New evidence of cryptic speciation in the family Longidoridae (Nematoda: Dorylaimida)

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Abstract. Longidorid nematodes comprise more than 500 species, and *Longidorus* and *Xiphinema* are the most diversified, abundant and cosmopolitan genera, which increases the risk of species misidentification. We conducted an integrative morphometric and genetic study on two longidorid species to elucidate the existence of new cases of cryptic speciation within the genera *Longidorus* and *Xiphinema*. Detailed morphological, morphometric, multivariate and genetic studies were carried out, as well as mitochondrial and nuclear haploweb analyses, to differentiate species within the *L. iliturgiensis*- and *X. hispanum*-complexes. Species delimitation using haplonet tools of *L. iliturgiensis*-species complex clearly separated *L. tabernensis* sp. nov. from *L. iliturgiensis* and *L. indalus*. Similarly, the haploweb analysis of *X. subbaetense* sp. nov. showed it as a unique and separate species from *X. hispanum* and *X. adenohystherum*. *D2-D3* expansion domains of *28S* rRNA, partial *18S* rRNA, and partial *coxI* region were used for inferring phylogenetic relationships. The present study provides new insights into the diversity of *Longidorus* and *Xiphinema* species detected in southern Spain, and new evidence of cryptic speciation in both genera. These results support our hypothesis that the biodiversity of Longidoridae in southern Europe is higher than previously supposed and is still not fully clarified.

1 | INTRODUCTION

Delineating taxonomic boundaries correctly in large species complexes is crucial for addressing practical and theoretical questions of evolution and conservation (Bickford et al., 2007; Dayrat, 2005). However, species delimitation based only on morphological studies may be a difficult task given the inconspicuous or nonexistent (e.g. pseudocryptic and cryptic speciation) differences among closely related species (Lajus, Sukhikh, and Alekseev, 2015). This phenomenon has been described extensively in many taxa such as nematodes both in marine and terrestrial ecosystems (Oliveira et al., 2017; Lee et al., 2017; Palomares-Rius, Cantalapiedra-Navarrete, and Castillo, 2014). There are several reasons that can be used to explain cryptic speciation in nematodes, such as genetic mutations and ecological adaptations by geographic location or host range (Palomares-Rius et al., 2014; Wellborn & Broughton, 2008).

Interest in cryptic species has increased significantly with the progress of molecular-based approaches that have revealed an exponential increase in the number of cryptic species over recent decades (Bickford et al., 2007; Lee & Oliver, 2016). This enormous acceleration in the identification of cryptic species suggests that traditional morphological techniques may be deficient for accurate species identification in some species groups (Bickford et al., 2007; Jörger & Schrödl, 2013). In fact, the application of molecular techniques to taxa delimitation has uncovered a remarkable number of unknown cryptic species and/or revealed species hidden under one species identity (Gutiérrez-Gutiérrez et al., 2011; Lee et al., 2017; Palomares-Rius et al., 2014; Pérez-Portela, Arranz, Rius, & Turon, 2013; Pfenninger & Schwenk, 2007). The conserved morphology that characterizes soil nematodes has led to the development of molecular methods using different fragments of nuclear (nc) ribosomal and mitochondrial DNA (mt) gene sequences to be used in DNA barcoding (Hebert, Ratnasingham, & de Waard, 2003; Palomares-Rius et al., 2014; Palomares-Rius et al., 2017a; Palomares-Rius et al., 2017b). However, molecular taxonomy frequently remains incomplete without standard descriptions of nematode species, through which species delimitation accuracy and consistency has been significantly improved when used with morphological data prior to DNA extraction. Thus, species discovery and description needs to be achieved through the combined use of morphological and molecular analyses (Dayrat, 2005; Padial et al., 2010) defined as “integrative taxonomy”. In addition, the use of multivariate methods using morphometric characters as complement to custom integrative taxonomy has proven to be the most common way of delimiting cryptic species and therefore, resolving the taxonomy of diverse groups of organisms (Bärmann et al., 2013; Kuta et al., 2014; Legendre & Legendre, 1998; Vďačný, Slovák, & Foissner, 2014) including nematodes (Archidona-Yuste et al., 2016a; Cantalapiedra-Navarrete et al., 2013; Cho & Robbins, 1991).

Deciphering the cryptic biodiversity of soil nematodes is an essential task to increase our knowledge about soil ecosystem functioning (Barnes et al., 2018). Many cryptic species of both free-living and plant-parasitic nematodes (PPNs) have been discovered (Lee et al., 2017; Palomares-Rius et al., 2014). In the case of PPNs, the discovery and unravelling of cryptic species has implications in food security, quarantine and agronomic management of crops (Palomares-Rius et al., 2014). In addition, the possibility of an interesting ecological phenomenon describing the coexistence of identical species sharing the same niche and on the same host enhances the significance of describing cryptic species of PPNs (Zhang, Lin, & Hanski, 2004). To cope with the number of candidate species with the same identity, several studies have widely emphasized the socio-economic benefits of the application of new technologies and careful examination using integrative taxonomy in species delimitation of the cryptic complexes of PPNs (Archidona-Yuste et al., 2016a; Cantalapiedra-Navarrete et al., 2013; Gutiérrez-Gutiérrez et al., 2010; Palomares-Rius et al., 2017b; Palomares-Rius et al., 2014; Qing et al., 2019).

One of the most economically important nematodes includes ectoparasitic species belonging to the family Longidoridae Thorne, 1935 (Thorne, 1935). The importance of this group of nematodes lies not only in their polyphagy and cosmopolitan distribution but also their status as vectors of plant viruses that causes significant damage to a wide range of agricultural crops (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d; Coomans, 1996; Decraemer & Robbins, 2007; Macfarlane, 2003; Taylor & Brown, 1997). The family Longidoridae includes more than 500 species (Coomans et al., 2001; Decraemer & Robbins, 2007), and *Xiphinema* Cobb, 1913 (Cobb, 1913) (i.e., 296 species) and *Longidorus* Micoletzky, 1922 (Micoletzky, 1922) (i.e., 181 species) are the most diversified, abundant and cosmopolitan genera (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d), enhancing the risk of species misidentification and therefore, highlighting the importance of using integrative taxonomy (Dayrat, 2005; Padial et al., 2010; Palomares-Rius et al., 2014). Some cryptic species have been recently discovered, particularly in the genus *Xiphinema*, showing the potential of the combined application of morphological and molecular analyses against traditional taxonomy (Archidona-Yuste et al., 2016a; Gutiérrez-Gutiérrez et al., 2010; Lazarova et al., 2019; Peraza-Padilla et al., 2016). Likewise, phenetic studies based on multivariate methods have proven a useful and additional tool for species discrimination in cryptic complexes in this group of nematodes (Archidona-Yuste et al., 2016a). These integrative studies also provide DNA sequence data mainly of two marker sequences for precise and unequivocal species identification: the nc ribosomal RNA (rRNA) gene sequences, e.g., *D2-D3* expansion domains of the *28S* rRNA gene, internal transcribed spacer (*ITS1*) and the *18S* rRNA gene, as well as the mt gene cytochrome c oxidase subunit I (*coxI*). In fact, the use of these molecular markers has made it possible to provide accurate identification of

118 species complexes and explain the phylogenetic relationships within the genera *Longidorus* and
119 *Xiphinema* (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016a; Gutiérrez-Gutiérrez et
120 al., 2010; He et al., 2005; Palomares-Rius et al., 2017b; Ye et al., 2004). Two prominent examples
121 of high cryptic species diversity in both genera are the *L. iliturgiensis*- and *X. hispanum*-complex
122 species (Archidona-Yuste et al., 2019a; Gutiérrez-Gutiérrez et al., 2010). The *Longidorus*
123 *iliturgiensis*-complex was recently described showing a highly conserved morphology with similar
124 anatomical characteristics among species such as lip region and tail shape or key morphometric
125 diagnostic characteristics (i.e., body length) (Archidona-Yuste et al., 2019a). The history of the
126 *Xiphinema hispanum*-complex has been a nematological hot topic of controversy since Lamberti et
127 al. (1992) first reported this cryptic complex. In that study, the *Xiphinema hispanum* complex was
128 described as including five new didelphic *Xiphinema* species from the Mediterranean Basin
129 characterized by a rounded tail in females with or without an inconspicuous bulge projecting
130 slightly ventrally and a uterus showing spiniform structures (Lamberti et al., 1992). Later, Baujard,
131 Luc & Loof (1996) and Loof, Luc & Baujard (1996) examined the paratypes of those species and
132 concluded that there were not enough morphological differences to differentiate those species from
133 each other, hence, they were proposed as junior synonyms. However Gutiérrez-Gutiérrez et al.
134 (2010) helped to clarify the identity and phylogenetic relationships of this complex *Xiphinema*
135 group by applying integrative taxonomical approaches that allowed us to verify these species as
136 valid. Finally, and equally important to emphasize, recent studies have revealed the coexistence of
137 both cryptic complexes in close natural and agricultural areas in southern Spain constrained by the
138 same abiotic and biotic characteristics (such as environmental factors and host species) (Archidona-
139 Yuste et al., 2019a; Archidona-Yuste et al., 2019b; Archidona-Yuste et al., 2020), highlighting the
140 difficult task of making an accurate species identification solely using classical taxonomy
141 approaches.

142 Intensive nematological surveys during the last decade in agricultural and natural ecosystems in
143 Andalusia, southern Spain, indicated a remarkable diversity within the family Longidoridae
144 including the presence of both cryptic species complexes as stated above (Archidona-Yuste et al.,
145 2019a; Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d; Cai et al., 2020). However,
146 we suspect that biodiversity of Longidoridae in southern Spain is still not fully clarified and needs
147 additional sampling efforts given the significant gaps in soil nematode biodiversity regarding the
148 large number of undescribed species (Cameron et al., 2018; Decaëns, 2010) and the hypothesis
149 suggesting the Iberian Peninsula as a possible centre of speciation for some groups of the family
150 Longidoridae (Archidona-Yuste et al., 2016b; Archidona-Yuste et al., 2016c; Archidona-Yuste et
151 al., 2016d; Coomans, 1996). In fact, recent surveys during 2019 in natural environments in
152 Andalusia revealed two populations of *Longidorus* and *Xiphinema* showing morphological and

morphometric traits quite similar to previously described species and the cryptic species groups mentioned above, such as the members of the *L. iliturgiensis*- and *X. hispanum*-complexes, respectively. Nevertheless, the application of integrative taxonomical approaches indicated that both populations belong to undescribed species.

Therefore, the objectives of this research were: (1) to elucidate the existence of new species belonging to cryptic complexes within the genera *Longidorus* and *Xiphinema* using an integrative species delineation approach based on multivariate morphometric analysis (Archidona-Yuste et al., 2016a; Reyment, 1982) and haplonet mt and nc haploweb tools (Flot, Couloux & Tillier, 2010) to differentiate species within the *L. iliturgiensis*- and *X. hispanum*-complex species; (2) to describe two new species of the genera *Longidorus* and *Xiphinema* (*L. tabernensis* sp. nov. and *X. subbaetense* sp. nov.) through integrative methods based on combination of morphological, morphometric and molecular data; and (3) to apply phylogenetic analyses to clarify the relationship of the identified *Longidorus* and *Xiphinema* species.

2 | MATERIAL AND METHODS

2.1 | Ethics statement

No specific permits were required for the indicated fieldwork studies. The soil samples were obtained in public areas, forests, and other natural areas and do not involve any species endangered or protected in Spain, nor are the sites protected in any way.

