for each gene were aligned using ClustalW (Thompson et al. 1994) with default parameters. Sequence alignments were manually edited using BioEdit (Hall 1999). Phylogenetic analysis of the sequence data sets were performed with maximum likelihood (ML) using a distant server (http://phylobench.vital-it.ch/raxml-bb/index.php) running the program RAxML-VI-HPC v. 4.0.0 (Randomized Accelerated Maximum Likelihood for High Performance Computing) (Stamatakis et al.

Table 1 Taxa sampled for longidorid species and sequences used in this study

Nematode species	Locality of sampling	GenBank accession		
		D2–D3	ITS1	partial 18S
X. adenohystherum Lamberti et al., 1992	Bollullos par del Condado (Huelva province)	GU725075[a]	GU725063[a]	GU725084[a]
X. hispidum Roca & Bravo, 1994	Montemayor (Córdoba province)	HM921366	–	HM921368
	Bollullos par del Condado (Huelva province)	HM921346	HM921367	–
X. index Thorne & Allen, 1950	Moriles, (Córdoba province)	HM921347	HM921334	–
	Jerez de la Frontera (Cádiz province)	HM921348	–	–
	Rociana (Huelva province)	HM921349	–	HM921342
X. italiae Meyl, 1953	Montemayor (Córdoba province)	HM921350	HM921341	–
	Bollullos par del Condado (Huelva province)	HM921351	HM921335	HM921343
X. lupini Roca & Pereira, 1993	Bollullos par del Condado (Huelva province)	HM921352	HM921336	–
X. nuragicum Lamberti et al., 1992	Puente Genil, (Córdoba province)	GU725067[a]	GU725056[a]	GU725079[a]
	Marchena, (Sevilla province)	GU725069[a]	GU725057[a]	GU725078[a]
X. pachtaicum (Tulaganov 1938) Kirjanova, 1951	Moriles, (Córdoba province)	HM921353	–	–
	Jerez de la Frontera (Cádiz province)	HM921354	HM921337	–
	Jerez de la Frontera (Cádiz province)	HM921355	–	–
	Bollullos par del Condado (Huelva province)	HM921356	–	–
X. rivesi Dalmasso, 1969	Moriles, (Córdoba province)	HM921357	–	–
	Bollullos par del Condado (Huelva province)	HM921358	HM921338	HM921344
X. turcicum Luc & Dalmasso, 1963	Moriles, (Córdoba province)	GU725077[a]	GU725064[a]	GU725086[a]
L. alvegus Roca, Pereira & Lamberti, 1989	Montemayor (Córdoba province)	HM921359	HM921339	–
	Bollullos par del Condado (Huelva province)	HM921360	–	–
L. magnus Lamberti, Bleve-Zacheo & Arias, 1982	Moriles, (Córdoba province)	HM921361	HM921340	HM921345
	Sanlucar de Barrameda (Cádiz province)	HM921362	–	–

[a] Sequenced in a previous study by Gutiérrez-Gutiérrez et al. (2010)

(–) Not obtained

2008) using 100 bootstraps. Bayesian inference (BI) was conducting using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The best fit model of DNA evolution was obtained using the program JModeltest ver. 0.1.1 (Posada 2008) with the Akaike Information Criterion (AIC). The Akaike-supported model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC were used in phylogenetic analyses. BI analysis under GTR + I + G model for each gene was initiated with a random starting tree and was run with four chains for 5.0×10^6 generations. For ITS1 sequences the model selected was TIM3 + G and 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees were visualised using TreeView program (Page 1996). In ML analysis the estimation of the support for each node was made using a bootstrap with 100 replicates. In order to test alternative tree topologies by constraining hypothetical monophyletic groups, we performed Shimodaira-Hasegawa test (SH-test) as implemented in PAUP (Swofford 2003) using RELL option. The tested

hypothetical monophyletic groups included the genera *Xiphidorus*, *Xiphinema* and *Paralongidorus*.

Results

Frequency of longidorids in vineyards in southern Spain

The overall prevalence of infestation by longidorids in vineyards in Southern Spain ranged from 90.8% (*X. pachtaicum*) to 1.3% (*X. adenohystherum*, *X. nuragicum*, *X. turcicum*). Nematode population densities ranged from 1 (*X. hispidum*) to 235 (*X. index*) nematodes per 500/cm^3 soil. *Xiphinema adenohystherum* was detected in a single vineyard in Bollullos par del Condado (Huelva province) out of the 77 sampled vineyards, with a population density of 4 females per 500/cm^3 soil. *Xiphinema hispidum* was detected in two localities of D.O. Montilla-Moriles (Montilla and Montemayor, Córdoba province) and one locality of Condado de Huelva (Bollullos par del Condado, Huelva province). Overall frequency of infestation by *X. hispidum* in this area was 7.9% (17.4% and 2.6% in D.O. Montilla-Moriles and D.O. Condado de Huelva, respectively), with a population density of 1 to 9 nematodes per 500/cm^3 soil. Biodiversity of longidorids inhabiting the grapevine rhizosphere in the three D.O. zones ranged from one species (53.2% of samples) or two species (35.1%) to five species (1.3%). The highest biodiversity was detected in D.O. Montilla-Moriles and D.O. Condado de Huelva, with nine and eight species, respectively; while only three species (*X. index*, *X. pachtaicum* and *L. magnus*) were found in D.O. Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda.

Xiphinema index was rather extensively distributed among the three studied D.O. zones, with an overall prevalence of infestation of 30.3% and a population density of 1 to 235 nematodes per 500/cm^3 soil. Frequency of infestation was higher (32.1 and 32.0%, respectively) in D.O. Jerez-Xérès-Sherry y Manzanilla-Sanlúcar de Barrameda and Condado de Huelva, followed by 26.1% in D.O. Montilla-Moriles.

Xiphinema italiae was distributed in a single locality at D.O. Montilla-Moriles (Montemayor) and three localities (Almonte, Bollullos par del Condado, and Rociana) at D.O. Condado de Huelva, with an overall prevalence of infestation of 13.2%, and a population density of 1 to 12 nematodes per 500/cm^3 soil. Prevalence of infestation was higher (32.0%) in D.O. Condado de Huelva and lower (8.7%) in D.O. Montilla-Moriles, while it was absent in D.O. Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda. *Xiphinema lupini* was detected in Bollullos par del Condado (Huelva province), in three out of the 77 vineyards sampled (3.9%), with a population density of 2 to 3 nematodes per 500/cm^3 soil. *Xiphinema nuragicum* was detected in a single vineyard at Puente Genil, Córdoba province out of the 77 sampled ones (1.3% overall prevalence of infestation), with a population density of 8 females per 500/cm^3 soil.

Xiphinema pachtaicum was also widespread among the three studied D.O. grapevine zones, with the highest overall prevalence of infestation (90.8%), and a population density of 1 to 84 nematodes per 500/cm^3 soil. Frequency of infestation was higher (96.4%) in D. O. Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda, followed by 91.3% in D.O. Montilla-Moriles, and 84.0% in D.O. Condado de Huelva.

Xiphinema rivesi was distributed in a locality of D. O. Montilla-Moriles (Moriles, Córdoba province) and another of D.O. Condado de Huelva (Bollullos par del Condado, Huelva province), with an overall prevalence of infestation of 2.6%, and a population density from 7 to 10 nematodes per 500/cm^3 soil.

Xiphinema turcicum was detected in a single vineyard at D.O. Montilla-Moriles (Moriles, Córdoba province) out of the 77 sampled ones (1.3%) with a population density of 3 nematodes per 500/cm^3 soil.

Longidorus alvegus was only detected from two vineyards in Bollullos par del Condado (Huelva province) and Montemayor (Córdoba province), with 3.6% and 4.3% prevalence of infestation, and 4 to 5 specimens per 500/cm^3 of soil population densities, respectively.

Finally, *L. magnus* was distributed to a certain extent in D.O. Montilla-Moriles (34.8% prevalence of infestation), including four localities (Aguilar de la Frontera, Montilla, Moriles and Monturque), and in Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda confined to Sanlúcar de Barrameda (3.6% frequency of infestation). The species showed an overall prevalence of infestation of 11.8% and a population density of 2 to 28 nematodes per 500/cm^3 soil.

Morphological and morphometrical study

The morphological and morphometrical data as well as molecular delineation of *Xiphinema adenohystherum*

Lamberti, Castillo, Gomez-Barcina & Agostinelli, 1992, *Xiphinema nuragicum* Lamberti, Castillo, Gomez-Barcina & Agostine1li, 1992, and *X. turcicum* were previously compared with original descriptions and paratype specimens within a study on the species complex for *Xiphinema pyrenaicum* Dalmasso, 1969, which re-established the validity of these species, based on comparative morphology and rDNA sequence analysis (Gutiérrez-Gutiérrez et al. 2010).

Xiphinema hispidum Roca & Bravo, 1994 (Fig. 1)

The Spanish populations of this species were characterised by a lip region rounded offset from the rest of the body by a wide depression, two equally developed female genital branches, vulva slightly anterior to mid-body, an indistinct pseudo-Z-organ with globular bodies (2–3 μm in diameter) close to the *pars dilatata uteri* (Fig. 1), spines in the uterus (4–5 μm), female tail short conical with subdigitate terminus, male tail digitate with precloacal pair of papillae preceded generally by three (rarely four) medioventral supplements (Fig. 1). The morphology and morphometrics of these populations agree closely with the original description of the species by Roca and Bravo (1994) and examined paratypes (Fig. 1, Table 2), except for a lower V ratio in females from Bollullos par del Condado (42.2 vs 47.5), a shorter odontostyle and odontophore length in males from Bollullos par del Condado (100.2, 52.8 vs 123.5, 66.0, respectively), and shorter spicule length in both populations (53.2, 42.8 vs 66.5, respectively). These differences are attributable to intraspecific variability, as confirmed by molecular analyses. The present record of *X. hispidum* is the first from Spain, and the second after the original description from Portugal (Roca and Bravo 1994). These data indicate that this species may be an Iberian endemism as suggested by Peña Santiago et al. (2003).

The alpha-numeric codes for *X. hispidum* to be applied to the polytomic identification key for *Xiphinema* species by Loof and Luc (1990) are: A 4, B 23, C 4, D 45 E 45, F 34, G 23, H 2, I 2, J 4, K 2, L 2.

Xiphinema index Thorne & Allen, 1950 (Fig. 2)

In all populations of *X. index* detected, except one in Moriles (Cordoba province), males were absent. It has been proven that *X. index* reproduction is of the meiotic parthenogenetic type (Dalmasso and Younes 1969). In fact, in the population from Moriles males were quite rare 0.5 to 1.0%, as already reported (Luc and Cohn 1982; Bileva and Chaleva 2009). Similarly, although a terminal digitate mucro (peg) on the female tail is one of the characteristics used in diagnosis of *X. index,* individuals without tail mucro (pegless) have been also observed (Barsi and Lamberti 2000; Tzortzakakis 2004). In this present survey, female pegless were also rare (1 to 2%) and concurrently detected with the amphimictic population in Moriles. The morphometrics of these populations agree closely with the species redescription conducted by Siddiqi (1974). An exception was the slightly higher c' ratio found in the Jerez de la Frontera population (Cádiz province). Female pegless showed also similar morphometrics to normal peg tail females, except for a higher c ratio and a lower c' ratio (Table 3), which were similar to those previously reported (Barsi and Lamberti 2000; Tzortzakakis 2004). Also, morphometrics of males were close to that of Luc and Cohn (1982) and Bileva and Chaleva (2009), including a precloacal pair of papillae preceded generally by four medioventral supplements (Fig. 2). The species has been widely reported in the Iberian Peninsula and Europe (Brown and Taylor 1987; Peña Santiago et al. 2003; Murillo-Navarro et al. 2005).

The alpha-numeric codes for *X. index* to be applied to the polytomic identification key for *Xiphinema* species by Loof and Luc (1990) are: A 4, B 4, C 56, D 56, E 34, F 3, G 23, H 2, I 3, J 4, K 3, L 1.