2.2 | Nematode populations and morphological studies

A total of 101 individuals including 51 adult and 50 juvenile specimens were used for morphological analyses. Nematodes were surveyed from March to June 2019 during the spring season in natural ecosystems in Andalusia, southern Spain (Table 1). Soil samples were collected for nematode analysis with a shovel randomly selecting four to five cores of each point, and considering the upper 5-50 cm depth of soil that closed to the active plant root at each sampling spot. Nematodes were extracted from a 500-cm^3 sub-sample of soil by centrifugal flotation and a modification of Cobb´s decanting and sieving methods (Flegg, 1967). For morphometric studies, *Longidorus* and *Xiphinema* specimens were killed and fixed by a hot solution of 4% formalin + 1% glycerol, then processed in pure glycerin (Seinhorst, 1962) as modified by De Grisse (1969). The light micrographs and measurements of each nematode population including important diagnostic characteristics (i.e. de Man indices, body length, odontostyle length, lip region, tail shape, amphid

shape and oral aperture-guiding ring) were performed using a Leica DM6 compound microscope with a Leica DFC7000 T digital camera. For the line drawings of each new species, CorelDraw software version X7 (Corel Corporation, London, UK) was used to redraw according to the selected light micrographs.

2.3 | DNA extraction, PCR and sequencing

For molecular analyses, in order to ensure the selected nematodes for extracting DNA are from the same species, two live nematodes from each sample were temporary mounted in a drop of 1M NaCl containing glass beads (to avoid nematode crushing/damaging specimens) to ensure specimens conformed to the unidentified populations of *Longidorus* and *Xiphinema*. Thus, 59 individuals collected from several sampling points in Spain were analyzed (Table 1). All necessary morphological and morphometric data by taking pictures and measurements using the above camera-equipped microscope were recorded. This was followed by DNA extraction from a single specimen and polymerase chain reaction (PCR) cycle conditions as previously described (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016d). Several sets of primers were used for PCR: the expansion domains of the *28S* rRNA gene (*D2-D3*) were amplified by using the D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (De Ley et al., 1999); a partial sequence of the 18S rRNA gene (*18S*) was amplified as previously described (Holterman et al., 2006) using primers 988F (5'-CTCAAAGATTAAGCCATGC-3'), 1912R (5'-TTTACGGTCAGAACTAGGG-3'), 1813F (5'-CTGCGTGAGAGGTGAAAT-3'), and 2426R (5'-GCTACCTTGTTACGACTTTT -3'); the ITS1 region (*ITS1*) was amplified using forward primer 18S (5'-TTGATTACGTCCCTGCCCTTT-3') (Vrain et al., 1992) and reverse primer rDNA1 5.8S (5'-ACGAGCCGAGTGATCCACCG-3') (Cherry et al., 1997). Finally, the portion of the *coxI* gene was amplified using the primers COIF (5'-GATTTTTTGGKCATCCWGARG-3') and COIR (5'-CWACATAATAAGTATCATG-3') (Lazarova et al., 2006). The newly obtained sequences were deposited in the GenBank database under accession numbers indicated on the phylogenetic trees and in Table 1.

2.4 | Species delimitation

Two independent strategies of species delimitation were applied to address the first objective of this study: multivariate morphometric and haplowebs methods. These methods were based on morphometric and molecular data, respectively. The recognition of the group of species used for both approaches was not only established as belonging to *L. iliturgiensis*- and *X. hispanum*-complex (that is, similar key morphometric characters) but also determined by phylogenetic relationships

provided in previous studies (Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d; Cai et al., 2020; Cai et al., 2019; Fouladvand et al., 2019) as well as species distribution (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2019b; Archidona-Yuste et al., 2020). In addition to the new taxa, *L. tabernensis* sp. nov. and *X. subbaetense* sp. nov., the selected species list was therefore as follows: *L. indalus* and *L. iliturgiensis* for *L. iliturgiensis*-complex, and *X. adenohystherum* and *X. hispanum* for *X. hispanum*-complex. Several nematode populations from natural and agricultural areas were used for some of the selected species (Table 1). All the nematode populations were selected based on the availability of molecular data in order to avoid misidentifications.

2.4.1 | Multivariate morphometric analysis

Overall, 44 and 73 female specimens were used in multivariate morphometric approach for *X. hispanum*- and *L. iliturgiensis*-complex, respectively. Species delineation using morphology was conducted with PCA in order to estimate the degree of association among species within the *L. iliturgiensis*- and *X. hispanum*-complex (Archidona-Yuste et al., 2016a; Legendre & Legendre, 2012). PCA was based upon the following morphological characters: L (body length), the ratios a, c, c', d, d', V [(distance from anterior end to vulva/body length) x 100], odontostyle and odontophore length, lip region width and hyaline region length (Table 2, Archidona-Yuste et al., 2016a; Jairajpuri & Ahmad, 1992). Prior to the statistical analysis, variables were tested for collinearity (Zuur et al., 2010). We used the collinearity test based on the values of the variance inflation factor (VIF) method that iteratively excludes numeric covariates showing VIF values > 10 as suggested by Montgomery and Peck (1992). PCA was performed by a decomposition of the data matrix amongst populations using the principal function implemented in the package 'psych' (Revelle, 2019). We used an orthogonal varimax raw rotation was used to estimate the factor loadings. Only factors with sum of squares (SS) loadings > 1 were extracted. All statistical analyses were performed using the R v. 3.5.1 freeware (R_Core_Team, 2019).

2.4.2 | Haplotype networks construction and species delimitation analyses

In order to clarify putative molecular species, haplotype network (briefly, haplonet) was constructed to each of the two separate dataset, *i.e.* the nc *D2-D3* region and the mt *coxI* region. Alignments were converted to the NEXUS format using DnaSP V.6 (Rozas et al., 2017); TCS networks (Clement et al., 2002) were applied in the program PopART V.1.7 (http://popart.otago.ac.nz). The haplonets obtained from nc marker were converted into haplotype web (briefly, haploweb) by Adobe illustrator to add connecting curves between the haplotypes found co-occurring in heterozygous individuals (Flot et al., 2010).

2.5 | Phylogenetic analysis

Different *Longidorus* spp. and *Xiphinema* spp. sequences applied in the present study as genetic markers (*28S*, *18S*, *coxI*) were obtained from GenBank and used for phylogenetic reconstruction. Outgroup taxa for each dataset were selected based on previous published studies (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016d). Multiple sequence alignments of the newly obtained and published sequences were made using the FFT-NS-2 algorithm of MAFFT V.7.450 (Katoh et al., 2019). Sequence alignments were visualised with BioEdit (Hall, 1999) and manually edited by Gblocks ver. 0.91b (Castresana, 2000) in Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). All alignments used (pre- and post-Gblocks), original tree files, and scripts for phylogenetic analyses are available at Zenodo repository (https://zenodo.org/record/3749246#.XpMzvZlS-Uk).

Phylogenetic analyses of the sequence datasets were conducted based on Bayesian inference (BI) using MRBAYES 3.2.7a (Ronquist & Huelsenbeck, 2003) The best-fit model of DNA evolution was calculated with the Akaike information (AIC) of JMODELTEST V.2.1.7 (Darriba et al., 2012). The best-fit model, the base frequency, the proportion of invariable sites, substitution rates and the gamma distribution shape parameters in the AIC were used for phylogenetic analyses. BI analyses were performed under a general time reversible, with a proportion of invariable sites and a rate of variation across sites (GTR + I + G) model for *D2-D3*, the partial *18S* rRNA, and the partial *coxI* gene. These BI analyses were run separately per dataset with four chains for 2×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples of 10% and evaluating convergence, the remaining samples were retained for more in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees from all analyses were edited by FigTree software V.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

3 | RESULTS

The identification of species boundaries within the *Longidorus* and *Xiphinema* genera was based upon the integrative application of morphological, morphometric, and molecular methods to unravel potential cryptic species diversity (Table 1). Species delimitation was carried out using two independent approaches based on morphometric (multivariate analysis) and molecular data using ribosomal and mt sequences (haploweb and haplonet). Multivariate morphometric and haploweb

289 methods were performed on the studied populations to verify species identifications. The
290 integration of this procedure with the analysis of nematode morphology allowed us to verify
291 *Longidorus tabernensis* sp. nov. and *Xiphinema subbaetense* sp. nov. as valid new species within
292 the *Longidorus iliturgiensis* and *Xiphinema hispanum* cryptic complexes. Additionally, we
293 maintained a consensus approach for the different species delimitation methods, including
294 concordant results in phylogenetic trees inferred from nc and mt markers and/or different
295 morphological or morphometric characteristics.
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297 3.1 | Species delimitation

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299 3.1.1 | Multivariate morphometric analysis of longidoridae cryptic-complexes

300 In the principal component analysis (PCA), the first three components (sum of squares (SS)
301 loadings > 1) accounted for 64.75% and 70.42% of the total variance in the morphometric
302 characters of the *L. iliturgiensis*-complex and *X. hispanum*-complex species, respectively (Table 2).
303 Table 2 includes the SS loadings for the three extracted factors, which were a linear combination of
304 all characters in the analysis. The eigenvectors for each character were used to interpret the
305 biological meaning of the factors. First, in the *L. iliturgiensis* complex principal component 1 (PC1)
306 was dominated by d (anterior to guiding ring/body diameter at lip region) and d' (body diameter at
307 guiding ring/body diameter at lip region) ratios with a high positive weight (eigenvector = 0.44 and
308 0.46, respectively) as well as with similar but negative weight for lip region width, relating this
309 component with the overall lip region shape. PC2 was dominated by high positive weight
310 (eigenvectors = 0.54, 0.43 and 0.56) for body length, and a (body length/maximum body width) and
311 c (body length/tail length) ratios, respectively. Finally, PC3 was mainly dominated by negative
312 positive weights for a and c' ratios (eigenvectors = -0.54 and -0.67, respectively; Table 2). These
313 results suggest that all of the extracted PCs were related to the overall size and shape of nematode
314 populations. In the case of the *X. hispanum* complex, PC1 was dominated by positive weights for
315 body and odontostyle length and d ratio (eigenvectors = 0.47, 0.46 and 0.44, respectively). PC2 was
316 dominated by a positive weight (eigenvector = 0.46) for the c' (tail length/body width at anus) ratio,
317 and high negative weights for the V ((distance from anterior end to vulva/body length) × 100) ratio
318 and odontophore length (eigenvectors = -0.51 and -0.54, respectively). According to the results,
319 both principal components were related to the overall nematode size and shape as well as stylet
320 length. Finally, PC3 was mainly dominated by a high positive weight for a ratio (eigenvector =
321 0.58), relating this component with the overall nematode body shape (Table 2).
322 The results of the PCA for both cryptic complexes were represented graphically in Cartesian
323 plots in which *Longidorus* and *Xiphinema* populations were projected on the plane of the *x*- and *y*-