Xiphinema italiae Meyl, 1953 (Fig 2)

This species is morphologically recognisable by a lip region separated by conspicuous constriction, vulva anterior to mid-body, without Z-organ or spines, and female tail shape commonly elongate-conoid with slight dorsal and ventral constrictions towards the terminus, sometimes bluntly conoid, and even almost subdigitate (Fig. 2). Morphological and morphometrical traits of populations from Montemayor and Bollullos par del Condado (Table 3) agree very well with those of Cohn (1977). The species has been widely reported in the Iberian Peninsula and Europe (Brown and Taylor 1987; Peña Santiago et al. 2003).

The alpha-numeric codes for *X. italiae* to be applied to the polytomic identification key for

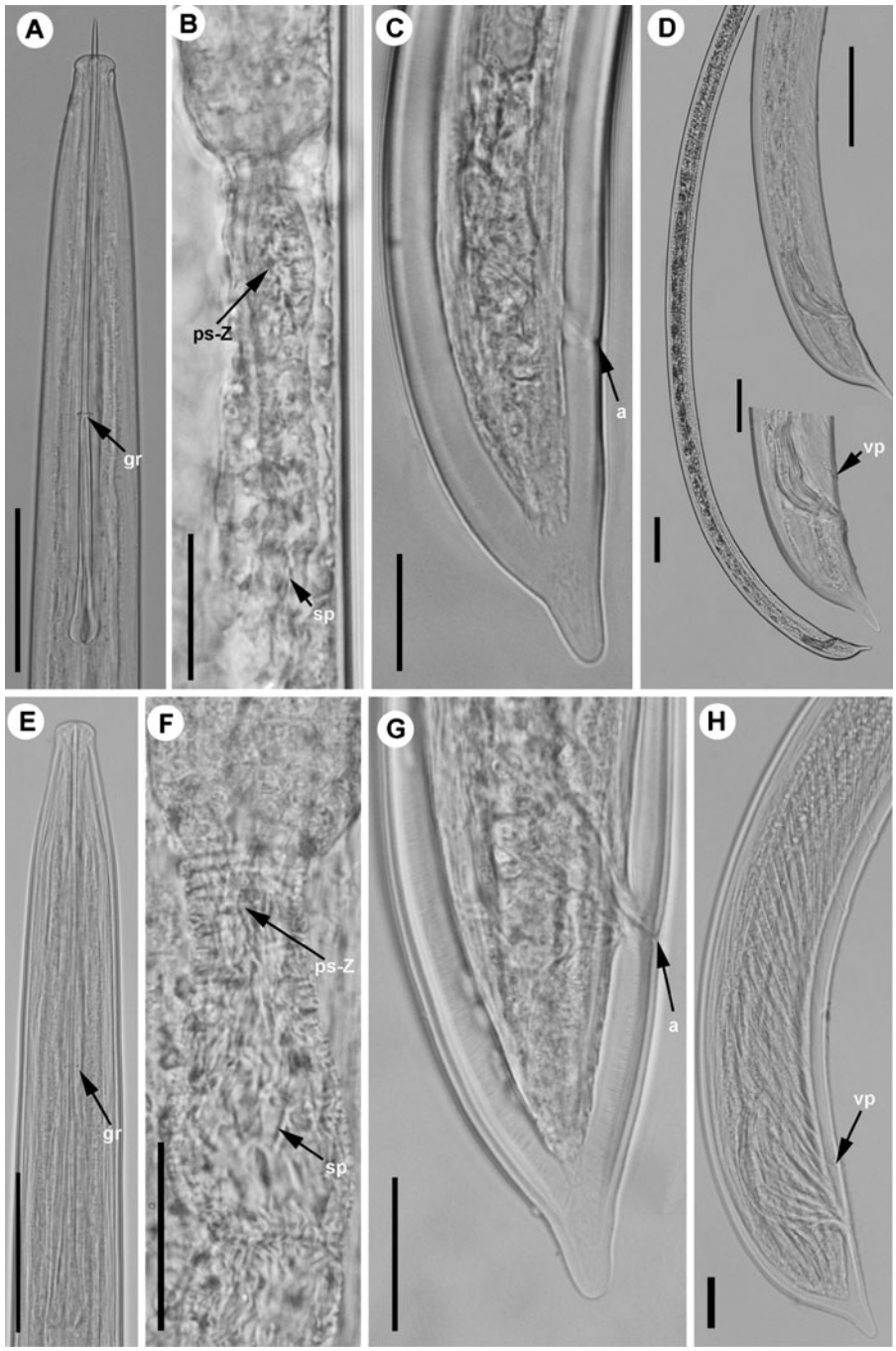

Fig. 1 Light micrographs of *Xiphinema hispidum* Roca & Bravo, 1994 infesting the grapevine rhizosphere in southern Spain (**a**–**d**), and paratypes from Portugal (**e**–**h**). **a**, **e** Female neck region; **b**, **f** Detail of the pseudo-Z-organ and spines; **c**, **g** Female tail region; **d**, **h** Male tail regions. Abbreviations: *a* anus; *gr* guiding ring; *sp* spines; *vp* ventromedian papillae. (*Scale bars*: **a**, **e** = 50 μm; **b**, **c**, **f**, **g**, **h** = 20 μm; **d** = 50 μm, 20 μm and 50 μm, respectively from *left* to *right*)

Table 2 Morphometrics of *Xiphinema hispidum* Roca & Bravo, 1994, *X. lupini* Roca & Pereira, 1993, and *X. rivesi* Dalmasso, 1969 infesting vineyards from southern Spain. All measurements in μm and in the format: mean ± s.d. (range)

Nematode species	*X. hispidum*				*X. lupini*		*X. rivesi*	
Locality	Montemayor (Córdoba province)		Bollullos par del Condado (Huelva province)		Bollullos par del Condado (Huelva province)		Moriles (Córdoba province)	Bollullos par del Condado (Huelva province)
Character	Females	Males	Females	Males	Females	Male	Females	Females
N	12	15	7	2	10	1	8	4
L	4151±249 (3870–4655)	4046±164 (3780–4380)	3800±147 (3530–4000)	3545±212 (3395–3695)	3766±205 (3450–4210)	3,450	1724±91 (1610–1890)	1920±92 (1835–2050)
a	97.9±8.4 (87.2–112.9)	104.0±6.8 (97.6–122.7)	100.5±7.1 (86.9–108.2)	107.5±4.8 (104.1–110.9)	99.6±7.6 (87.0–110.9)	98.6	44.1±3.2 (37.7–48.1)	49.0±4.3 (45.1–55.1)
B	8.9±0.4 (8.3–9.7)	8.8±0.6 (8.0–10.2)	10.9±0.9 (9.7–12.0)	9.4±0.1 (9.3–9.4)	8.9±0.7 (7.9–10.1)	8.5	6.8±0.5 (6.2–7.6)	7.2±0.3 (6.8–7.6)
C	76.9±4.3 (70.1–86.6)	87.4±5.8 (78.6–99.6)	84.6±8.5 (72.5–94.8)	90.4±7.5 (85.1–95.7)	74.7±7.7 (63.2–89.9)	95.8	52.8±3.2 (47.6–57.5)	53.3±2.8 (50.9–56.8)
c′	1.9±0.11 (1.8–2.2)	1.6±0.1 (1.4–1.8)	1.7±0.2 (1.4–2.1)	1.7±0.1 (1.7–1.8)	1.8±0.20 (1.5–2.1)	1.5	1.4±0.1 (1.2–1.5)	1.4±0.03 (1.38–1.44)
V or T	48.6±1.3 (45–50)	41.3±6.9 (33–47)	42.2±0.9 (41–44)	44.5±3.7 (42–47)	42.8±2.8 (38–46)	44	53.3±0.7 (52–54)	53.1±0.3 (53–54)
Odontostyle length	124.9±3.7 (116–131)	123.3±5.1 (112–131)	115.7±5.2 (107–121)	100.2±2.0 (99–101)	116.6±3.3 (112–121)	100	89.0±7.7 (72–96)	93.7±4.7 (88–98)
Odontophore length	67.5±3.1 (61–72)	66.0±1.5 (63–68)	62.6±4.0 (59–71)	52.8±0.8 (52–53)	64.2±2.5 (60–68)	57	49.1±2.8 (45–53)	46.7±3.7 (44–52)
Lip region width	12.4±1.0 (11.5–15.0)	12.4±0.9 (10.5–15.0)	11.7±0.3 (11.5–12.0)	10.3±0.5 (9.9–10.5)	12.4±0.5 (12.0–13.5)	11.5	10.1±0.5 (9.5–11.0)	9.8±0.8 (8.6–10.5)
Oral aperture–guiding ring	114.4±5.8 (102–122)	109.7±5.3 (100–116)	97.3±6.4 (88–104)	85.8±2.8 (84–88)	106.9±5.7 (97–116)	91	71.9±2.8 (67–76)	72.7±5.1 (66–77)
Tail length	53.6±3.0 (50–60)	46.4±3.2 (41–53)	45.4±5.6 (37–53)	39.3±0.9 (39–40)	50.7±4.1 (44–51.6)	36	32.8±3.0 (28–36)	36.0±0.8 (35–37)
Spicules	–	53.2±3.6 (47–60)	–	42.8±2.3 (41–44)	–	45	–	–
Lateral accessory piece	–	12.2±0.9 (11–14)	–	13.0±1.4 (12–14)	–	11	–	–

Abbreviations are defined in Jairajpuri and Ahmad (1992)

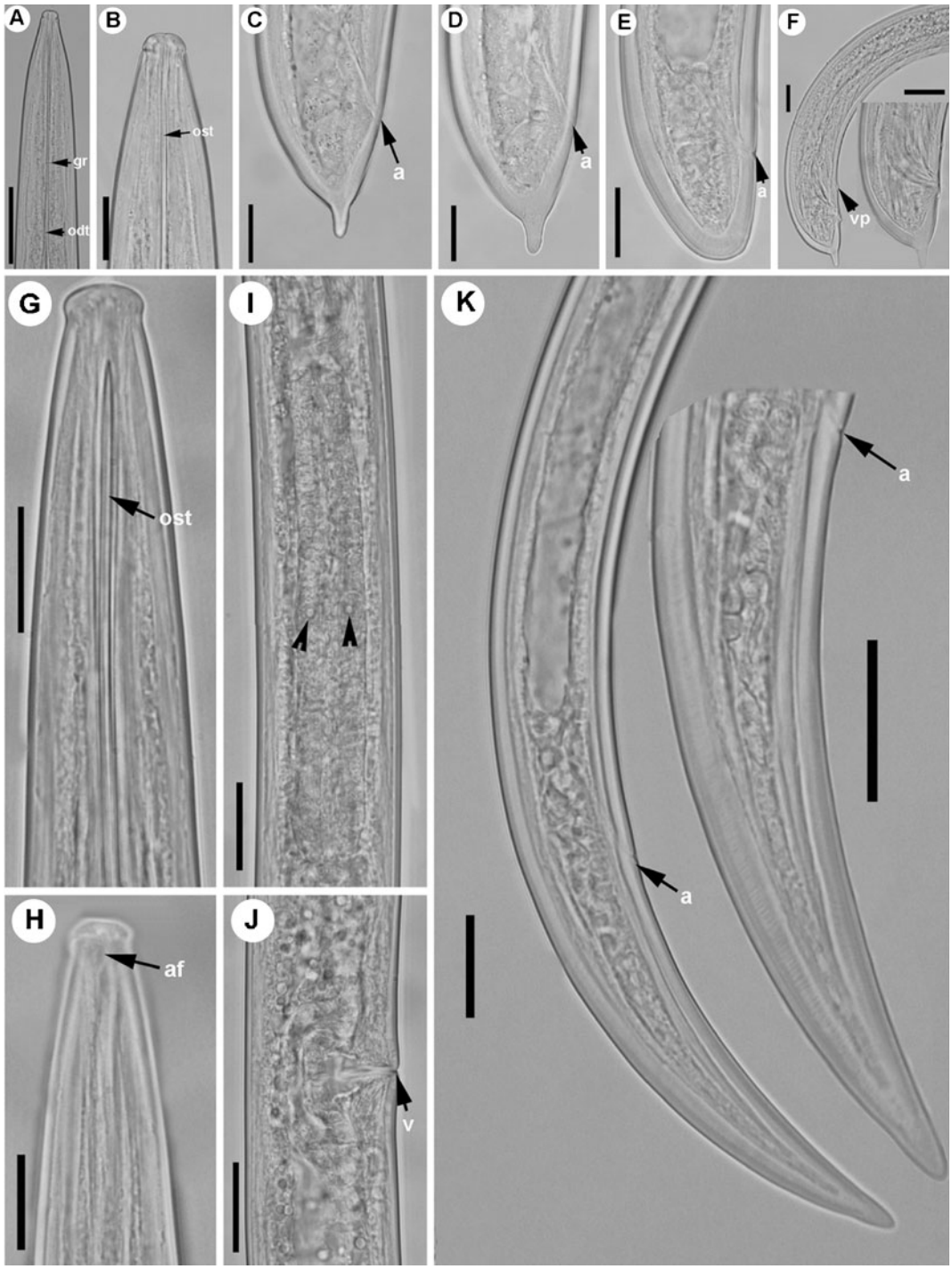

Fig. 2 Light micrographs of *Xiphinema index* Thorne & Allen, 1950 (**a**–**f**) and *Xiphinema italiae* Mey1, 1953 (**g**–**k**) infesting the grapevine rhizosphere in southern Spain. **a**, **g** Female neck regions; **b**, **h** Anterior regions; **c**, **d**, **k** Female tail regions; **e** Female tail pegless; **f** Male tails; **i** Detail of basal bulb showing ventrosublateral gland nuclei (*arrowed*); **j** Vulval region. Abbreviations: *a* anus; *gr* guiding ring; *odt* odontophore; *ost* odontostyle; *vp* ventromedian papillae. (*Scale bars*: **a** = 50 μm; **b**–**k** = 20 μm)

Table 3 Morphometrics of *Xiphinema index* Thorne & Allen, 1950 and *X. italiae* Meyl, 1953 infesting vineyards from southern Spain. All measurements in μm and in the format: mean ± s.d. (range)

Nematode species	*X. index*					*X. italiae*	
Locality	Moriles (Córdoba province)			Jerez de la Frontera (Cádiz province)	Rociana (Huelva province)	Montemayor (Córdoba province)	Bollullos par del Condado (Huelva province)
Character	Females	Females (peg less)	Males	Females	Females	Females	Females
N	12	10	2	8	7	8	5
L	3007±211 (2700–3380)	2930±187 (2740–3380)	3055±37 (3030–3080)	2970±172 (2735–3210)	2830±238 (2550–3140)	2819±113 (2580–2920)	2968±294 (2720–3295)
a	56.5±6.9 (45.7–68.6)	57.8±5.2 (48.1–62.7)	61.9±2.0 (60.5–63.4)	60.7±3.6 (55.8–65.1)	55.8±3.8 (49.7–60.4)	84.9±6.0 (77.6–96.3)	96.4±16.6 (83.5–115.2)
B	6.1±0.8 (5.1–7.3)	6.4±0.6 (5.8–7.0)	6.7±0.3 (6.5–7.0)	7.1±1.0 (5.9–9.1)	8.1±0.9 (6.4–8.9)	7.3±0.3 (6.8–7.7)	8.0±1.2 (6.8–9.1)
C	74.9±7.7 (64.5–89.0)	98.5±7.0 (87.3–108.9)	71.1±0.1 (71.0–71.2)	69.5±4.5 (65.2–77.1)	76.6±6.1 (70.2–85.0)	34.0±1.8 (32.6–38.1)	42.4±6.4 (38.3–49.8)
c′	1.1±0.12 (1.0–1.2)	0.8±0.1 (0.8–0.9)	0.98±0.02 (0.97–1.00)	1.2±0.1 (1.0–1.3)	1.0±0.1 (0.8–1.1)	3.7±0.3 (3.3–4.0)	3.5±0.2 (3.3–3.7)
V or T	41.4±1.6 (39–45)	42.2±0.6 (41–43)	56.7±4.9 (53–60)	39.9±1.3 (38–42)	42.0±1.5 (40–44)	45.4±0.9 (44–47)	47.7±1.5 (47–49)
Odontostyle length	131.3±5.9 (122–142)	129.5±3.0 (126–134)	133.9±3.9 (131–137)	125.5±6.5 (115–134)	127.9±3.1 (123–131)	98.1±1.8 (94–99)	96.4±4.6 (93–102)
Odontophore length	72.4±6.4 (60–83)	69.6±3.9 (63–74)	74.5±7.7 (69–80)	69.8±3.4 (66–77)	65.4±4.0 (60–72)	59.8±2.9 (56–65)	56.5±3.1 (54–60)
Lip region width	13.2±0.7 (12.0–15.0)	13.4±1.2 (12.5–16.5)	14.0±1.9 (13.0–15.0)	13.4±0.9 (12.0–14.5)	13.5±0.5 (12.5–14.0)	11.9±1.3 (10.5–14.0)	10.4±0.4 (10.0–11.0)
Oral aperture–guiding ring	113.0±12.8 (92–127)	117.0±15.3 (102–150)	120.1±14.2 (110–130)	113.2±7.6 (100–125)	103.9±7.9 (94–115)	92.3±1.9 (91–96)	85.7±1.8 (84–88)
Tail length	40.3±2.9 (35–45)	29.9±3.0 (27–36)	42.9±0.5 (42–44)	42.8±1.6 (40–45)	37.1±3.5 (30–40)	83.1±5.5 (74–88)	70.3±3.9 (66–74)
Spicules	–	–	62.6±5.0 (59–66)	–	–	–	–
Lateral accessory piece	–	–	12.5±0.7 (12–13)	–	–	–	–

Abbreviations are defined in Jairajpuri and Ahmad (1992)

Xiphinema species by Loof and Luc (1990) are: A 4, B 4, C 2, D 34, E 45, F 23, G 12 H 32, I 3, J 2, K 2, L 1.

Xiphinema lupini Roca & Pereira, 1993 (Fig 3)

The Spanish population of this species was characterised by a lip region rounded offset from the rest of the body by a conspicuous constriction, two equally developed female genital branches, vulva anterior to mid-body, a rudimentary pseudo-Z-organ with small granular bodies (2–3 μm in diameter) close to the *pars dilatata uteri* (Fig. 3), spines in the uterus (4–5 μm), female tail conoid with ventral profile almost straight, dorsal profile regularly curved and rounded tip (Fig. 3). The morphology and morphometrics of these populations agree closely with the original description of the species by Roca and Pereira (1993) and examined paratypes (Fig. 3, Table 2), except for lower a, c', and V ratios (99.6, 1.8, 42.8 vs 128.5, 2.4, 51.3, respectively), a shorter tail in females (50.7 vs 55.5) and lower L, a, c', tail length, and spicules length in males (3450, 98.6, 1.5, 36, 45 *vs* 4300, 139, 1.8, 45, 50, respectively) (Table 2). Nevertheless, these differences further expand and do not exceed the intraspecific variation.

The present record of *X. lupini* is the second from southern Spain, after Murillo-Navarro et al. (2005) in Guadiamar river basin (Sevilla province), and the third after original description from Portugal (Roca and Pereira 1993). These data suggest that this species may be an Iberian endemism as reported by Peña Santiago et al. (2003).

The alpha-numeric codes for *X. lupini* to be applied to the polytomic identification key for *Xiphinema* species by Loof and Luc (1990) are: A 4, B 23, C 34, D 34, E 456, F 45, G 2, H 3, I 2, J 34, K 2, L 12.

Xiphinema pachtaicum (Tulaganov 1938) Kirjanova, 1951 (Fig. 4)

The Spanish populations of this species were characterised by a body forming a close C to coiled spiral, lip region expanded and offset from the rest of the body, two equally developed female genital branches, vulva posterior to mid-body and female tail conoid with acute rounded tip (Fig. 4). Morphology and morphometrics of females closely agree with the description of Lamberti and Siddiqi (1977) and with other populations described in the literature (Lamberti et al. 1993; Fadaei et al. 2003; Kumari et al. 2005). Among 70 populations and more than 700 specimens examined in the present study, presence of males was extremely rare (0.1%), and only a single male was detected from Moriles, in agreement with data already reported (Vovlas and Avgelis 1988; Kumari et al. 2005). Male morphology concurs with the general description of the species (L = 1.72 mm; odontostyle = 84 μm; odontophore = 45 μm; spicules = 36 μm).

Analysis of variance of morphometrical characters and ratios of females from eight populations infesting grapevines in southern Spain showed that body length, lip region width, and tail length, as well as ratios c, c', and V were significantly ($P<0.05$) different among them in relation to sample studied (Table 4). However, these differences do not exceed the intraspecific variation showed for the thoroughly studied populations (Lamberti and Siddiqi 1977; Lamberti et al. 1993; Fadaei et al. 2003; Kumari et al. 2005). This species is widespread in the Iberian Peninsula and countries bordering the Mediterranean sea, central and Eastern Europe and southern Russia (Brown and Taylor 1987; Peña Santiago et al. 2003; Murillo-Navarro et al. 2005).

The alpha-numeric codes for *X. pachtaicum* to be applied to the polytomic identification key for the *Xiphinema americanum group* species by Lamberti et al. (2000) are: A 2, B 2, C 12, D 32, E 32, F 21, G 21, H 23, I 21, J 1.

Xiphinema rivesi Dalmasso, 1969 (Fig. 5)

The Spanish populations of this species were characterised by a lip region rounded continuous with the rest of the body, two equally developed female genital branches, vulva slightly posterior to mid-body, and female tail conoid with widely rounded tip (Fig. 5). Morphological and morphometrical traits (Table 2) agree well with original description by Dalmasso (1969) and other descriptions (Wojtowicz et al. 1982; Ebsary et al. 1984; Urek et al. 2005). Also, this species can be differentiated from *X. americanum* Cobb, 1913 in a longer odontostyle and distance from guiding ring to anterior end (72–96, 67–76 μm vs 63–73, 49–66 μm), and from *X. inaequale* Khan & Ahmad, 1977 by the shape of the lip region (with a slight depression *vs* continuous with body contour). This species has been reported in several localities from Spain (Bello et al. 2005) and Portugal (Lamberti