axes, respectively, as pairwise combinations of PC1 to 3 (Fig. 1). In the graphic representation of the *L. iliturgiensis*-complex, the specimens of each species were projected showing a wide distribution for all combinations of components owing to their wide morphometric variation within species and/or populations, which was more pronounced for *L. indalus* where a high number of populations were considered (Tables 1 and 2). With the exception of the projection on the plane of PC2 and PC3 where specimens of species were randomly situated, a wide spatial separation amongst the three *Longidorus* species was observed for the remaining pairwise combinations (Fig. 1). This spatial separation was mainly dominated by the PC1 (33.9% of the total of variance) grouping of species according to the position of the guiding ring and lip region width (Table 2). Thus, *L. indalus* specimens having a posterior guiding ring and narrower lip region were located on the right side, and on the opposite side was *L. tabernensis* sp. nov., which is characterized by an anterior position of the guiding ring and wider lip region (Fig. 1). However, specimens of *L. iliturgiensis* were located in the middle part of the plane and randomly grouped with specimens of *L. indalus* and *L. tabernensis* sp. nov., having an intermediate position of the guiding ring and a lip region width between these two species (Fig. 1). In the case of the *X. hispanum*-complex and with few exceptions, specimens and populations of each species were projected close to each other, except for *X. subbaetense* sp. nov. which showed a wide distribution for all pairwise combinations of components owing to their wide morphometric variation amongst populations (Fig. 1). However, it is necessary to highlight that this fact was not found in the remaining species where only one population was analysed (Tables 1 and 2). According to their relative position along the x-axis (PC1), the odontostyle and body length as well as body width at the guiding ring level increased from left to right, grouping species with a longer odontostyle and body and wider body at the guiding ring level on the right side (Fig. 1). According to their position along the y-axis (PC2), the length of the female tail (> c′ ratio) increased, and the position of the vulva and odontophore length decreased from bottom to top along the y-axis. Then, when projected on the plane of PC1 and PC2 (57.71% of the total variance), species with a longer odontostyle, body and female tail, shorter odontophore and an anterior position of the vulva were located on the right-top side, with a clear distinction of *X. adenohystherum* (Fig. 1). The *Xiphinema hispanum* population, having a longer female tail, anterior vulva position and shorter odontophore, was located in the top quadrants (above y = 0), compared to most specimens of *X. subbaetense* sp. nov., which were located on the bottom quadrant owing their shorter female tail, posterior vulva position and longer odontophore. However, we found some specimens of these species were mixed up given the wide morphometric variation observed for *X. subbaetense* sp. nov. as stated above (Fig. 1). A similar pattern was observed when projected on the plane of the pairwise combination among PC1 and PC3, where no clear graphic separation of any of the three species studied was observed. Finally, the clearest

graphic separation of *X. subbaetense* sp. nov. specimens from the remaining species was detected when projected on the plane of PC2 and 3 (37.5% of total variance) with most of the specimens of the new species situated on the left side because of their shorter female tail, posterior vulva position and longer odontophore (PC2; Fig. 1).

3.1.2 | Mitochondrial haplonet and nuclear haploweb

Species delimitation using haplonet methods in *L. iliturgiensis*-complex species revealed that the *28S* rRNA and *coxI* alignments contained 9 and 18 sequences with five and four different haplotypes, respectively (Table 1, Fig. 2). Moreover, no differences among sequenced individuals were found in nc *28S* rRNA sequences for *L. tabernensis* sp. nov., and in *L. indalus* and *L. iliturgiensis*, and only two haplotypes were found differing in 3 nucleotides (Table 1, Fig. 2). For this reason, haploweb was not suitable for analysis and individuals were simply classified as haplogroups. The *28S* rRNA and the *coxI* haplonets agreed with each other indicating that *L. tabernensis* sp. nov., *L. iliturgiensis* and *L. indalus* were clearly differentiated as distinct haplogroups (Fig. 2). With the *coxI* marker, two haplotypes were found for *L. iliturgiensis* with 3 nucleotide differences among both haplotypes in each species (Table 1, Fig. 2) and only one haplotype in *L. tabernensis* sp. nov. (Table 1, Fig. 2). For *X. hispanum*-complex species, the *28S* rRNA and *coxI* alignments contained 35 and 32 sequences with 18 and 10 different haplotypes, respectively (Table 1, Fig. 2). The TCS haplotype analysis inferred from the nc *28S* region showed three well-differentiated haplogroups, corresponding to three different main lineages (clades I-III) (Figure 2). Clades I and III separately consisted of *X. adenohystherum* and *X. hispanum* (Fig. 2). The two studied populations from *X. subbaetense* sp. nov., the Alto Pandera (APP) and Prado Pandera (PPP) populations located at 1800 m a.s.l. and 1352 m a.s.l., respectively, constituted clade II with 11 different haplotypes (Table 1, Fig. 2), and haplotypes from APP and PPP were separated, with 5 haplotypes from APP and 6 from PPP (Table 1, Fig. 2). However, in mt *coxI* haplonet, only two haplotypes separated by one nucleotide difference were found in *X. subbaetense* sp. nov., with one haplotype (co-sub1) comprising both populations (APP and PPP), and only one mutated position within these two haplotypes (co-sub1 vs co-sub2), the latter found only in the APP population. Additionally, these two haplotypes did not change the protein amino acid composition. The *coxI* haplonet resolved *X. subbaetense* sp. nov., *X. hispanum* and *X. adenohystherum* as separate and genetically isolated lineages in accordance with the *28S* haploweb, except for a *coxI* haplotype of *X. hispanum* (KY816614) (co-his3) from the type locality (419-Andújar), which was far away from the other two haplotypes of *X. hispanum* (co-his1 and co-his2), belonging to the 419-Andújar and AR52-Andújar populations. The 419-Andújar population showed two very different haplotypes (co-his3 and co-his 2). In addition, this sampling point (419-Andújar) showed all four

haplotypes detected for this species with the D2-D3 marker (his1-his4). With coi-his3, the individual of this population had a unique haplotype for the D2D3 marker (his4), but with scarce differences from the other haplotypes. This individual was collected several years ago and, as for the other individuals, all markers came from the same DNA extraction of a single nematode.

3.2 | Systematic description

3.2.1 | *Longidorus tabernensis* sp. nov.

ZooBank (zoobank.org) identifier: urn:lsid:zoobank.org:act:E465E695-9B4B-4ABF-9EE8-494EA5B55B2B

(Figures 3-5, Table 3)

Material examined

Morphometric measurements were taken for 50 individuals, 20 females, 9 males and 21 juveniles from first-stage (J1) to fourth-stage (J4), Table 3.

Holotype

Adult female was collected by A. Archidona-Yuste on March 16, 2019; mounted in pure glycerin and deposited in the nematode collection at Institute for Sustainable Agriculture (IAS) of Spanish National Research Council (CSIC), Córdoba, Spain (Slide number TAB-02).

Type locality

Nematodes were found in the rhizosphere of yellow broom (*Retama sphaerocarpa* L.) from Tabernas, Almería province, Spain (GPS: 37°07′25.4″E; 2°21′39.3″W) at 550 m a.s.l.

Referenced specimens

Female, male and juvenile paratypes were collected from the same soil samples as the holotype; mounted in pure glycerin and deposited in Institute for Sustainable Agriculture (IAS) of Spanish National Research Council (CSIC), Córdoba, Spain (Slide number TAB-03-TAB-06); one female and one male at Istituto per la Protezione delle Piante (IPP) of Consiglio Nazionale delle Ricerche (C.N.R.), Sezione di Bari, Bari, Italy (TAB-07); and one female and one male at the USDA Nematode Collection (P-7359p).

Etymology

The specific epithet refers to the type locality as well as the name of the desert, Tabernas, where the species was detected.

Description

Female

Body moderately long and thin, open C-shape when heated relaxed, slightly tapering towards both ends. Cuticle 2.0 ± 0.5 (1.5-2.5) µm thick at mid body, but thicker (8.9 ± 0.6 (8.0-10.0) µm) at tail tip. Lip region expanded and rounded, distinctly set off from the rest of body, 5.6 ± 0.3 (4.5-6.0) µm high. Amphidial fovea pouch-shaped with slightly asymmetrical lobes, occupying 2/5 part of distance from oral aperture to guiding ring. Guiding ring single, located 2.3-2.8 times lip region diameter from anterior end. Odontostyle 1.9-2.4 times as long as odontophore; odontophore weakly developed, with slight basal swellings. Pharynx extending to a terminal pharyngeal bulb with dorsal (DN) gland nucleus and ventrosublateral (SVN) gland nuclei separately located at 33.4 ± 2.8 (30.4-38.6) % and 54.7 ± 1.7 (52.3-56.8) % of distance from anterior end of pharyngeal bulb, respectively. Basal bulb cylindrical, 58.8 ± 1.7 (55.5-61.0) µm long and 12.6 ± 1.2 (11.0-14.0) µm in diam. Glandularium 54.1 ± 2.8 (50.0-58.0) µm long. Cardia conoid. Reproductive system with both genital branches almost equally developed with reflexed ovaries, 452.1 ± 55.8 (395.0-532.0) µm long each one. Vulva slit-like, situated at 45.2-48.9% of body length, vagina 15.4 ± 2.2 (12.5-17.5) µm long, perpendicular to body axis *ca* less than half of corresponding body width, surrounded by constrictor muscles. Sperm cells absent in the genital branch from all female specimens examined. Rectum 18.2 ± 1.9 (15.0-20.5) µm long. Tail moderately long, dorsally convex and ventrally flattened conoid, with two or three pairs of caudal pores on each side.

Male

Common, as frequent as female. Morphologically similar to female except for genital system and secondary sexual features. Male genital tract diorchic with testes opposed, containing multiple rows of spermatogonia. Tail dorsally convex-conoid, with thickened ventral outer cuticular layer. Adanal supplements paired, preceded anteriorly by a row of 6-8 irregularly spaced ventromedians supplements. Spicules paired, dorylaimoid, short, 33.4 ± 1.3 (32.0-36.0) µm long and ventrally curved, approximately 0.70-0.74 times shorter than tail length. Lateral guiding pieces with a curved proximal end.

Juveniles

Four juvenile stages were found and distinguished by relative body lengths, tail shape, odontostyle and replacement odontostyle length. The first-stage juvenile was characterized by a bluntly conoid

tail (c'=2.9, 3.0), ending with a small bulge, and the replacement odontostyle inserted into odontophore base. Morphologically the second-, third- and fourth-juvenile stages were similar to female, except for their shorter body length, immature sexual characteristics (developing genital primordium 15.5-85.5 µm long) and tail shape (Table 3, Fig. 5).

Diagnosis

Longidorus tabernensis sp. nov. is an amphimictic species characterized by a moderate long body (4.3-5.5 mm); lip region rounded distinctly offset by constriction, 9.5-10.5 µm wide and 4.5-6.0 µm high; amphidial fovea slightly asymmetrically bilobed; relatively short odontostyle (60.0-64.5 µm); guiding ring located 22.0-28.0 µm from anterior end; vulva located at 45.2-48.9 % of body length; female tail 42.0-53.0 µm long, dorsally convex and ventrally flattened conoid (c' =1.8-2.4), with two or three pairs of caudal pores. Males frequent (1:2 ratio), with very short spicules (32.0-36.0 µm) and 1 + 6-8 ventromedian supplements. Four developmental juvenile stages were found, the 1st-stage juvenile showed a conoid tail, ending with a small bulge. According to the polytomous key by Chen et al. (1997), supplement by Loof & Chen, 1999 and the addition of some characters by Peneva et al. (2013), codes for the new species are (codes in parentheses are exceptions): A2-B1-C2-D4-E3-F23-G3(24)-H6(5)-I2-J1-K6.