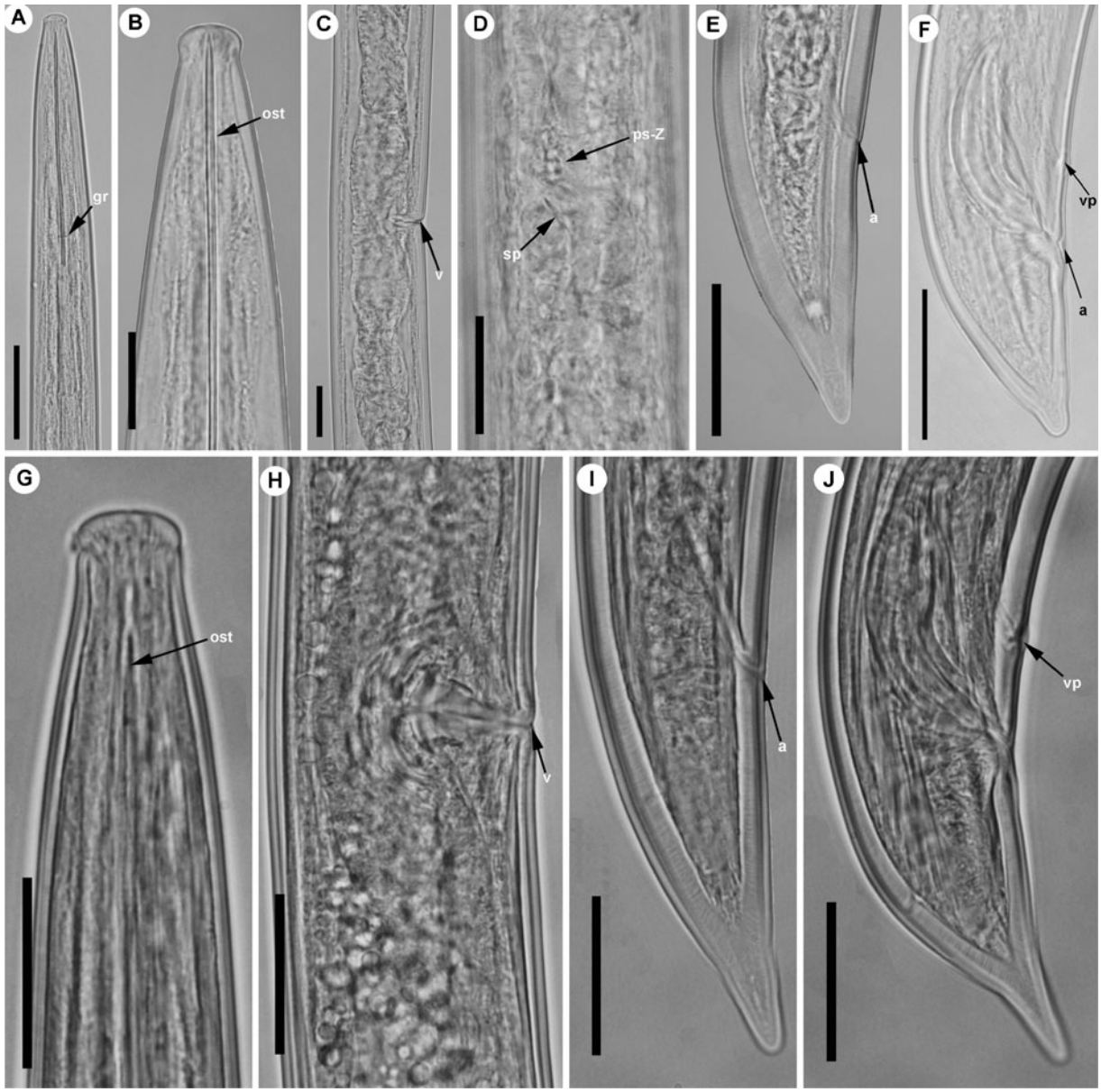

Fig. 3 Light micrographs of *Xiphinema lupini* Roca & Pereira, 1993 infesting the grapevine rhizosphere in southern Spain (**a**–**f**), and paratypes from Portugal (**g**–**j**). **a** Female neck region; **b**, **g** Anterior regions; **c**, **h** Vulval regions; **d** Detail of the pseudo-Z-organ and spines; **e**, **i** Female tail regions; **f**, **j** Male tails. Abbreviations: *a* anus; *gr* guiding ring; *v* vulva. (*Scale bars*: **a** = 50 μm; **b**–**j** = 20 μm)

et al. 1994); and it has been reported from several localities in North America (Ebsary et al. 1984; Robbins 1993), and Central and South American countries (Doucet et al. 1998).

The alpha-numeric codes for *X. rivesi* to be applied to the polytomic identification key for the *Xiphinema americanum group* species by Lamberti et al. (2000) are: A 1, B 2, C 12, D 23, E 12, F 1, G 1, H 23, I 32, J 2.

Longidorus alvegus Roca, Pereira & Lamberti, 1989 (Fig. 6)

As longidorid males have features that are useful taxonomic characters, the male of *L. alvegus* is described here, for the first time, from two amphimictic populations infesting grapevines in southern Spain. The male was common, similar to female except for

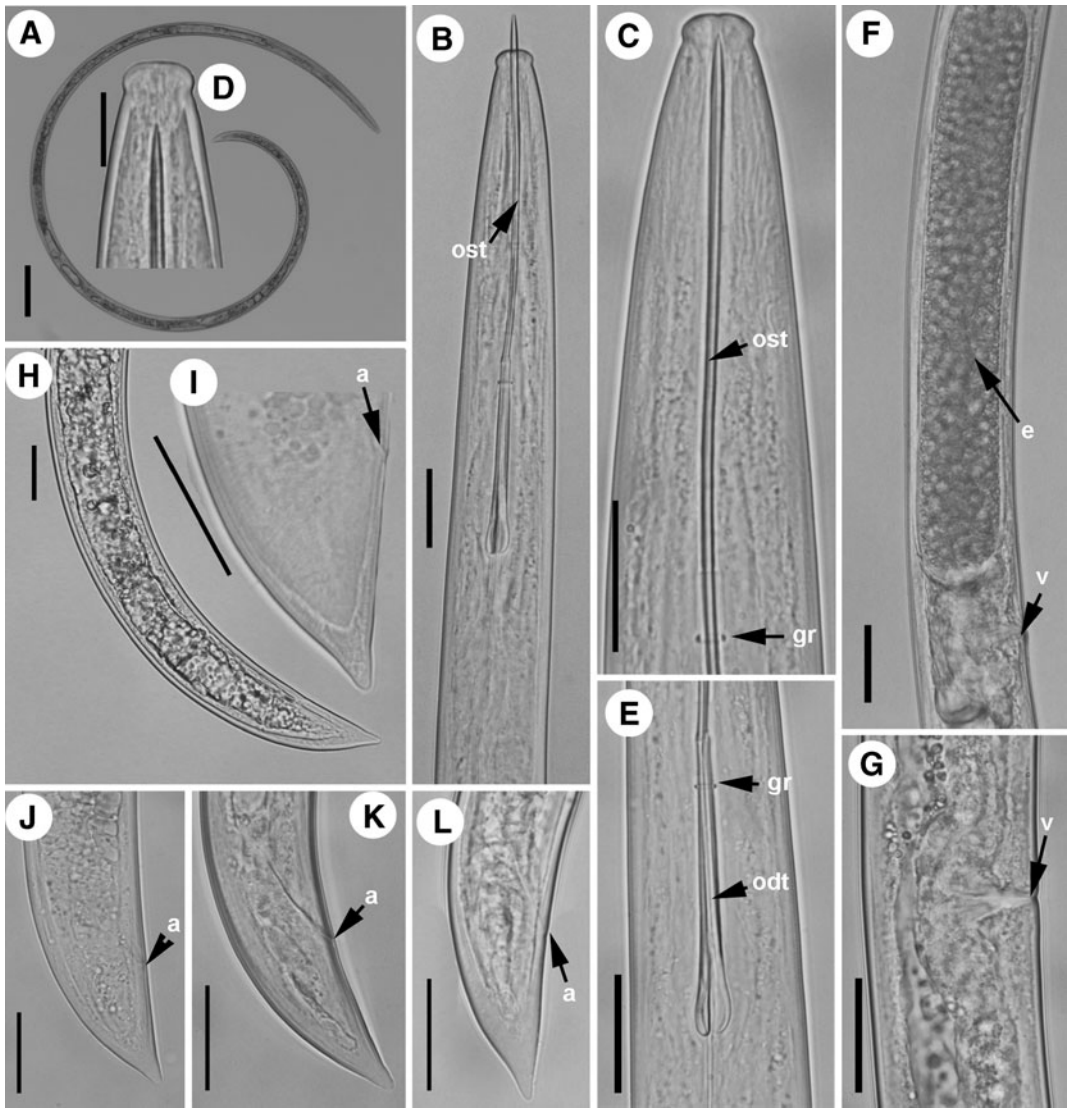

Fig. 4 Light micrographs of *Xiphinema pachtaicum* (Tulaganov 1938) Kirjanova, 1951 infesting the grapevine rhizosphere in southern Spain. **a** Whole female; **b**, **c** Female neck region; **d** Anterior region; **e** Detail of odontophore; **f**, **g** Vulval regions with egg; **h**–**l** Female tail regions. Abbreviations: *a* anus; *e* egg; *odt* odontophore; *ost* odontostyle; *v* vulva; *vp* ventromedian papillae. (*Scale bars*: **a** = 100 μm; **b**–**c**, **e**–**l** = 20 μm; **d** = 10 μm)

reproductive system and almost as abundant as female. Amphidial fovea pouch-shaped, asymmetrically bilobed, body ventrally arcuate, more strongly curved in posterior region due to well developed copulatory muscles (Fig. 6e). Eight to ten (usually) ventromedian precloacal papillae at equal distance from each other, and anterior to the adanal pair (Fig. 6e). Spicules well sclerotized and massive, lateral accessory pieces somewhat straight or slightly ventrally curved, 12.5 (11–14) μm long (Table 5). Copulatory muscles and spicule protractor and retractor muscles well developed (Fig. 6e). Sperm cells oval-rounded 4.5 (4–5) μm long. Tail dorsally convex, ventrally concave, narrowly conoid to a rounded terminus with two or rarely three pores on each side, similar to that of female (Fig. 6).

Morphological and morphometrical traits of both populations are broadly similar among them, except for a higher maximum body width and lower a ratio in the population from Montemayor than in population from Bollullos par del Condado (Table 5). Similarly, morphological and morphometrical traits of both populations agree very well with the original description by

Table 4 Morphometrics of *Xiphinema pachtaicum* (Tulaganov 1938) Kirjanova, 1951 infesting vineyards from southern Spain. All measurements in μm and in the format: mean ± s.d. (range)

Locality	Moriles (Córdoba province)	Montillla (Córdoba province)	Jerez de la Frontera (Cádiz province) 398	Jerez de la Frontera (Cádiz province) 401	Jerez de la Frontera (Cádiz province) 403	Jerez de la Frontera (Cádiz province) 406	Bollullos par del Condado (Huelva province) 155	Bollullos par del Condado (Huelva province) 426
Character	Females	Females	Females	Females	Females	Females	Females	Females
N	10	10	10	10	10	10	10	10
L	1785 B[a]±136 (1580–1980)	1718 AB±138 (1480–1880)	1956 A±196 (1715–2130)	1759 AB±69 (1680–1900)	1801 AB±156 (1555–2070)	1711 AB±69 (1635–1800)	1785 AB±136 (1585–1980)	1830 A±87 (1670–2000)
A	59.1 A±3.8 (53.3–65.1)	60.5 A±4.6 (54.2–66.1)	54.2 A±13.9 (34.0–63.9)	57.7 A±2.6 (54.7–62.1)	60.1 A±4.8 (50.0–65.9)	55.9 A±2.2 (53.4–58.8)	59.1 A±3.8 (53.3–65.1)	62.9 A±4.7 (51.1–67.7)
B	8.1 A±1.6 (6.1–10.7)	6.6 A±1.3 (5.4–8.2)	8.2 A±1.2 (7.1–9.4)	7.6 A±1.4 (6.1–10.4)	6.5 A±0.8 (5.4–7.6)	6.8 A±0.8 (6.0–7.6)	8.1 A±1.6 (6.1–10.7)	8.0 A±0.9 (7.2–9.3)
C	57.9 B±7.5 (47.5–70.9)	59.2 B±4.4 (54.2–66.1)	70.1 A±1.2 (68.8–71.1)	62.9 AB±6.1 (54.2–74.5)	59.7 B±5.1 (49.7–65.9)	57.9 B±2.9 (54.5–61.4)	57.9 B±7.5 (47.5–70.9)	64.7 AB±4.9 (56.9–75.2)
c′	1.60 AB±0.1 (1.60–1.90)	1.67 AB±0.1 (1.60–1.80)	1.49 B±0.02 (1.47–1.50)	1.49 B±0.1 (1.30–1.60)	1.63 AB±0.1 (1.40–1.80)	1.54 AB±0.1 (1.50–1.60)	1.75 A±0.1 (1.60–1.90)	1.60 AB±0.1 (1.50–1.70)
V	57.2 AB±1.4 (55–60)	56.4 B±1.4 (55–58)	58.1 AB±0.8 (57–59)	57.2 AB±2.1 (54–61)	59.4 A±1.4 (57–62)	56.4 B±1.3 (55–58)	57.9 AB±1.7 (55–61)	57.4 AB±1.6 (55–60)
Odontostyle length	84.1 A±2.9 (80–87)	89.0 A±2.8 (85–92)	88.3 A±0.9 (87–89)	86.6 A±3.4 (79–91)	85.1 A±3.5 (79–89)	86.1 A±3.2 (83–89)	86.2 A±4.5 (78–91)	83.9 A±2.5 (79–88)
Odontophore length	48.7 A±2.7 (45–54)	47.7 A±5.5 (37–53)	47.1 A±0.9 (46–48)	51.9 A±3.0 (49–58)	48.8 A±1.5 (47–52)	48.6 A±2.1 (46–51)	48.2 A±2.9 (45–53)	49.0 A±3.2 (45–54)
Lip region width	8.3 B±0.4 (8.0–8.5)	8.7 AB±0.3 (8.5–9.5)	9.1 A±0.6 (8.5–10.0)	8.7 AB±0.4 (8.0–9.5)	8.6 AB±0.1 (8.5–9.0)	9.1 A±0.4 (8.5–9.5)	8.7 AB±0.4 (8.0–9.5)	8.8 AB±0.5 (8.0–9.5)
Oral aperture–guiding ring	70.6 A±5.7 (59–79)	75.6 A±1.1 (74–77)	76.2 A±2.5 (73–78)	75.0 A±2.1 (72–77)	74.0 A±2.1 (72–76)	75.0 A±2.1 (72–77)	74.0 A±4.0 (65–78)	74.0 A±4.0 (65–78)
Tail length	28.1 B±1.7 (25–31)	29.1 AB±2.1 (27–32)	29.0 AB±1.5 (27–30)	28.1 B±2.3 (24–33)	30.8 AB±2.3 (28–33)	29.6 AB±2.1 (27–31)	31.1 A±1.9 (28–34)	28.4 AB±1.3 (27–31)

Abbreviations are defined in Jairajpuri and Ahmad (1992)

[a] Means within rows followed by the same upper-case letter do not differ ($P<0.05$) according to Tukey HSD test

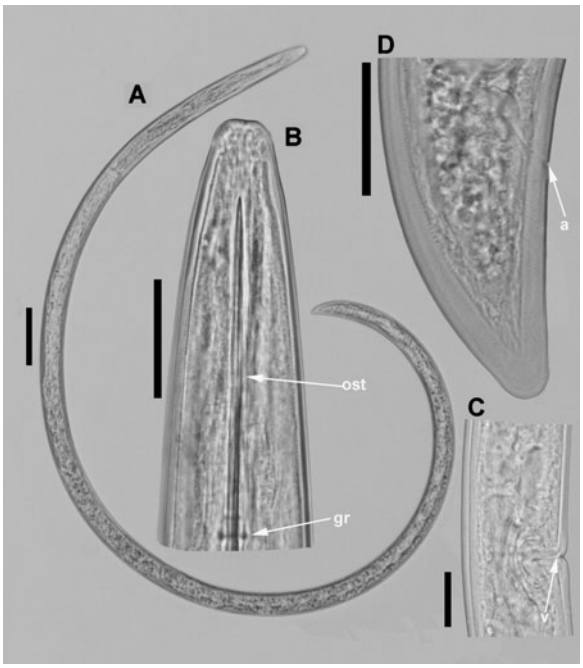

Fig. 5 Light micrographs of *Xiphinema rivesi* Dalmasso, 1969 infesting the grapevine rhizosphere in southern Spain. **a** Whole female; **b** Female neck region; **c** Vulval region; **d** Female tail region. Abbreviations: *a* anus; *odt* odontophore; *ost* odontostyle; *v* vulva. (*Scale bars*: **a** = 100 μm; **b**–**d** = 20 μm)

Roca et al. (1989), except for a lower a ratio, and a higher c' ratio in the population from Montemayor (140.7, 2.6 vs 188.4, 2.3, respectively); and a slightly shorter odontostyle and odontophore in the population from Bollullos par del Condado (78.0, 38.5 vs 87.2, 46.5, respectively), which do not exceed the intraspecific variations as showed for these Spanish populations (Table 5), and as confirmed by molecular analyses. The present record of *L. alvegus* is the first from Spain and the second in the Iberian Peninsula after the original description from central Portugal, and suggests that this species may be an Iberian endemism (Peña Santiago et al. 2003).

The alpha-numeric codes for *L. alvegus* to be applied to the polytomic identification key for *Longidorus* species by Chen et al. (1997) are: A 23, B 23, C 23, D 2, E 3, F 34, G 345, H 56, I 12.

Longidorus magnus Lamberti, Bleve-Zacheo & Arias, 1982 (Fig. 6)

The Spanish populations of this species were characterised by a lip region subacute, rounded and continuous with the rest of the body, amphidial fovea pouch-shaped, asymmetrically bilobed, two equally developed female genital branches, vulva slightly posterior to mid-body, female tail short, bluntly rounded bearing three caudal pores (Fig. 6). First-stage juveniles (J1) were positively identified by the replacement odontostyle which lay mostly within the odontophore, and showed a tail elongate conoid, dorsally convex, with the terminus separated by a slight constriction (Fig. 6). The morphology of the amphidial fovea and the female tail of the first-stage juvenile clearly differentiate this species from *L. macrosoma* (with not lobed amphidial fovea and subdigitate tail of J1), and confirmed the diagnosis of this species, which previously was identified as *L. macrosoma*, since was only based in third and fourth stage-juveniles (Téliz et al. 2007). Morphological and morphometrical traits of females of both populations agree closely with the original description of the species from Malta by Lamberti et al. (1982), except for a larger odontostyle (122–144 vs 100–118 μm). Nevertheless, the *L. magnus* population associated with grapevines in northern Italy (Roca et al. 1986) also showed a higher variability in odontostyle length (95–131 μm) than type population, which indicated that the Spanish populations of this species do not exceed the intraspecific variations (Table 5). The present record of *L. magnus* is the first from Spain and the third after original description from Malta and central Italy.

The alpha-numeric codes for *L. magnus* to be applied to the polytomic identification key for *Longidorus* species by Chen et al. (1997) are: A 45, B 345, C 4, D 1, E 3, F 5, G 12, H 1, I 12.

Phylogenetic relationships of longidorids infesting vineyards in southern Spain with other Longidoridae species

The amplification of D2–D3 expansion segments of 28S rDNA, partial 18S and ITS1 region yielded a single fragment of approximately 800 bp, 1,600 bp and 1,030 bp, respectively. However, sequences of 900 bp were found for ITS1 in *X. rivesi* and *L. magnus*. Sequence size was based on gel electrophoresis. Sequences from other species of Longidoridae obtained from NCBI were used for further phylogenetic studies. Sequences for *X. adenohystherum*, *X. nuragicum* and *X. turcicum* were obtained and

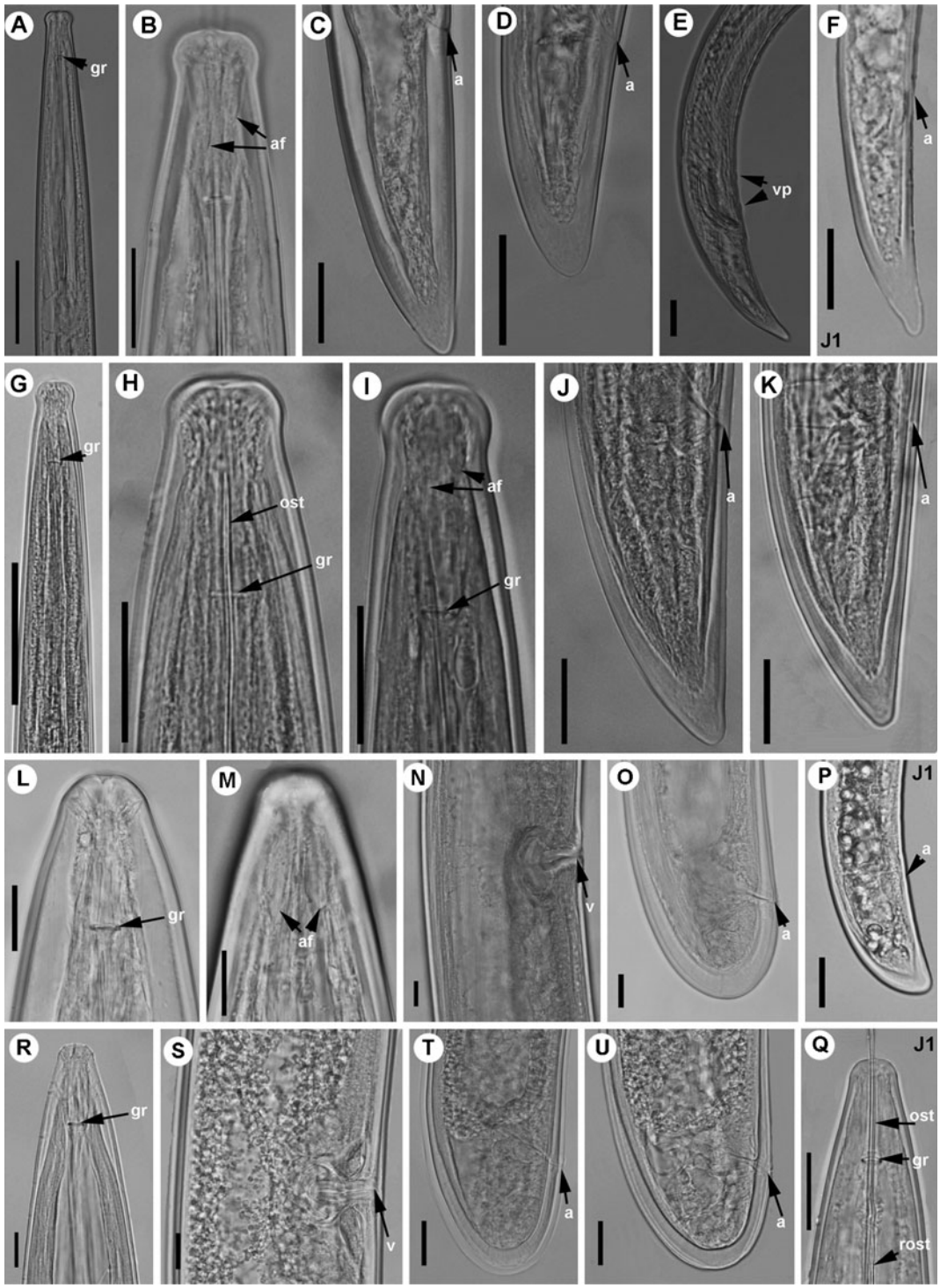

Fig. 6 Light micrographs of *Longidorus alvegus* Roca, Pereira & Lamberti, 1989 and *Longidorus magnus* Roca & Pereira, 1993 infesting the grapevine rhizosphere in southern Spain (**a**–**f**, **l**–**q**, respectively), and paratypes from Portugal and Malta (**g**–**k**, **r**–**u**, respectively). **a**, **g** Female neck regions; **b**, **h**, **i**, **l**, **m**, **r** Anterior regions; **c**, **d**, **j**, **k**, **o**, **t**, **u** Female tail regions; **e** Male tail region; **f**, **p** Tail region of first-stage juvenile; **n**, **s** Vulval regions; **q** neck regions of first-stage juvenile. Abbreviations: *a* anus; *af* amphidial fovea; *gr* guiding ring; *v* vulva; *vp* ventromedian papillae. (*Scale bars*: **a**, **g** = 50 μm; **b**–**f**, **h**–**u** = 20 μm)