Relationships

According to odontostyle and body length, lip region shape, ratios a, c and c′, distance of guiding-ring from anterior body end, amphidial fovea, female and male tail shape and the abundance of males (in this order), *L. tabernensis* sp. nov. is closely related to *L. iliturgiensis* Archidona-Yuste et al. (2019a), from which it can only be differentiated by the J1 tail shape (tail digitate for *L. tabernensis* sp. nov.) and a shorter odontophore (25.5-34.0 *vs* 29.5-47.5 µm long) (Archidona-Yuste et al., 2019a), which agrees with the hypothesis that both *Longidorus* spp. may be considered as cryptic species (Fig. 2). Another species found in a close area and morphologically similar to *L. tabernensis* sp. nov. is *L. indalus* Archidona-Yuste et al. (2016d); however, the latter can be differentiated by a combination of morphological traits but particularly by a slightly longer odontostyle (60.0-64.5 *vs* 54.0-59.5 µm), the common *vs* rare presence of males and higher number of ventromedian supplements in the male tail (7-9 *vs* 5) (Archidona-Yuste et al., 2016d). In addition, *L. tabernensis* sp. nov. is molecularly related to *L. alvegus* Roca et al. (1989) and *L. rubi* Tomilin and Romanenko 1993 (Romanenko, 1998). From *Longidorus alvegus* can be mainly distinguished by a thinner lip region width (9.5-10.0 *vs* 13.1-17.0 µm), and shorter body and odontostyle lengths (4.3-5.5 *vs* 5.7-7.8 mm, 60.0-64.5 *vs* 80.0-92.5 µm; respectively) than the other species (Gutiérrez-Gutiérrez et al., 2011; Roca et al., 1989). Finally, the new species mainly differs

from *L. rubi* in having a shorter odontostyle length (60.0-64.5 *vs* 82.0-90.0 μm), a shorter spicule length (32.0-36.0 *vs* 40.0-45.0 μm) and a lower number of ventromedian supplements in the male tail (7-9 *vs* 11-12) than the other species (Gutiérrez-Gutiérrez et al., 2013; Romanenko, 1998).

3.2.2 | *Xiphinema subbaetense* sp. nov.

ZooBank (zoobank.org) identifier: urn:lsid:zoobank.org:act: 940F9643-68E0-4706-92E8-5DCF063D18CF

(Figures 5-7, Table 4)

Material examined

Morphometric measurements were taken for 51 individuals, 20 females and 20 juveniles from J1 to J4 from the type locality at 1800 m a.s.l. (APP population), and 11 females from a pasture in the same locality at 1352 m a.s.l. (PPP population), Table 4.

Holotype

Adult female was collected by R. Cai on June 9, 2019; mounted in pure glycerin and deposited in the nematode collection at Institute for Sustainable Agriculture (IAS) of Spanish National Research Council (CSIC), Córdoba, Spain (Slide number XPAND-02).

Type locality

Nematodes were found in the rhizosphere of asphodel (*Asphodelus ramosus* L.) at 1800 m a.s.l. from Valdepeñas de Jaén, Jaén province, Spain (GPS: 37º 37' 56.31" N; 3°46′24.57″W).

Referenced specimens

Female and juvenile paratypes were collected from the same soil sample as the holotype; mounted in pure glycerin and deposited in Institute for Sustainable Agriculture (IAS) of Spanish National Research Council (CSIC), Córdoba, Spain (Slide numbers XPAND-03-XPAND-06); one female at Istituto per la Protezione delle Piante (IPP) of Consiglio Nazionale delle Ricerche (C.N.R.), Sezione di Bari, Bari, Italy (XPAND-07); one female at the USDA Nematode Collection (P-7360p).

Etymology

The specific epithet refers to the Latin word Subbaetica, the mountain chain of the Iberian Peninsula where the species was found, particularly in the highest peak of this mountain range.

Description

Female

Body cylindrical, slightly tapering towards anterior end, in an open C-shape when heat relaxed. Cuticle with fine transverse striae visible in tail region, 3.6 ± 0.4 (3.0-4.0) µm thick at mid body, but thicker just posterior to anus. Lateral cord 19. ± 1.7 (17.0-21.0) µm wide, occupying *ca*. 25% of corresponding body diam. Lip region hemispherical, slightly offset from body contour by a depression, 9.4 ± 1.9 (8.0-15.0) µm high. Odontostyle moderately long, 1.3-1.6 times longer than odontophore, the latter with well-developed flanges (16.0-18.0 µm wide). Double guiding ring variable in length depending on degree of protraction/retraction of stylet. Pharynx composed by a slender narrow flexible part 304-499 µm long, and a posterior muscular, cylindrical and expanded part with three gland nuclei. Terminal pharyngeal bulb variable in length, 118.5-142.0 µm long and 22.0-35.5 µm wide. Glandularium 110.5-129.5 µm long. DN located at beginning of basal bulb (10.5-41.1%), SVN situated ca halfway along bulb (46.9-59.2%) (position of gland nuclei calculated as described by Loof & Coomans, 1968). In some specimens studied, vestigium (tip of reserve odontostyle), 2.5 µm long, observed in anterior region of slender part of pharynx. Cardia conoid-rounded and variable in length, 11.5-14.5 µm long. Intestine simple, prerectum variable in size 232-600 µm long. Rectum 31.0-44.5 µm long ending in anus as a small rounded slit. Reproductive system didelphic-amphidelphic with two equally developed branches. Each branch composed of a 120-154 µm long ovary, a reflexed oviduct 103-144 µm long, with well-developed *pars dilatata oviductus,* a sphincter, a well-developed *pars dilatata uteri*, and a 208-301 µm long uterus having pseudo Z-differentiation containing well discernible crystalloid bodies (7.5-10.0 µm long) and spines (Figs. 6-7); a 27.5-38.0 µm long vagina perpendicular to body axis (having 37-42% corresponding body diam.), ovejector well-developed 32.5-43.0 µm wide, *pars distalis vaginae* 18.1 ± 1.7 (16.0-20.0) µm long, and *pars proximalis vaginae* 14.3 ± 1.8 (12.0-16.0) µm long and 14.8 ± 1.0 (13.5-15.5) µm wide, and vulva a transverse slit. Tail short, broadly convex-conoid, dorso-ventrally convex and bearing 2 caudal pores, ending in a rounded and broad terminus.

Male

Not detected.

Juveniles

Four developmental juvenile stages were detected and distinguished by relative body length, odontostyle and replacement odontostyle length (Fig. 5). Morphologically similar to female, except for their size and sexual characteristics (Fig. 7). The first-stage juvenile was characterized by the replacement odontostyle inserted into odontophore base and tail elongate-conoid with characteristic subdigitate rounded terminus (c′ ratio 2.6-3.1). Tail of developmental stages becoming progressively shorter and wider after each moult.

Diagnosis

Xiphinema subbaetense sp. nov. is an apparently parthenogenetic species belonging to morphospecies Group 5 from the *Xiphinema* non-*americanum*-group species (Loof & Luc, 1990). It is characterized by a moderate long body (4.0-4.7 mm), assuming an open C-shaped when heat-relaxed; lip region hemispherical, separate from the body contour by a depression, 15.5-19.5 µm wide; a relatively long odontostyle 121.5-138.0 µm; vulva located at 49-54% of body length; female reproductive system didelphic-amphidelphic having both branches about equally developed, pseudo Z-differentiation containing almost 4-5 granular bodies, uterus tripartite with small crystalloid bodies and spines in low number, and presence of prominent wrinkles in the uterine wall that may be confused with spiniform structures; female tail short and broadly convex-conoid, dorso-ventrally convex and bearing 2 caudal pores; c´ ratio (0.6-0.9); males not found. Four developmental juvenile stages were identified, the 1st-stage juvenile with tail elongate-conoid with characteristic subdigitate rounded terminus (c´ ratio 2.6-3.1). According to the polytomous key of (Loof & Luc, 1990) and the updating of (Peraza-Padilla et al., 2018), codes for the new species are (codes in parentheses are exceptions): A4-B23-C6-D6-E6(5)-F4(5)-G3-H2-I3-J6-K2-L1.

Relationships

Morphologically and according to the polytomous key by Loof & Luc (1990) and matrix codes reported by Peraza-Padilla et al. (2018): A (type of female genital apparatus), C (tail shape), D (c' ratio), E (vulva position), F (body length), and G (total stylet length), *X. subbaetense* sp. nov. is closely related to *X. hispanum* Lamberti et al., 1992, *X. adenohystherum* Lamberti et al., 1992, *X. sphaerocephalum* Lamberti et al., 1992 and *X. cohni* Lamberti *et al.*, 1992. *Xiphinema subbaetense* sp. nov. is morphologically almost undistinguishable from *X. hispanum*, from which it differs in J1 tail shape (elongate-conoid with characteristic subdigitate rounded terminus *vs* elongate-conoid without terminal swelling), and female tail shape (broadly convex-conoid with rounded tip *vs* conoid with a central bulge); however, it can be clearly differentiated by the specific molecular markers *28S*, *ITS1* rRNA and *coxI* sequences. *Xiphinema subbaetense* sp. nov. can be differentiated from *X. adenohystherum* by its shorter odontostyle (121.5-138.0 *vs* 143.0-152.0 µm), longer tail (30.0-41.5 *vs* 29.0-35.0 µm), a wider lip region (15.5-18.5 *vs* 13.0-15.0 µm), and slightly lower a ratio (49.0-64.3 *vs* 65.2-73.3). It can be differentiated from *X. sphaerocephalum* by its shorter odontostyle (121.5-138.0 *vs* 143.5-168.0 µm), shorter oral aperture-guiding ring distance (106.5-131.5 *vs* 126.0-162.0 µm), and the absence of males. Finally, *X. subbaetense* sp. nov. can be differentiated from *X. cohni* by its shorter odontostyle (121.5-138.0 *vs* 149-174 µm), shorter oral aperture-guiding ring distance (106.5-131.5 *vs* 137.0-161.0 µm), slightly shorter tail (30.0-41.5 *vs*

36.5-48.0 μm), and slightly higher c ratio (101.9-139.4 *vs* 82.6-115.2) than those of *X. cohni*. Although some morphometric differences were detected between APP and PPP populations, in body and odontostyle length (4.0-4.7 mm, 121.5-138.0 μm *vs* 4.6-5.3 mm, 138.0-149.5 μm, respectively), no significant molecular differences were detected among both populations for the *coxI* marker and only a few molecular differences were found for marker *D2-D3*.

In addition, *X. subbaetense* sp. nov. is molecularly related to *X. celtiense* Archidona-Yuste et al. (2016c), but it can be clearly differentiated by its shorter body length (4.0-4.7 *vs* 4.7-5.5 mm), shorter odontostyle and odontophore length (121.5-138.0 *vs* 145.0-167.0 μm, 82.0-92.0 *vs* 89.0-103.0 μm, respectively), slightly wider lip region (15.5-18.5 *vs* 13.5-16.0 μm), shorter oral aperture-guiding ring distance (106.5-131.5 *vs* 132.0-155.0 μm), pseudo-Z-differentiation containing almost 4-5 granular bodies *vs* 15, lower a ratio (49.0-63.4 *vs* 67.4-81.0), than those of *X. celtiense* as well as the lack of males in the new species (absent *vs* present).

3.3 | Molecular characterisation of *Longidorus tabernensis* sp. nov. and *Xiphinema subbaetense* sp. nov.

The amplification of *D2-D3* expansion domains of *28S* rRNA, partial *18S* rRNA, *ITS1* rRNA and partial *coxI* regions yielded single fragments of *ca* 900 bp, 1800 bp, 1100 bp and 500 bp, respectively, based on gel electrophoresis, and after discarding primer sequences and ambiguously aligned regions from the alignment. Sequences from *L. tabernensis* sp. nov. and *X. subbaetense* sp. nov. obtained in this study, and from other species of *Longidorus* and *Xiphinema* collected from GenBank were used for further phylogenetic analyses. The low similarity of the ITS1 region and low coverage from *L. tabernensis* sp. nov. and *X. subbaetense* sp. nov. and the rest of ITS1 sequences deposited in GenBank made impossible to perform phylogenetic analyses for this molecular marker.

The DNA sequences of *D2-D3* expansion domains of *28S*, *18S* rRNA, *ITS1* rRNA and partial *coxI* for *L. tabernensis* sp. nov. were deposited in GenBank under the accession numbers MK941194-MK941197, MK941261, MK941256-MK941257 and MK937587-MK937588, respectively. The *D2-D3* expansion domains of *28S* for *L. tabernensis* sp. nov. (MK941194-MK941197) differed from the closest related species, *L. iliturgiensis* (MH430012) by 18 different nucleotides and 0 indels (98% similarity), from *L. rubi* (JX445116) by 39 different nucleotides and 4 indels (95% similarity), and from *L. indalus* (KT308853) by 62 different nucleotides and 5 indels (91% similarity). The *ITS1* of *L. tabernensis* sp. nov. (MK941256, MK941257) showed a low intraspecific variability within this population with only one different nucleotide, 0 indel (99% similarity), and the closest species was *L. iliturgiensis* (MH429988, 79% similarity, 196 different nucleotides, 112 indels). The partial *18S* sequence of *L. tabernensis* sp. nov. (MK941261) showed a

high level of similarity with several *Longidorus* species, such as *L. elongatus* (EU503141), *L. uroshis* (EF538760), and *L. piceicola* (AY687993), and to a lesser extent *L. indalus* (KT308894), by 6 nucleotides and 0 indels (99% similarity). Finally, the partial *coxI* sequences of *L. tabernensis* sp. nov. (MK937587-MK937588, MT040266-MT040270) showed low intraspecific variability within this population with 1-4 different nucleotides and 1 indel (99.7-98.9% similarity), and the closest species were *L. iliturgiensis*, *L. cretensis, L. pseudoelongatus*, and *L. indalus*, differing in 78, 83, 86, and 96 nucleotides, 0 to 2 indels, and showing 76, 78, 77, and 74% similarity, respectively.

The DNA sequences of *D2-D3* expansion domains of *28S*, *18S* rRNA, *ITS1* rRNA and partial *coxI* for *X. subbaetense* sp. nov. were deposited in GenBank under the accession numbers MT039104-MT039124, MT039135-MT039140, MT026293-MT026295 and MT040280-MT040300, respectively. The *D2-D3* expansion domains of *28S* (MT039104-MT039124) showed a low intraspecific variability with 2-8 different nucleotides and 0 indels (99% similarity). The molecular diversity of this marker within APP (5-7 nucleotides, 0 indels) and PPP (1-2 nucleotides, 0 indels) populations was similar to that detected between APP and PPP populations (2-8 nucleotides, 0 indels). Also, differed from the closest related species, *X. hispanum* (KX244905, MT039125-MT039134) by 22-25 different nucleotides and 1-3 indels (97% similarity), and from *X. adenohystherum* (KC567164, KX244898, GU725075, KX244897) by 23-24 different nucleotides and 2 indels (97% similarity). The *ITS1* of *X. subbaetense* sp. nov. (MT026293-MT026295) showed moderate intraspecific variability within this population with only 14-37 different nucleotides and 4-19 indels (98-97% similarity), and the closest related species were *X. hispanum* (GU725061, 88% similarity, 131 different nucleotides, 25 indels), and *X. adenohystherum* (GU725063, 87% similarity, 138 different nucleotides, 39 indels). No intraspecific variability was found in partial *18S* rRNA sequences of *X. subbaetense* sp. nov. (MT039135-MT039140) and a high level of similarity (99% similarity)was found with several *Xiphinema* species, such as *X. celtiense* (KX244943), *X. pyrenaicum* (GU725085) and *X. vuittenezi* (AY552979). Finally, the partial *coxI* sequences of *X. subbaetense* sp. nov. (MT040280-MT040300) showed low intraspecific variability with 1-5 different nucleotides and 0 indels (99-98% similarity). The molecular diversity of this marker within APP (1 nucleotide, 0 indels) and PPP (5 nucleotides, 0 indels) populations was similar to that detected between APP and PPP populations (1-5 nucleotides, 0 indels). Additionally, the closest species were *X. vuittenezi*, *X. hispidum* and *X. celtiense*, showing similarity values of 83% with all of them (from 57 to 65 nucleotides and 0 to 6 indels).

3.4 | Phylogenetic relationships

The phylogenetic relationships among *Longidorus* and *Xiphinema* species inferred from analyses of *D2-D3* expansion domains of *28S* rRNA gene sequences using BI are given in Figs. 8, 9. The *D2-D3* tree of *Longidorus* spp. based on a multiple edited alignment including 116 sequences and 742 total characters revealed four highly supported major clades (marked with roman numerals from I to IV) (Fig. 8). Clade I is well-supported (PP = 1.0), including 39 species. The majority of these species were from the Iberian Peninsula and shared a short hemispherical to bluntly conoid tail (c' = 1.0), and the lip region anteriorly rounded, continuous or slightly depressed from body contour, except for a well-supported subclade (PP = 1.00) including *L. tabernensis* sp. nov. (MK941194-MK941197), *L. iliturgiensis* and *L. alvegus*, with a rounded lip region distinctly offset by a constriction, and a long dorsally convex and ventrally flattened conoid female tail (c' = 1.8-2.9) (Fig. 8). The *D2-D3* tree of *Xiphinema* spp. based on a multiple edited alignment including 102 sequences and 752 total characters showed two clearly separate clades (Fig. 9). Clade I was well supported (PP = 1.00), including 43 species from all morphospecies groups, half of them belonging to morphospecies Group 5, and the majority of these species were reported from the Iberian Peninsula and included *X. subbaetense* sp. nov. (MT039104-MT039124) but also other species belonging to morphospecies Group 1 (*X. brasiliense, X. chambersi, X. hangzhouense, X. hunaniense, X. naturale*), Group 2 (*X. costaricense*), Group 3 (*X. poasense*), Group 4 (*X. ifacolum, X. oleae*), Group 6 (*X. afratakhtehnsis*, *X. azarbaijanensis*, *X. robbinsi, X. zagrosense*), Group 7 (*X. barense*, *X. elongatum*, *X. insigne*, *X. israeliae*, *X. italiae, X. lupini, X. savanicola, X. setariae*), and Group 8 (*X. granatum*, *X. vuittenezi*) (Fig. 9). Morphospecies groups were based on the structural diversity of the female reproductive system and female tail shape (Loof & Luc, 1990). *Xiphinema subbaetense* sp. nov. (MT039104-MT039124) occupies a superior position within clade I clustering with *X. hispanum*, *X. celtiense*, *X. cohni* and *X. histriae* (Fig. 9) in a well-supported subclade (PP = 0.99). Clade II was also well supported (PP = 0.99), including 21 species belonging mostly to morphospecies Group 5, except for *X. bakeri*, and *X. index* which belonged to Groups 7, and 8, respectively (Fig. 9).

The phylogenetic relationships among *Longidorus* and *Xiphinema* species inferred from analyses of partial *18S* rRNA gene sequences using BI are given in Figs. 10, 11. Based on the 50% majority rule consensus of *Longidorus* spp., the BI tree based on a multiple edited alignment including 83 sequences and 1728 total characters showed several major clades (Fig. 10). *Longidorus tabernensis* sp. nov. (MK941261) clustered with *L. iliturgiensis* (MH430002) and *L. kheiri* (EU503142) in a low supported subclade (Fig. 10). The partial *18S* rRNA tree of *Xiphinema* spp. based on a multiple edited alignment including 61 sequences and 1676 total characters also showed several major clades (Fig. 11). *Xiphinema subbaetense* sp. nov. (MT039135-MT039140) clustered with *X. hispanum* and *X. adenohystherum*, and other species from morphospecies Group 5 (Fig. 11).

Finally, the phylogenetic relationships among *Longidorus* and *Xiphinema* species inferred from analyses of partial *coxI* gene sequences using BI are given in Figs. 12, 13. The *coxI* region of *Longidorus* spp. using a multiple alignment of 108 sequences and 289 characters showed several clades that were not well defined, and *L. tabernensis* sp. nov. (MK937587-937588, MT040266-MT040270) clustered with *L. laevicapitatus* (MH430002) in a well-supported clade (PP = 0.90), and clearly separated from *L. iliturgiensis* (MH454065, MT040271-MT040275) and *L. indalus* (KY816675, MT040276-MT040279) in different subclades (Fig. 12). Similarly, the *coxI* region of *Xiphinema* spp. using a multiple alignment of 82 sequences and 338 characters showed several clades that were not well defined (Fig. 13). *Xiphinema subbaetense* sp. nov. (MT039104-MT039124) clustered with *X. hispanum* (KY816614, Mt040301-MT040305), *X. adenohystherum* (KY816588-KY816592), and other species from morphospecies Group 5 (Fig. 13).

4 | DISCUSSION

This study aimed to obtain knowledge and a better understanding of the presence of cryptic species complexes within the genera *Longidorus* and *Xiphinema*, assessing the potential of using diagnostic morphological, allometric, and molecular markers to differentiate species within the *L. iliturgiensis*- and *X. hispanum*-species complexes. In fact, we have described here two new species, *Longidorus tabernensis* sp. nov. and *Xiphinema subbaeten*se sp. nov. during additional surveys in natural environments in southern Spain which closely resembled to the morphological features describing *L. iliturgiensis* and *X. hispanum*species complexes. There are few distinguishing features that can identify each of the new species, such as the J1 tail shape in *X. subbaetense* sp. nov. *vs X. hispanum*, as well as in *L. tabernensis* sp. nov. *vs L. iliturgiensis* (tail digitate for *L. tabernensis* sp. nov.). This supports the concept that juvenile stages, particularly J1s of Dorylaimida, including Longidoridae, have a decisive practical significance when distinguishing closely related species (Hunt, 1993). . Multivariate morphometric analyses have proven to be useful tools for species delimitation within the genera *Longidorus* and *Xiphinema* (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d). The results of the multivariate analysis identified the overall lip region shape described by the position of the guiding ring and lip region width as key morphometric characters to differentiate some closely related species (*L. iliturgiensis*, *L. indalus* and *L. tabernensis* sp. nov.) within *L. iliturgiensis*-complex (Table 2). This result is in agreement with the taxonomic statement describing the position of the guiding ring as fundamental feature in combination with lip region shape in the identifying species within the genus *Longidorus* (Archidona-Yuste et al., 2016d; Loof & Luc, 1990; Loof et al., 1996). Although some specimens for some species, such as *L. iliturgiensis* share similar values for most of the morphological characters with the remaining species included in this study, multivariate analysis allowed us to

differentiate species within this cryptic complex using a discrete number of characters (Table 2, Fig. 1). Indeed, specimens of *L. tabernensis* sp. nov. and *L. indalus* form two clearly distinct groups and those of *L. iliturgiensis* are located in an intermediate position, supporting the naming of this complex group through this species (Fig. 1). On the other hand, multivariate principal component analysis revealed body and stylet length as well as the position of the vulva and female tail shape as key morphometric features for species delimitation within closely related species (*X. hispanum*, *X. adenohystherum* and *X. subbaetense* sp. nov.) of the *X. hispanum*-complex (Table 2). As in the *L. iliturgiensis*-complex but even more evident, some specimens, particularly *X. subbaetense* sp. nov. showed values outside the overall mean value of the species for some morphometric characters, making their accurate identification difficult and suggesting membership to another different species. In fact, some morphometric characteristics and ratios apparently showed significant differences between APP and PPP individuals of *X. subbaetense* sp. nov. (*viz.* body and odontostyle length, Table 4). However, multivariate analysis also supports the idea of a unique species clearly separated from *X. adenohystherum* (Fig. 1). It is relevant to point out that *X. hispanum* and *X. subbaetense* sp. nov. could resemble the same species given the wide morphometric variation in some characters observed in the new taxa as stated above. However, surprisingly and based on this statistical analysis, specimens of both species formed two distinct groups (particularly when using PC2 and 3, Fig. 1), delimiting both species when a combination of morphometric characters was used (Table 2). However, some specimens showed values outside the overall mean value of the species for some morphometric characters, making their accurate identification difficult and suggesting membership to another different species.