Table 5 Morphometrics of *Longidorus magnus* Lamberti, Bleve-Zacheo & Arias, 1982 and *L. alvegus* Roca, Pereira & Lamberti, 1989 infesting vineyards from southern Spain. (All measurements in μm and in the format: mean ± s.d. (range)

Nematode species	*L. magnus*		*L. alvegus*			
Locality	Moriles (Córdoba province)	Sanlucar de Barrameda (Cádiz province)	Montemayor (Córdoba province)		Bollullos par del Condado (Huelva province)	
Character	Females	Females	Females	Males	Females	Males
N	8	4	7	5	6	3
L	10357±747 (9290–11320)	10510±640 (9980–11400)	6105±290 (5670–6610)	5440±233 (5155–5720)	6605±678 (5880–7400)	5560±611 (4980–6200)
a	76.6±8.8 (66.3–91.4)	73.8±5.8 (67.9–81.4)	140.7±10.5 (127.0–150.7)	155.9±10.2 (147.5–173.7)	206.2±39.7 (158.8–246.7)	183.3±14.7 (172.0–200.0)
B	17.0±1.9 (14.8–19.9)	16.9±0.8 (16.2–17.9)	17.6±1.7 (15.3–20.0)	17.9±1.0 (17.2–19.6)	17.6±1.6 (15.3–20.0)	18.1±1.7 (16.2–19.6)
C	168.5±12.9 (145.9–187.1)	170.0±16.8 (153.5–193.3)	85.5±13.8 (70.4–107.3)	76.7±6.9 (70.6–84.1)	107.2±11.5 (97.6–119.9)	91.7±7.4 (86.0–100.0)
c′	0.7±0.1 (0.6–0.8)	0.68±0.02 (0.66–0.70)	2.6±0.4 (1.9–3.0)	2.4±0.3 (2.2–2.8)	2.4±0.6 (1.8–3.0)	2.7±0.3 (2.5–3.0)
V or T	53.2±2.3 (50–56)	51.8±1.0 (51–53)	49.3±2.1 (46–51)	40.4±5.3 (35–49)	48.8±0.5 (48–49)	31.3±3.5 (28–35)
Odontostyle length	130.7±6.8 (122–144)	130.3±1.3 (129–132)	86.9±4.5 (80–91)	94.0±1.4 (92–95)	78.0±4.8 (73–83)	82.7±2.5 (80–85)
Odontophore length	78.6±8.0 (67–92)	76.5±5.1 (69–80)	51.3±8.0 (42–65)	43.6±1.1 (42–45)	38.5±5.3 (34–46)	42.0±1.0 (41–43)
Lip region width	23.6±1.9 (21.0–27.5)	24.5±0.6 (24.0–25.0)	16.3±1.0 (15.0–17.0)	16.5±0.5 (16.0–17.0)	14.3±0.5 (14.0–15.0)	13.7±0.6 (13.0–14.0)
Oral aperture–guiding ring	47.6±2.0 (44–49)	49.0±0.8 (48–50)	29.4±1.4 (28–32)	29.6±1.1 (28–31)	26.8±1.0 (26–28)	26.7±0.6 (26–27)
Tail length	61.6±4.5 (57–67)	62.0±2.9 (59–65)	72.9±11.1 (58–84)	71.2±3.7 (67–76)	61.3±11.2 (49–71)	60.7±4.2 (56–64)
Spicules	–	–	–	44.4±3.0 (41–48)	–	39.0±1.0 (38–40)
Lateral accessory piece	–	–	–	12.8±1.3 (11–14)	–	11.0±1.0 (10–12)

Abbreviations are defined in Jairajpuri and Ahmad (1992)

characterized by Gutiérrez-Gutiérrez et al. (2010). Sequences for *X. hispidum*, *X. lupini*, *L. alvegus* and *L. magnus* were obtained for these species in this study. Sequences for *X. index*, *X. italiae*, *X. pachtaicum* and *X. rivesi* matched well with former sequences deposited in GenBank, extending the molecular diversity of these species to newly studied areas. Some difficulties were experienced in the sequencing of partial 18S for *X. pachtaicum* and *L. alvegus* and for this reason some of these sequences were not included (Table 1).

Xiphinema index matched well with sequences deposited in GenBank, being 100% similar with accessions AY584243 and AY601628. However, intra-specific variations detected in our populations ranged from 1 to 2 nucleotides among these sequences and those from Jerez de la Frontera, Rociana, and Moriles populations. Partial 18S and ITS1 also agree with results obtained from D2–D3 region. For partial 18S, sequences were identical with AY687997 and mostly matched with EF207249 (4 nucleotides-99% similarity). ITS1 were similar to sequences deposited in GenBank for *X. index* with 99% of similarity and nucleotide differences from 5 to 8 nucleotides (AJ437026, AY584243 and AY430175). Additionally, female pegless and male specimens were also sequenced for D2–D3 region, which confirmed their molecular identification as *X. index*. These sequences were also deposited under GenBank accession numbers (HM921364 and HM921363, respectively).

Xiphinema italiae showed a high homogeneity for the D2–D3 region (100% similarity) in the sampled populations (Montemayor and Bollullos par del Condado). However, this homogeneity is not well represented with sequences deposited in GenBank, which showed a 98% similarity with FJ713153 and AY601613. These small differences were also represented in partial 18S with 99% similarity with FJ713154 and for ITS1 was 94% similarity with AJ437029.

Xiphinema pachtaicum showed coincidence with the sequences deposited in GenBank, but low intra-specific diversity in the D2–D3 region was found between our samples and sequences deposited in GenBank. Similarity was of 93% (AY601607) or 99% (AY601606). ITS1 also showed some similarity (98%) with the sequence AY430178. Additionally, one male specimen was also sequenced for D2–D3 region, which confirmed morphological identification as *X. pachtaicum* (HM921365).

The *Xiphinema rivesi* D2–D3 region matched closely with other species of *X. americanum*-group such as *X. americanum* (AY580056, 99% similarity, 6 nucleotide differences), as well as *X. rivesi* (AY210845, 99% similarity, 7 nucleotide differences). Intra-specific differences were not found between our populations (Moriles and Bollullos par del Condado). Similar results were detected for other markers as ITS1 and partial 18S. In fact, ITS1 showed even more similarities with many *X. americanum*-group species as *X. inaequale* Khan & Ahmad, 1977 (GQ231530, 98% similarity), *X. thornei* Lamberti & Golden, 1986 (AY430176, 97%), and *X. rivesi* (AY430186, 94%). However, nucleotide differences were produced mainly by insertions and deletions in the sequence. Using partial 18S, many similar sequences were found, some of them *X. rivesi* entries (AM086673) differing in 5 nucleotides with a 99% similarity; however, other species showed a similar sequence (100% similarity) in this region as *X. georgianum* Lamberti & Bleve-Zacheo, 1979 (AM086688), *X. floridae* Lamberti & Bleve-Zacheo, 1979 (AM086687) and *X. citricolum* Lamberti & Bleve-Zacheo, 1979 (AM086686) indicating that this sequence is not adequate for species delimitation.

Xiphinema hispidum was closely related in D2–D3 sequence to other *Xiphinema* species in the Iberian Peninsula such as *X. turcicum* (GU725077) and *X. sphaerocephalum* Lamberti, Castillo, Gómez-Barcina & Agostinelli, 1992 (GU725076), with 98% and 95% similarity values, respectively. However, there was an intra-species diversity of five nucleotides between the two *X. hispidum* populations sampled in Montemayor and Bollullos par del Condado, respectively. For partial 18S, the maximum similarities were related to *X. index*, in which only 1 nucleotide difference was found with our *X. index* population sequence (HM921342) and from 1 to 5 nucleotide for sequences AY687997 and EF207249, respectively. *X. hispidum* ITS1 sequence showed homology with *X. hispanum* Lamberti, Castillo, Gómez-Barcina & Agostinelli, 1992 (GU725061, 84% similarity), *X. turcicum* (GU725064, 83% similarity), *X. vuittenezi* Luc, Lima, Weischer & Flegg, 1964 (AJ437028, 81% similarity), *X. iranicum* Pedram, Niknam, Robbins, Ye & Karegar, 2009 (EU477386, 80% similarity), *X. aceri* Chizhov, Tiev & Turkina, 1986 (EU471385, 79% similarity) and *X. italiae* (AJ437029, 78% similarity).

Regarding the D2–D3 region, the *X. lupini*-closest species was *X. turcicum* (GU725077) with 87% similarity, but there was a high degree of differences with other *Xiphinema* spp. ITS1 sequences from *X. lupini* did not show any homology with other sequences compared in this study.

Longidorus alvegus presented four nucleotides differences for the D2–D3 region within the two populations sampled at Montemayor and Bollullos par del Condado, respectively. Other close species were *L. breviannulatus* Norton & Hofmann, 1975 (91% similarity) followed by *L. kuiperi* Brinkman, Loof & Barbez, 1987 (AM911623, 91% similarity) and *L. dunensis* Brinkman, Loof & Barbez, 1987 (AY593056, 90% similarity). No homologies in the GenBank were found for ITS1 sequences in this species.

Longidorus magnus presented also a low nucleotide divergence for the D2D3 region between the two population sampled (Moriles and Sanlucar de Barrameda), with only 2 nucleotides of difference. The closest species in relation to this marker were *L. goodeyi* Hooper, 1961 (AY601581, 95% similarity); *L. vineacola* Sturhan & Weischer, 1954 (AY283169, 99%), *L. orientalis* Loof, 1982 (GQ988721, 99%) and *L. fragilis* Thorne, 1974 (AY283172, 98%). However, *L. orientalis* sequence was shorter than the sequences obtained for *L. magnus.* For this reason, more differences are also possible. No homologies in the Genbank database were found for ITS1 sequences in this species.

Figure 7 presents the phylogenetic position of needle and dagger nematodes found in southern Spain vineyards with other *Xiphinema* and *Longidorus* species based on D2–D3 region of 28S of a multiple edited alignment of 787 total characters. The phylogenetic analysis showed well supported groups at major and close clades to the species level in both analyses (BI and ML). The phylogenetic tree resolved three major clades: *a) Longidorus* and *Paralongidorus*; *b) Xiphinema americanum*-group including *Xiphidorus minor* Rashid, Coomans & Sharma, 1986; and *c)* the other *Xiphinema* species. However, the grouping of clades a) with b) was not well supported in our analysis. New sequences obtained in this study showed a clear relationship with the species identification conducted by morphometrical studies. In this regard, *X. rivesi* (HM921357 and HM921358) was placed in a position between the clade formed by *X. americanum*-group species that was not well supported. However, specific phylogenetic analysis of this group is not well supported in the majority of the species. *X. pachtaicum* populations (HM921353-HM921356) were well resolved between the most similar GenBank sequence (AY601606) with a good support in BI and ML analyses. The group of *X. americanum* species: *X. pachtaicum* (AY601606 and AY601607), *X. pachydermum* Sturhan, 1983 (AY601608), *X. simile* Lamberti, Cho1eva & Agostinelli, 1983 (AY601609) and *X. brevisicum* Lamberti, Bravo, Agostinelli & Lemos, 1994 (AY601609) were placed in a well supported group differentiated from the rest of *X. americanum*-group species. *X. index* populations were well resolved and formed a well supported clade with the single available GenBank entry (AY601628). However, the clade of *X. index* was included within one of the sub-clades for the non-americanum group of *Xiphinema* spp. *X. italiae* (HM921350 and HM921351) was within the same cluster with another *X. italiae* sequence (AY601613). This cluster was included with one of the sub-clades for the non-americanum group of *Xiphinema* spp. New obtained sequences for species without prior GenBank entries, such as *X. lupini* (HM921352) and *X. hispidum* (HM921346 and HM921366), were well separated in different groups. *X. lupini* was associated phylogenetically with *X. turcicum* (GU725077). This clade was related to other major clades with the non-americanum group and was relatively well supported by BI with good posterior probabilities (85), but not supported by ML analysis. *X. hispidum* was related phylogenetically to *X. adenohystherum* (GU725075) and *X. hispanum* (GU725074). However, this clade was related to other clade within the non-americanum group, and relatively well supported by BI with good posterior probabilities (84) and with a low bootstrap value for ML analysis (57). To our knowledge, this is the most complete phylogeny of species belonging to Longidoridae for D2–D3 region.