In this regard, the haplonet results of *L. iliturgiensis*-complex species clearly separated *L. tabernensis* sp. nov. from *L. iliturgiensis* and *L. indalus*. Similarly, the haploweb analysis of the *X. hispanum* complex, showed that *X. subbaetense*. nov. is a unique and separate species from *X. hispanum* and *X. adenohystherum*. Consequently, the important differences found in the morphometric analysis between APP *vs* PPP populations of *X. subbaetense*. nov. in body and odontostyle length (4.0-4.7 mm, 121.5-138.0 µm *vs* 4.6-5.3 mm, 138.0-149.5 µm, respectively) must be considered intraspecific variation of the species and populations. There was not a link between the morphometric differences and molecular differences within these *X. subbaetense* sp. nov. populations using the molecular markers *coxI* and *D2-D3*. The APP sampling point for *X. subbaetense* sp. nov. at 1800 m. a.s.l. seems to be a more restrictive habitat for nematode survival during the winter with respect to the PPP sampling point at 1352 m a.s.l. because of low temperatures, in addition, there are differences in the vegetation between these sites, with the former composed mainly of asphodel and the latter composed mainly of graminaceous grasses. To date, nematodes of the family Longidoridae have shown higher diversity for *coxI* marker than

774 ribosomal markers (Palomares-Rius et al., 2017b). In some species of this family, even the *coxI*
775 marker displayed a similarity lower than 90% (Palomares-Rius et al., 2017b), and in the case of
776 *Longidorus orientalis* Loof (1982), this high variability (15.5% intraspecific *coxI* variability, only
777 1% intraspecific amino acid variation) was not associated with ribosomal variability (Subbotin et
778 al., 2015). Surprisingly, for the *L. iliturgiensis* complex and *X. hispanum* complex, the variability of
779 *28S* rRNA was higher than that of mtDNA, even using direct sequencing of the PCR product, in
780 which the sequence obtained was the majority among the different copies of the rRNA gene array in
781 the genome (Bik et al., 2013). This was also found when the ITS1 region was used, even with the
782 few sequences obtained for this study. We found a similar scenario in *X. hispanum*, with four
783 haplotypes for *28S* rRNA and two haplotypes for *coxI*. On the other hand, some PPNs do not have a
784 unique major copy of the ribosomal genes in their genome, as is the case in the genus *Rotylenchulus*
785 (Palomares-Rius et al., 2018; Van Den Berg et al., 2016). In this study, the *28S* rRNA haplotypes
786 were specific to each sampling point, and they were not shared among sites for this marker.
787 However, this is not the same for the *coxI* marker, in which the same haplotype was shared between
788 the APP and PPP sampling sites. A similar situation of high intraspecific and intrapopulation
789 diversities has been found in *Cephalenchus* spp., in which the variation of rRNA surpassed the mt
790 gene *coxI* using a clone sequencing strategy per individual (Pereira & Baldwin, 2016). Pereira &
791 Baldwin (2016) suggested that the high levels of intraspecific polymorphism could be mostly due to
792 intragenomic variation with functional rRNA copies, and this variability was suggested by the
793 potential cross-fertilization in some *Cephalenchus* spp. Recently, another paper by Qing et al.
794 (2020) found that levels of variation varied widely across rRNA loci and species in a wide study
795 across 30 terrestrial nematode species, with some taxa observed to lack rRNA polymorphism
796 entirely. In our case, direct PCR sequencing could lead to sequencing of the major haplotype for
797 each individual, which, with our data, seems to be different for some individuals in each population.
798 However, we did not use several clones per individual to sequence this region and the intragenomic
799 variability was not determined. Additionally of cross-fertilization in some species, the distribution
800 of rRNA gene arrays in different regions of the genome can also affect the ability of concerted
801 evolution if they are found in different chromosomes (Fenton et al., 1998; Keller et al., 2006). In the
802 case of *Caenorhabditis elegans*, rRNA repeat numbers are all in chromosome 1 and could vary
803 from 56 to 32, as estimated by Bik et al. (2013).

804 The differential haplotype diversity detected between the two longidorid populations of the *L.*
805 *iliturgiensis* and *X. hispanum* complexes prompted us to perform a species separation analysis based
806 on the *28S* rRNA and *coxI* markers. In our case, for the *L. iliturgiensis* complex, all species had good
807 congruence between the taxonomy applied for this group and the species separation analyses
808 obtained. Similarly, in the *X. hispanum* complex, the presence of only one haplotype for *coxI* shared

between both populations of *X. subbaetense* sp. nov. (APP and PPP), strongly supports the idea of a unique species. On the other hand, topotype specimens of *X. hispanum* (419-Andújar) and one additional population (AR52-Andújar) of this species were also studied showing different results depending on the molecular marker used in the study. In this case, the presence of a different haplotype for *coxI* (KY816614), not detected in the most recent sampling, may suggest a selection excluding these individuals from this population. However, only one individual was selected in the first sampling and a conclusion about this hypothesis requires additional sampling and sequencing.

Genomic and mt markers have already been extensively used in species identification of longidorid nematodes (Archidona-Yuste et al., 2019a; Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d). In general, the phylogenetic relationships inferred in this study support most of the previously reported lineages within *Longidorus* and *Xiphinema* (Archidona-Yuste et al., 2016c; Archidona-Yuste et al., 2016d; Cai et al., 2020) except for those inferred with *coxI*. Phylogenetic inferences based on the *D2-D3* expansion domains of *28S* and *18S* rRNA genes suggest that *L. tabernensis* sp. nov. and *L. iliturgiensis*, as well as, *X. subbaetense* sp. nov. and *X. hispanum* are related species, although results of all analyses on both species were consistent and clearly separated them by phylogenetic and species delimitation methods (Figs. 2, 8-13).

5 | CONCLUSIONS

Through this study, we clarify and provide new insights into the diversity of *Longidorus* and *Xiphinema* species detected in southern Spain. We found evidence for cryptic species within *L. iliturgiensis*- and *X. hispanum*-complex species and the utilized integrative taxonomic approaches can clearly separate them within these groups. In addition, our results suggest that the genera *Longidorus* and *Xiphinema* have a greater tendency than which other genera for cryptic speciation. These results also support our hypothesis that the biodiversity of *Longidorus* and *Xiphinema* in this region is higher than that previously supposed, and needs additional sampling efforts to be fully clarified. This study emphasizes the need for using integrative taxonomic approaches, including morphological, multivariate, molecular and species delimitation analyses, to better understand and decipher the cryptic diversity of this important and complex group of PPNs.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTIONS

AAY, JEPR, and PC conceived the ideas and designed methodology; RC, AAY, CCN, JEPR, and PC collected the data; RC, AAY, JEPR, and PC analysed the data; RC, AAY, CCN, JEPR, and PC led the writing of the manuscript. All authors contributed to the final discussion data, and read and approved the final manuscript.

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867 **REFERENCES**

868

869 Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Castillo, P., & Palomares-Rius J. E. (2019a).
870 Molecular phylogenetic analysis and comparative morphology reveals the diversity and
871 distribution of needle nematodes of the genus *Longidorus* (Dorylaimida: Longidoridae) from
872 Spain. *Contributions to Zoology* 88, 1-41. doi.org/10.1163/18759866-20191345
873 Archidona-Yuste, A., Navas-Cortés, J.A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., &
874 Castillo, P. (2016a). Cryptic diversity and species delimitation in the *Xiphinema americanum*-
875 group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular
876 markers. *Zoological Journal of the Linnean Society* 176, 231-265. doi.org/10.1111/zoj.12316
877 Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., &
878 Castillo, P. (2016b). Molecular phylogenetic analysis and comparative morphology resolve
879 two new species of olive-tree soil related dagger nematodes of the genus *Xiphinema*
880 (Dorylaimida: Longidoridae) from Spain. *Invertebrate Systematics* 30, 547-565.
881 doi.org/10.1071/IS16002
882 Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., &
883 Castillo, P. (2016c). Remarkable diversity and prevalence of dagger nematodes of the genus
884 *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) in olives revealed by integrative
885 approaches. *PLOS ONE* 11, e0165412. doi.org/10.1371/journal.pone.0165412
886 Archidona-Yuste, A., Navas-Cortés, J. A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., &
887 Castillo, P. (2016d). Unravelling the biodiversity and molecular phylogeny of needle
888 nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and a description of
889 six new species. *PLOS ONE* 11, e0147689. doi.org/10.1371/journal.pone.0147689
890 Archidona-Yuste, A., Wiegand, T., Castillo, P., & Navas-Cortés, J. A. (2019b). Dataset on the
891 diversity of plant-parasitic nematodes in cultivated olive trees in southern Spain. *Data in Brief*
892 27, 104658. doi.org/10.1016/j.dib.2019.104658
893 Archidona-Yuste, A., Wiegand, T., Castillo, P., & Navas-Cortés, J. A. (2020). Spatial structure and
894 soil properties shape local community structure of plant-parasitic nematodes in cultivated
895 olive trees in southern Spain. *Agriculture, Ecosystems & Environment* 287, 106688.
896 doi.org/10.1016/j.agee.2019.106688
897 Bärmann, E. V., Wronski, T., Lerp, H., Azanza, B., Börner, S., Erpenbeck, D., Rössner, G. E., &
898 Wörheide, G. (2013). A morphometric and genetic framework for the genus *Gazella* de
899 Blainville, 1816 (Ruminantia: Bovidae) with special focus on Arabian and Levantine
900 mountain gazelles. *Zoological Journal of the Linnean Society* 169, 673-696.
901 doi.org/10.1111/zoj.12066

902 Barnes, A. D., Jochum, M., Lefcheck, J. S., Eisenhauer, N., Scherber, C., O'Connor, M. I., de
903 Ruiter, P., & Brose, U. (2018). Energy Flux: The Link between multitrophic biodiversity and
904 ecosystem functioning. *Trends in Ecology & Evolution* 33, 186-197.
905 doi.org/10.1016/j.tree.2017.12.007
906 Baujard, P., Luc, M., & Loof, P. A. A. (1996). *Xiphinema pyrenaicum* Dalmasso, 1964 and its
907 synonyms (Nematoda: Longidoridae). *Fundamental and Applied Nematology* 19, 293-296.
908 Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., &
909 Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology*
910 *& Evolution* 22, 148-155. doi.org/10.1016/j.tree.2006.11.004
911 Bik, H. M., Fournier, D., Sung, W., Bergeron, R. D., & Thomas, W. K. (2013). Intra-genomic
912 variation in the ribosomal repeats of nematodes. *PLOS ONE* 8, e78230.
913 doi.org/10.1371/journal.pone.0078230
914 Cai, R., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., & Castillo, P.
915 (2020). Integrative descriptions and molecular phylogeny of two new needle nematodes of the
916 genus *Longidorus* (Nematoda: Longidoridae) from Spain. *European Journal of Plant*
917 *Pathology* 156, 67-86. doi.org/10.1007/s10658-019-01862-4
918 Cai, R., Archidona-Yuste, A., Cantalapiedra-Navarrete, C., Palomares-Rius, J. E., Zheng, J., &
919 Castillo, P. (2019). Integrative taxonomy of *Xiphinema histriae* and *Xiphinema lapidosum*
920 from Spain. *Journal of Nematology* 51, e2019-2037. doi: 10.21307/jofnem-2019-037
921 Cameron, E. K., Martins, I. S., Lavelle, P., Mathieu, J., Tedersoo, L., Gottschall, F., Guerra, C. A.,
922 Hines, J., Patoine, G., Siebert, J., Winter, M., Cesarz, S., Delgado-Baquerizo, M., Ferlian, O.,
923 Fierer, N., Kreft, H., Lovejoy, T. E., Montanarella, L., Orgiazzi, A., Pereira, H. M., Phillips,
924 H. R. P., Settele, J., Wall, D. H., & Eisenhauer, N. (2018). Global gaps in soil biodiversity
925 data. *Nature Ecology & Evolution* 2, 1042-1043. doi.org/10.1038/s41559-018-0573-8
926 Cantalapiedra-Navarrete, C., Navas-Cortés, J. A., Liébanas, G., Vovlas, N., Subbotin, S. A.,
927 Palomares-Rius, J.E., & Castillo, P. (2013). Comparative molecular and morphological
928 characterisations in the nematode genus *Rotylenchus*: *Rotylenchus paravitis* n. sp., an example
929 of cryptic speciation. *Zoologischer Anzeiger - A Journal of Comparative Zoology* 252, 246-
930 268. doi.org/10.1016/j.jcz.2012.08.002
931 Castresana, J. (2000). Selection of Conserved Blocks from Multiple Alignments for Their Use in
932 Phylogenetic Analysis. *Molecular Biology and Evolution* 17, 540-552.
933 doi:10.1093/oxfordjournals.molbev.a026334
934 Chen, Q. W., Hooper, D. J., Loof, P. A. A., & Xu, J. (1997). A revised polytomous key for the
935 identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda:
936 Dorylaimoidea). *Fundamental and Applied Nematology* 20, 15-28.

Cherry, T., Szalanski, A. L., Todd, T. C., & Powers, T. O. (1997). The internal transcribed spacer region of *Belonolaimus* (Nemata: Belonolaimidae). *Journal of Nematology* 29, 23-29.

Cho, M. R., & Robbins, R. T. (1991). Morphological variation among 23 *Xiphinema americanum* populations. *Journal of Nematology* 23, 134-144.

Clement, M., Snell, Q., Walker, P., Posada, D., & Crandall, K. (2002). TCS: Estimating gene genealogies. 6th, International parallel and distributed processing symposium. Fort Lauderdale, FL: IEEE Computer Society, 184.

Cobb, N. A. (1913). New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of the Washington Academy of Sciences* 3, 432-444.

Coomans, A. (1996). Phylogeny of the Longidoridae. *Russian Journal of Nematology* 4, 51-60.

Coomans, A., Huys, R., Heyns, J., & Luc, M. (2001). Character analysis, phylogeny, and biogeography of the genus *Xiphinema* Cobb, 1973 (Nematoda, Longidoridae). Vol. 287, Annales du Musée Royal de l'Afrique Centrale (Zoologie), Tervuren, Belgique.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772. doi:10.1038/nmeth.2109

Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85, 407-415. doi.org/10.1111/j.1095-8312.2005.00503.x

De Grisse, A. T. (1969). Redescription ou modification de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen Rijksfakulteit Landbouwwetenschappen* 34, 351.

De Ley, P., Felix, M. A., Frisse, L. A., Nadler, S., Sternberg, P., & Thomas, W. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1, 591-612. doi.org/10.1163/156854199508559

Decaëns, T. (2010). Macroecological patterns in soil communities. *Global Ecology and Biogeography* 19, 287-302. doi.org/10.1111/j.1466-8238.2009.00517.x

Decraemer, W., & Robbins, R. T. (2007). The Who, What and where of Longidoridae and Trichodoridae. *Journal of Nematology* 39, 295-297.

Fenton, B., Malloch, G., & Germa, F. (1998). A study of variation in rDNA ITS regions shows that two haplotypes coexist within a single aphid genome. *Genome* 41, 337-345. doi.org/10.1139/g98-030

Flegg, J. J. M. (1967). Extraction of *Xiphinema* and *Longidorus* species from soil by a modification of Coob´s decanting and sieving technique. *Annals of Applied Biology* 60, 429-437.

Flot, J. F., Couloux, A., & Tillier, S. (2010). Haplowebs as a graphical tool for delimiting species: a revival of Doyle's "field for recombination" approach and its application to the coral genus

Pocillopora in Clipperton. *BMC Evolutionary Biology* 10, 372. doi.org/10.1186/1471-2148-10-372

Fouladvand, Z. M., Pourjam, E., Castillo, P., & Pedram, M. (2019). Genetic diversity, and description of a new dagger nematode, *Xiphinema afratakhtehnsis* sp. nov., (Dorylaimida: Longidoridae) in natural forests of southeastern Gorgan, northern Iran. *PLOS ONE* 14, e0214147. doi.org/10.1371/journal.pone.0214147

Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Montes Borrego, M., Palomares-Rius, J. E., & Castillo, P. (2013). Molecular phylogeny of the nematode genus *Longidorus* (Nematoda: Longidoridae) with description of three new species. *Zoological Journal of the Linnean Society* 167, 473-500. doi:10.1111/zoj.12019

Gutiérrez-Gutiérrez, C., Palomares-Rius, J. E., Cantalapiedra-Navarrete, C., Landa, B. B., Esmenjaud, D., & Castillo, P. (2010). Molecular analysis and comparative morphology to resolve a complex of cryptic *Xiphinema* species. *Zoologica Scripta* 39, 483-498. doi.org/10.1111/j.1463-6409.2010.00437.x

Gutiérrez-Gutiérrez, C., Palomares-Rius, J., Cantalapiedra-Navarrete, C., Landa, B. B., & Castillo, P. (2011). Prevalence, polyphasic identification, and molecular phylogeny of dagger and needle nematodes infesting vineyards in southern Spain. *European Journal of Plant Pathology* 129, 427-453. doi:10.1007/s10658-010-9705-y

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95-98.

He, Y., Subbotin, S. A., Rubtsova, T. V., Lamberti, F., Brown, D. J. F., & Moens, M. (2005). A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology* 7, 111-124. doi:10.1163/1568541054192108

Hebert, P. D. N., Ratnasingham, S., de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270, S96-S99. doi: 10.1098/rsbl.2003.0025

Holterman, M., Van Der Wurff, A., Van Den Elsen, S., Van Megen, H., Bongers, T., Holovachov, O., Bakker, J., & Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Phylogenetic Evolution* 23, 1792-1800. doi:10.1093/molbev/msl044

Hunt, D. J. (1993). Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics. CAB International.

Jairajpuri, M.S., & Ahmad, W. (1992). Dorylaimida: Free-Living, Predaceous and Plant-Parasitic Nematodes. New Delhi; Oxford: IBH Publishing Co.

1006 Jörger, K. M., & Schrödl, M. (2013). How to describe a cryptic species? Practical challenges of
1007 molecular taxonomy. *Frontiers in Zoology* 10, 59. doi.org/10.1186/1742-9994-10-59
1008 Katoh, K., Rozewicki, J., & Yamada, K.D. (2019). MAFFT online service: multiple sequence
1009 alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20,
1010 1160-1166. doi: 10.1093/bib/bbx108
1011 Keller, I., Chintauan-Marquier, I. C., Veltsos, P., & Nichols, R. A. (2006). Ribosomal DNA in the
1012 grasshopper *Podisma pedestris*: escape from concerted evolution. *Genetics* 174, 863-874. doi:
1013 10.1534/genetics.106.061341
1014 Kuta, E., Jędrzejczyk-Korycińska, M., Cieślak, E., Rostański, A., Szczepaniak, M., Migdałek, G.,
1015 Wąsowicz, P., Suda, J., Combik, M., & Słomka, A. (2014). Morphological versus genetic
1016 diversity of *Viola reichenbachiana* and *V. riviniana* (sect. *Viola*, Violaceae) from soils
1017 differing in heavy metal content. *Plant Biology* 16, 924-934. doi.org/10.1111/plb.12143
1018 Lajus, D., Sukhikh, N., & Alekseev, V. (2015). Cryptic or pseudocryptic: can morphological
1019 methods inform copepod taxonomy? An analysis of publications and a case study of the
1020 *Eurytemora affinis* species complex. *Ecology and Evolution* 5, 2374-2385.
1021 doi:10.1002/ece3.1521
1022 Lamberti, F., Castillo, P., Gómez-Barcina, A., & Agostinelli, A. (1992). Descriptions of six new
1023 species of *Xiphinema* (Nematoda, Dorylaimida) from the Mediterranean region. *Nematologia*
1024 *Mediterranea* 20, 125-139.
1025 Lazarova, S., Oliveira, C.M.G., Prior, T., Peneva, V., & Kumari, S. (2019). An integrative approach
1026 to the study of *Xiphinema brevicolle* Lordello and Da Costa 1961, supports its limited
1027 distribution worldwide (Nematoda: Longidoridae). *European Journal of Plant Pathology* 153,
1028 441-464. doi.org/10.1007/s10658-018-1571-z
1029 Lazarova, S. S., Malloch, G., Oliveira, C. M. G., Hübschen, J., & Neilson, R. (2006). Ribosomal
1030 and mitochondrial DNA analyses of *Xiphinema americanum*-group populations. *Journal of*
1031 *Nematology* 38, 404–410.
1032 Lee, M.R., Canales-Aguirre, C.B., Nuñez, D., Pérez, K., Hernández, C.E., & Brante, A. (2017). The
1033 identification of sympatric cryptic free-living nematode species in the Antarctic intertidal.
1034 *PLOS ONE* 12, e0186140. doi.org/10.1371/journal.pone.0186140
1035 Lee, M.S.Y., & Oliver, P.M. (2016). Count cryptic species in biodiversity tally. *Nature* 534, 621.
1036 doi.org/10.1038/534621a
1037 Legendre, P., & Legendre, L. (1998). Numerical Ecology. Elsevier.
1038 Legendre, P., & Legendre, L. (2012). Numerical Ecology. Elsevier.
1039 Loof, P. A. A. (1982). Two new species of Longidoridae (Dorylaimida) from Saudi Arabia.
1040 *Nematologica* 28, 307-317. doi.org/10.1163/187529282X00358

1041 Loof, P. A. A., & Chen, Q. (1999). A revised polytomous key for the identification of the species of
1042 the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). Supplement 1.
1043 *Nematology* 1, 55-59. doi:10.1163/156854199507974
1044 Loof, P. A. A., & Coomans, A. (1968). On the development and location of the oesophageal gland
1045 nuclei in the Dorylaimina. *Nematologica* 14, 596. doi.org/10.1163/187529268X00345
1046 Loof, P. A. A., & Luc, M. (1990). A revised polytomous key for the identification of species of the
1047 genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the *X.*
1048 *americanum*-group. *Systematic Parasitology* 16, 35-66. doi.org/10.1007/BF00009600
1049 Loof, P.A.A., Luc, M., & Baujard, P. (1996). A revised polytomous key for the identification of
1050 species of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae) with exclusion of the
1051 *X. americanum*-group: Supplement 2. *Systematic Parasitology* 33, 23-29.
1052 doi.org/10.1007/BF00009717
1053 Macfarlane, S.A. (2003). Molecular determinants of the transmission of plant viruses by nematodes.
1054 *Molecular Plant Pathology* 4, 211-215. doi: 10.1046/j.1364-3703.2003.00164.x
1055 Micoletzky, H. (1922). The free-living soil nematodes. *Archiv für Naturgeschichte* 87, 1-650.
1056 Montgomery, D.C., & Peck, E.A. (1992). Introduction to Linear Regression Analysis. Wiley.
1057 Oliveira, A.S.D., Decraemer, W., Moens, T., dos Santos, G.A.P., & Derycke, S. (2017). Low
1058 genetic but high morphological variation over more than 1000 km coastline refutes
1059 omnipresence of cryptic diversity in marine nematodes. *BMC Evolutionary Biology* 17, 71.
1060 doi.org/10.1186/s12862-017-0908-0
1061 Padial, J.M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy.
1062 *Frontiers in Zoology* 7, 16. doi.org/10.1186/1742-9994-7-16
1063 Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., Archidona-Yuste, A., Blok, V.C., & Castillo, P.
1064 (2017a). Mitochondrial genome diversity in dagger and needle nematodes (Nematoda:
1065 Longidoridae). *Scientific Reports* 7, 41813. doi.org/10.1038/srep41813
1066 Palomares-Rius, J.E. Cantalapiedra-Navarrete, C. Archidona-Yuste, A. Subbotin, S. A., & Castillo,
1067 P. (2017b). The utility of mtDNA and rDNA for barcoding and phylogeny of plant-parasitic
1068 nematodes from Longidoridae (Nematoda, Enoplea). *Scientific Reports* 7, 10905. doi:
1069 10.1038/s41598-017-11085-4.
1070 Palomares-Rius, J. E., Cantalapiedra-Navarrete, C., Archidona-Yuste, A., Tzortzakakis, E. A.,
1071 Birmpilis, I. G., Vovlas, N., Subbotin, S. A., & Castillo, P. (2018). Prevalence and molecular
1072 diversity of reniform nematodes of the genus *Rotylenchulus* (Nematoda: Rotylenchulinae) in
1073 the Mediterranean Basin. *European Journal of Plant Pathology* 150, 439-455.
1074 doi.org/10.1007/s10658-017-1292-8