Figure 8 presents the phylogenetic position of needle and dagger nematodes found in southern Spain vineyards with other *Xiphinema* and *Longidorus* species based on partial 18S of a multiple edited alignment of 1,627 total characters. Similarly to D2–D3 region, three major clades were found in the phylogenetic tree of partial 18S: *a) Longidorus* and *Paralongidorus*; *b) Xiphinema americanum*-group including *Xiphidorus* spp.; and *c)* the other *Xiphinema*

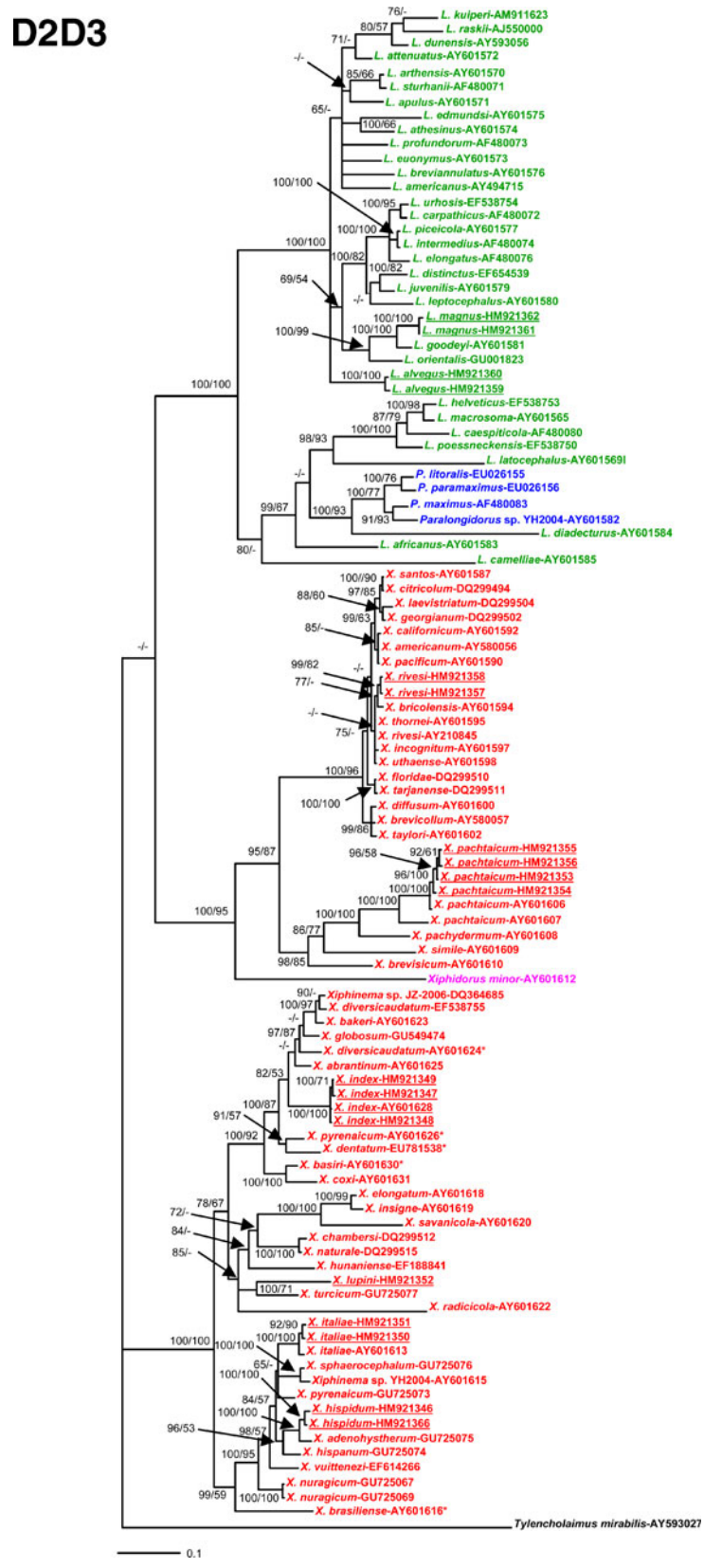

Fig. 7 Phylogenetic relationships within Longidoridae family. Bayesian 50% majority rule consensus trees as inferred from D2 and D3 expansion segments of 28S rRNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 65% are given for appropriate clades (in *bold letters*); bootstrap values greater than 50% are given on appropriate clades in ML analysis. Newly obtained sequences in this study are *underlined*. *: populations identified only on the basis of general morphology in He et al. (2005)

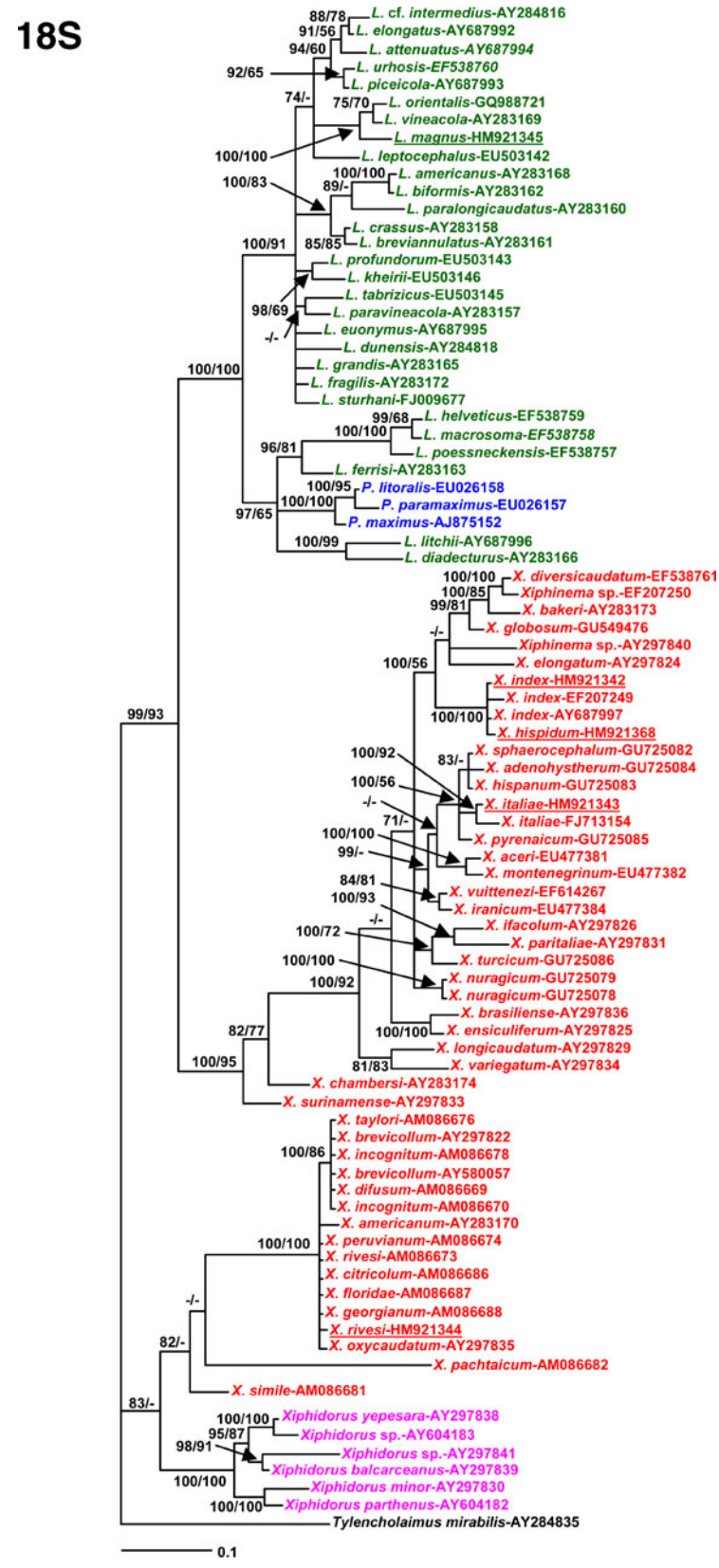

Fig. 8 Phylogenetic relationships within Longidoridae family. Bayesian 50% majority rule consensus trees as inferred from (A) 18S rRNA gene sequence alignments under the GTR + I + G model. Posterior probabilities more than 65% are given for appropriate clades (in *bold letters*); bootstrap values greater than 50% are given on appropriate clades in ML analysis. Newly obtained sequences in this study are *underlined*

species. However, species positions for this marker were not well defined as occurred for D2–D3 region. *X. rivesi* (HM921344) also occupied a not well supported position between the clade formed by *X. americanum*-group species. *X. pachtaicum* (AM86682) was included in the main clade, but the position between the genus *Xiphidorus* and the *X. americanum* group was not well supported by ML analysis for this marker. *X. index* (HM921342) and *X. hispidum* (HM921368) were placed in a well defined clade between other *X. index* GenBank accessions (EF207249 and AY687997). *X. italiae* (HM921343) was closely related to others GenBank accession for *X. italiae* (FJ713154), *X. hispanum* (GU725083), *X. adenohystherum* (GU725084), *X. sphaerocephalum* (GU725082), and *X. pyrenaicum* (GU725083). However, these clades were well supported by BI good posterior probabilities, but, not with appropriate bootstrap values for ML analyses. *L. magnus* (HM921345) was well phylogenetically related with *L. orientalis* (GQ988721) and *L. vineacola* (AY283169). To our knowledge, this is the most complete phylogeny of species belonging to Longidoridae for partial 18S gene.

Figure 9 presents the position of homologous and related sequences for ITS1 including *X. hispidum* (HM921367), *X. adenohystherum* (GU725063), *X. nuragicum* (GU725056 and GU725057) and *X. italie* (HM921335 and HM921341) with other sequences with shared homology from GenBank. *X. nuragicum* was placed at a basal position in the tree while the other species were grouped with a low BI good posterior probability and a high bootstrap value. Inside this clade several subgroups are formed: a) *X. italiae* (HM921335 and HM921341) with *X. italiae* (AJ437029) were closely related to *X. sphaerocephalum* (GU725062) and all these species with *X. aceri* (EU477385); b) *X. hispidum* was closely related to *X.*

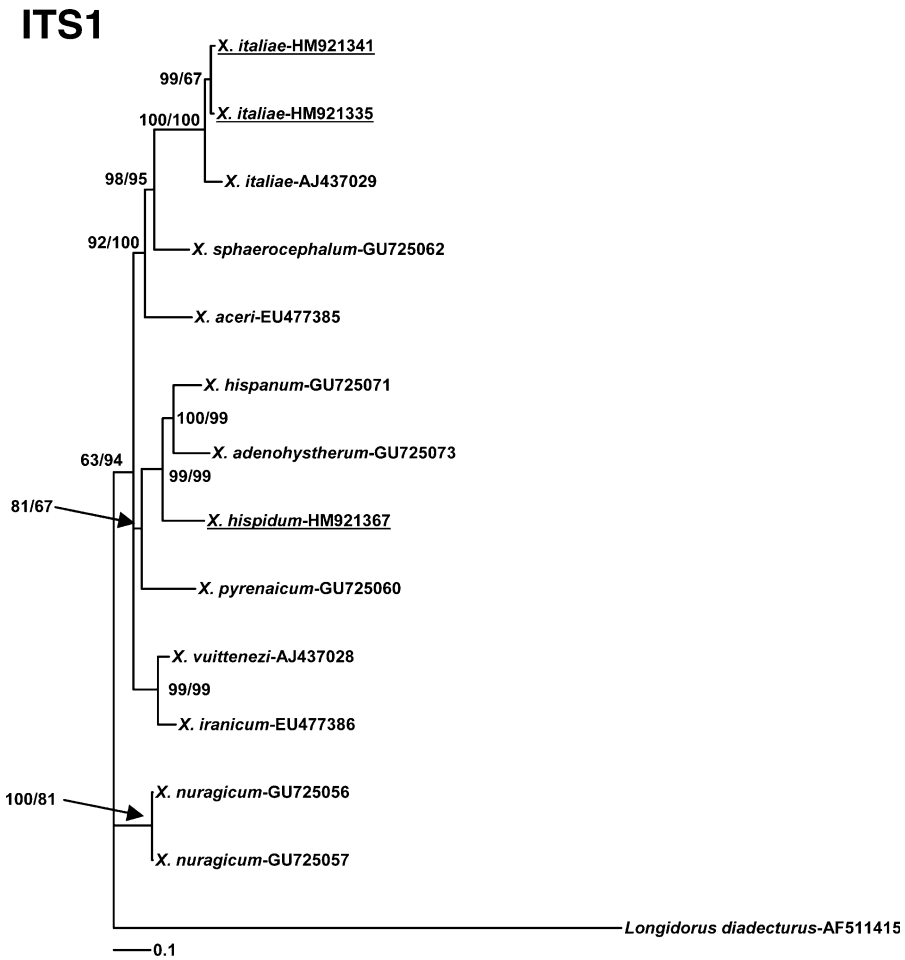

Fig. 9 Phylogenetic relationships within close related *Xiphinema* species. Bayesian 50% majority rule consensus trees as inferred from ITS1 under the TIM3 + G model. Posterior probabilities more than 65% are given for appropriate clades (in *bold letters*); bootstrap values greater than 50% are given on appropriate clades in ML analysis. Newly obtained sequences in this study are *underlined*

hispanum (GU725071) and *X. adenohystherum* (GU725073), and with *X. pyrenaicum* (GU725060) although with low support values; and c) *X. vuittenezi* (AJ437028) and *X. iranicum* (EU477386).

The tree topologies studied by SH-test did not refute the monophyly of the genus *Xiphinema* even though it was split into two major clades (D2–D3 region, P=0.517; and partial 18S, P=0.750). The genus *Paralongidorus* was rejected as a group outside the genus *Longidorus* (D2–D3 region, P=0.001; partial 18S, P=0.01). Finally, the genus *Xiphidorus* has an inconclusive position outside of genus *Xiphinema* (D2–D3 region, P=0.194; partial 18S, P=0.333).

Discussion

The primary objective of this study was to determine the prevalence, and to identify and molecularly characterize the dagger and needle nematodes infesting grapevine in provinces at Andalusia, southern Spain belonging to the "wine denomination of origin (D.O.) zones". Our survey indicated that in each of all 77 sampled vineyards at least one longidorid species was detected, which confirm why longidorids are considered one of the 10 most economically important nematode groups globally (Sasser and Freckman, 1987). Maximum biodiversity of up to eight longidorid species were found in some localities of the "wine denominations of origin (D.O.) zones" studied, although the majority of samples showed a range of one or two species of longidorids. The lowest levels of biodiversity detected in Jerez-Xérès-Sherry & Manzanilla-Sanlúcar de Barrameda D.O. are difficult to explain. Nevertheless, longidorids, as other large dorylaimids, are very sensitive to environmental stresses because of their permeable cuticle to pollutants and other soil disturbances (Bongers and Bongers 1998). Consequently, some physical or chemical soil characteristics, including soil perturbations could be responsible for this, which need further studies.

The predominance of *X. pachtaicum* in vineyards in southern Spain was also reported by Téliz et al. (2007) with similar frequency of infestation (90.8% vs. 79.7%), being also one of the most widely distributed *Xiphinema* species in Europe together with *X. diversicaudatum* (Micoletzky 1927) Thorne, 1939 (Brown and Taylor 1987). *X. pachtaicum* has fewer ecological requirements and a wider range of habitats and host range than other *Xiphinema* species (Navas et al. 1988). *X. index* was the second species in prevalence of infestation with 30.3%. This species has been widespread detected in grapevine-growing areas in a wide range of soil textures and pH, even in soils with high percentage of carbonates (Arias and Fresno 1994).

Some studies have developed species-specific primers that reliably discriminate some longidorid nematode species typical of grapevine soils in Europe (Wang et al. 2003; Hübschen et al. 2004a, b). However, the limited extensions of the species for which specific PCR protocols have been developed make necessary knowledge of longidorid biodiversity in other grapevine-growing regions, as well as the development of specific primers for a correct nematode identification in quarantine or replant situations. This study contribute to morphological and molecular diagnostic of longidorid species infesting grapevine in southern Spain, which may be useful as a diagnostic tool for this group with high phenotypic plasticity, for which diagnosis is complex and time-consuming. In our case, the presence of Iberian endemism, such as *X. hispidum*, *X. adenohystherum*, *X. nuragicum*, *X. lupini* and *L. alvegus*, make necessary the development of well characterized molecular markers confirmed by morphological and morphometrical characterizations in an integrative identification. In this regards, molecular analyses confirmed that the previous identification of *L. magnus* as *L. macrosoma* based only in third- and fourth-stage juveniles (Téliz et al. 2007) was a misidentification, since all three rDNA regions clearly separated the Spanish populations of *L. magnus* from those of *L. macrosoma* deposited in GenBank database. Similarly, these findings confirmed that the report of *X. americanum* by Weiland-Ardaiz and Pérez-Camacho (1995) in vineyards from Condado de Huelva (Huelva province) must be disproved in the surveyed area, as previously suggested by Bello et al. (2005).

The species identification based on sequencing of rRNA regions and BLAST analysis was congruent with species identification based on morphometrical studies. D2–D3 expansion segments of 28S rDNA and ITS1 were most useful for species identification than partial 18S, since they showed more variability than partial 18S. In fact, partial 18S sequences of different species only showed one or few nucleotide

differences (e.g., *X. hispidum* and *X. index*). Some sequences, such as that of *X. pachtaicum* (AY601607), were very different compared to that in our populations and the other entry for this species in GenBank (AY601606). Since our *X. pachtaicum* populations and that for AY601606 were obtained from southern Europe (Spain and Italy, respectively), whereas AY601607 was obtained from specimens from Eastern Europe (Moldava), our results suggest that close morphological and morphometrical but different species may be present in this latter region. For this reason additional molecular markers from the population from Moldava are needed to clarify the *X. pachtaicum* species definition.

Xiphinema americanum-group species presented low molecular variability for the markers studied, mainly in the *X. americanum* subgroup. However, for some species, discrimination has been possible, as the case of specific primers targeting the ITS1 for *X. brevicollum* and *X. diffusum* (Oliveira et al. 2005), or the well species separation for other subgroups, as *X. pachtaicum* subgroup (He et al. 2005; Lazarova et al. 2006). For this reason, identification based on sequence identities in the *X. americanum* group is difficult, and is only useful for specific species. These results agree with those obtained by He et al. (2005) and Lazarova et al. (2006). This limited difference and complexity has been observed in *X. rivesi*, for which the sequence comparisons between our sequences and sequences deposited in GenBank did not resolve their identification in spite of the agreement with our morphometrical studies.

Phylogeny of D2–D3 region of 28S gene tree showed a topology similar to those obtained in other studies (He et al. 2005; Palomares-Rius et al. 2008, 2010; Cantalapiedra-Navarrete et al. 2010). Main groups are well defined and supported in our analysis. However, *Longidorus* and the *X. americanum* group clustering together were not well supported by BI and ML for D2–D3 region, while in other studies the clustering between the *Longidorus* group and the other non-americanum *Xiphinema* species was well supported (He et al. 2005). Longidorid species found in southern Spain were well represented between all major clades for D2–D3 region and partial 18S. The comparison between these two markers showed similar trees and congruent positions between the species. *X. americanum* group comprised a lineage well differentiated from the other species, as previously demonstrated in other studies (Lazarova et al. 2006; Ye et al. 2004; Neilson et al. 2004; Pedram et al. 2009). Similar results were obtained with the grouping of *X. americanum* group and *Xiphidorus* species, which was not strongly supported. However, the grouping of *Longidorus* and *Paralongidorus* species with the rest of species is well supported in our case. Nevertheless, tree topology analysis by Shimodaira-Hasegawa test of D2–D3 and partial 18S of our broad a newly obtained sequences did not refute the monophyly of the genus *Xiphinema*, which agree with the results obtained by He et al. (2005). The genus *Paralongidorus* was not accepted as a valid taxon in our analysis, which also agrees with He et al. (2005) but disagrees with a more restricted study with fewer sequences conducted by Palomares-Rius et al. 2008. The genus *Xiphidorus* showed different results depending of the marker considered (D2–D3 or partial 18S). However, the result obtained with more *Xiphidorus* sequences of partial 18S did not accept it as a valid taxon, which agrees also with He et al. (2005). However, additional sequences of *Paralongidorus* spp. and *Xiphidorus* spp. from multiple origins need to be considered for clarifying their position within the family Longidoridae.

Therefore, the Iberian Peninsula should be considered as a centre of origin for a group of *Xiphinema* species, mainly, some species in the clade formed by *X. italiae*, *X. sphaerocephalum*, *X. pyrenaicum*, *X. hispidum*, *X. adenohystherum*, *X. hispanum*, *X. vuittenezi* and *X. nuragicum*. However, *X. brasiliense* Lordello, 1951 is difficult to be included morphologically in the *Xiphinema* clade for the presence of one ovary branches, and it was previously discussed (Gutiérrez-Gutiérrez et al. 2010). This grouping was well supported by the homologies between the ITS1, which could indicate a recent origin of these species. Morphological characteristics of these species included two well developed genital branches. However, other characters are difficult to link with the phylogeny of this group and with species with high plasticity of characters as in the genera *Xiphinema*. These suggestions agree with the results obtained for the phylogeny and biogeography of the closed genus *Longidorus* in the Euro-mediterranean region (Navas et al. 1993), in which a dispersalist model is one of the primary explanations for the large groups of *Longidorus* species found in that region. However, *X. lupini* is closely related to *X. turcicum*, which is

another of the Iberian Peninsula species. These close related species are well separated by sequence nucleotide differences D2–D3 and ITS. Both species showed similarities of two equally developed genital branches, the presence of pseudo-Z-organ and similar lip region morphology, but the tail varied considerably between a regularly hemispherical for *X. turcicum versus* conoid tail for *X. lupini*.

Longidorus magnus showed a good relationship with *L. orientalis*, *L. goodeyi* and *L. vineacola*. All these species are characterized by round tails, but the asymmetrically lobed amphidial fovea of *L. magnus* is more associated to *L. goodeyi* and *L. vineacola* than *L. orientalis*. This character is not well associated with the phylogenetic analysis of *L. magnus*, in spite of the correspondence between this morphological character and the phylogenetic trees inferred from the molecular data (Rubstova et al. 2001; He et al. 2005). *L. alvegus* was placed at a basal position in the subclade, and the relationship with other species was difficult to determine. However, the asymmetrically lobed amphidial fovea is well correlated with the main subclade in which is inserted.

In summary, this present study establishes the prevalence of infestation and biodiversity of longidorids found in commercial vineyards at southern Spain, as well as their polyphasic diagnosis and phylogenetical relationships within longidorids. *Xiphinema pachtaicum* and *X. index* are the most frequently dagger nematodes found. The high prevalence of *X. index* makes this species a severe threat to grapevine production in southern Spain, especially if the presence of *Grapevine Fanleaf Virus* (*GFLV*) is detected in the vineyard or it is introduced with non-certified planting material. Also, the importance of using polyphasic identification was highlighted for the difficulty of a correct and timely identification of this group of nematode species. This fact is particularly more important when endemism of species occurs, as it is shown in the present study. The high level of nematode endemism and their phylogenetic grouping suggest a common origin for several of the longidorid species found and the Iberian Peninsula as their potential centre of origin.

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