1075 Palomares-Rius, J.E., Cantalapiedra-Navarrete, C., & Castillo, P. (2014). Cryptic species in plant-
1076 parasitic nematodes. *Nematology* 16, 1105-1118. doi.org/10.1163/15685411-00002831
1077 Peneva, V., Lazarova, S., De Luca, F., & Brown, D. J. F. (2013) Description of *Longidorus*
1078 *cholevae* sp. n. (Nematoda, Dorylaimida) from riparian habitat in the Rila Mountains,
1079 Bulgaria. *ZooKeys* 330, 1–26. doi: doi.org/10.3897/zookeys.330.5750
1080 Peraza-Padilla, W., Archidona-Yuste, A., Ferris, H., Zamora-Araya, T., Cantalapiedra-Navarrete,
1081 C., Palomares-Rius, J. E., Subbotin, S. A., & Castillo, P. (2016). Molecular characterization
1082 of pseudomonodelphic dagger nematodes of the genus *Xiphinema* Cobb, 1913 (Nematoda:
1083 Longidoridae) in Costa Rica, with notes on *Xiphinema setariae* Tarjan, 1964. *European*
1084 *Journal of Plant Pathology* 148, 739-474. doi.org/10.1007/s10658-016-1124-2
1085 Peraza-Padilla, W., Cantalapiedra-Navarrete, C., Zamora-Araya, T., Palomares-Rius, J. E., Castillo,
1086 P., & Archidona-Yuste, A. (2018). A new dagger nematode, *Xiphinema tica* n. sp.
1087 (Nematoda: Longidoridae), from Costa Rica with updating of the polytomous key of Loof and
1088 Luc (1990). *European Journal of Plant Pathology* 150, 73-90. doi.org/10.1007/s10658-017-
1089 1253-2
1090 Pereira, T. J., & Baldwin, J. G. (2016). Contrasting evolutionary patterns of 28S and ITS rRNA
1091 genes reveal high intragenomic variation in *Cephalenchus* (Nematoda): Implications for
1092 species delimitation. *Molecular Phylogenetics and Evolution* 98, 244-260.
1093 doi:10.1016/j.ympev.2016.02.016
1094 Pérez-Portela, R., Arranz, V., Rius, M., & Turon, T. (2013). Cryptic speciation or global spread?
1095 The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. *Scientific*
1096 *Reports* 3, 3197. doi.org/10.1038/srep03197
1097 Pfenninger, M., & Schwenk, K. (2007). Cryptic animal species are homogeneously distributed
1098 among taxa and biogeographical regions. *BMC Evolutionary Biology* 7, 121.
1099 doi.org/10.1186/1471-2148-7-121
1100 Qing, X., Bert, W., Gamliel, A., Bucki, P., Duvrinin, S., Alon, T., & Braun Miyara, S. (2019).
1101 Phylogeography and molecular species delimitation of *Pratylenchus capsici* n. sp., a new
1102 root-lesion nematode in Israel on pepper (*Capsicum annuum*). *Phytopathology* 109, 847-858.
1103 doi:10.1094/PHYTO-09-18-0324-R
1104 Qing, X., Bik, H., Yergaliyev, T. M., Gu, J., Fonderie, P., Brown-Miyara, S., Szitenberg, A., &
1105 Bert, W. (2020). Widespread prevalence but contrasting patterns of intragenomic rRNA
1106 polymorphisms in nematodes: Implications for phylogeny, species delimitation and life
1107 history inference. *Molecular Ecology Resources* 20, 318-332. doi.org/10.1111/1755-
1108 0998.13118

1109 R_Core_Team. 2019. R: a language and environment for statistical computing. R Foundation for
1110 Statistical Computing, Vienna, Austria.
1111 Revelle, W. (2019). psych: Procedures for Psychological, Psychometric, and Personality Research.
1112 Reyment, R.A. (1982). Multivariate morphometrics Handbook of Statistics: Elsevier. 721-745.
1113 Roca, F., Pereira, M.J., & Lamberti, F. (1989). *Longidorus alvegus* sp. n. (Nematoda, Dorylaimida)
1114 from Portugal. *Nematologia Mediterranea* 17, 1-4.
1115 Romanenko, N. D. 1998. Redescription of *Longidorus rubi* Tomilin & Romanenko in Romanenko,
1116 1993 (Nematoda: Longidoridae) associated with raspberry in the Poltava region, Ukraine.
1117 *Russian Journal of Nematology* 6, 185-187.
1118 Ronquist, F., & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under
1119 mixed models. *Bioinformatics* 19, 1572-1574. doi:10.1093/bioinformatics/btg180
1120 Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins,
1121 S.E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large
1122 data sets. *Molecular Biology and Evolution* 34, 3299-3302. doi:10.1093/molbev/msx248
1123 Seinhorst, J.W. (1962). On the killing, fixation and transferring to glycerin of nematodes.
1124 *Nematologica* 8, 29-32. doi.org/10.1163/187529262X00981
1125 Subbotin, S. A., Stanley, J. D., Ploeg, A. T., Maafi, Z. T., Tzortzakakis, E. A., Chitambar, J. J.,
1126 Palomares-Rius, J. E., Castillo, P., & Inserra, R. N. (2015). Characterisation of populations of
1127 *Longidorus orientalis* Loof, 1982 (Nematoda: Dorylaimida) from date palm (*Phoenix*
1128 *dactylifera* L.) in the USA and other countries and incongruence of phylogenies inferred from
1129 ITS1 rRNA and coxI genes. *Nematology* 17, 459-477. doi.org/10.1163/15685411-00002881
1130 Taylor, C. A., & Brown, D. J. F. (1997). *Nematode Vectors of Plant Viruses*. (CAB International:
1131 Wallingford, UK).
1132 Thorne, G. (1935). Notes on free-living and plant-parasitic nematodes. II. *Proceedings*
1133 *Helminthological Society of Washington* 2, 96-98.
1134 Van Den Berg, E., Palomares-Rius, J. E., Vovlas, N., Tiedt, L. R., Castillo, P., & Subbotin, S. A.
1135 (2016). Morphological and molecular characterisation of one new and several known species
1136 of the reniform nematode, *Rotylenchulus* Linford & Oliveira, 1940 (Hoplolaimidae:
1137 Rotylenchulinae), and a phylogeny of the genus. *Nematology* 18, 67-107.
1138 doi.org/10.1163/15685411-00002945
1139 Vďačný, P., Slovák, M., Foissner, W. (2014). Multivariate morphometric analyses of the predatory
1140 ciliate genus *Semispathidium* (Ciliophora: Litostomatea), with description of *S. longiarmatum*
1141 nov. spec. *European Journal of Protistology* 50, 329-344. doi.org/10.1016/j.ejop.2014.03.003

Vrain, T. C., Wakarchuk, D. A., Levesque, A. C., & Hamilton, R. I. (1992). Intraspecific rDNA Restriction Fragment Length Polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology* 15, 563-573.

Wellborn, G. A., & Broughton, R. E. (2008). Diversification on an ecologically constrained adaptive landscape. *Molecular Ecology* 17, 2927-2936. doi:10.1111/j.1365-294X.2008.03805.x

Ye, W., Szalanski, A.L., & Robbins, R.T. (2004). Phylogenetic relationships and genetic variation in *Longidorus* and *Xiphinema* species (Nematoda: Longidoridae) using ITS1 sequences of nuclear ribosomal DNA. *Journal of Nematology* 36, 14-19.

Zhang, D-Y., Lin, K., & Hanski, I. (2004). Coexistence of cryptic species. *Ecology Letters* 7, 165-169. doi.org/10.1111/j.1461-0248.2004.00569.x

Zuur, A.F., Ieno, E.N., & Elphick, C.S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* 1, 3-14. doi.org/10.1111/j.2041-210X.2009.00001.x

FIGURE LEGENS

FIGURE 1 Principal component on morphometric characters to characterize *Longidorus iliturgiensis*- and *Xiphinema hispanum*-complex.

FIGURE 2 Construction of haplonets and haploweb. A: *28S* haploweb of *Longidorus tabernensis* sp. nov.; B: *coxI* haplonet of *L. tabernensis* sp. nov.; C: *28S* haplonet of *Xiphinema subbaetense* sp. nov.; D: *coxI* haplonet of *X. subbaetense* sp. nov. Coloured circles represent haplotypes and their diameter are proportional to the number of individuals sharing the same haplotype. Black short lines on the branches indicate the number of mutated positions in the alignment that separate each haplotype. Co-occurring haplotypes are enclosed in black dashes. Abbreviations: APP = Alto Pandera Population; PPP = Prado Pandera Population; 419-Andújar = *X. hispanum* topotype population; AR52-Andújar = another *X. hispanum* population.

FIGURE 3 Line drawings of holotype for *Longidorus tabernensis* sp. nov. A, Pharyngeal region; B-C, Details of lip region; D-E, Female tails; F, Male tail; G, Tail of first-stage juvenile (J1).

FIGURE 4 Light micrographs of *Longidorus tabernensis* sp. nov. Females: A, Pharynx holotype; B, Anterior region holotype; C, D, Anterior regions paratypes; E-F, Detail of basal bulb; G, Tail region holotype; H-J, Tail region of paratypes; K, Vulval region; L-O, Tail region of 1st, 2nd, 3rd and 4th stage juveniles; P, Tail region of male. Abbreviations: a = anus; af = amphidial fovea; dn = dorsal nucleus; gr = guiding ring; sp = spicule; spl = ventromedian supplements; svn = ventrosublateral nucleus; v = vulva. Scale bars: A = 50µm; B-P = 20µm.

FIGURE 5 Relationship of body length to length of functional and replacement odontostyle in four developmental stages and mature adults of *Longidorus tabernensis* sp. nov. (A), and *Xiphinema subbaetense* sp. nov.

FIGURE 6 Line drawings of holotype for *Xiphinema subbaetense* sp. nov. A, Pharyngeal region; B, Detail of lip region; C-E, Female tails; F, G, Details of uterine pseudo Z-differentiation; H, Tail of first-stage juvenile (J1).

FIGURE 7 Light micrographs of *Xiphinema subbaetense* sp. nov. Females: A, Pharynx holotype; B-C, Anterior regions of holotype and paratype, respectively; D-F, Detail of female genital track showing Z-differentiation; G, Detail of anterior female genital branch; H, Tail region of holotype; I-

M, Tail regions of paratypes; N, Detail of first-stage anterior region; O-R, Tail region of 1st, 2nd, 3rd and 4th stage juveniles. Abbreviations: a = anus; cb = crystalloid bodies; gb = granular bodies; gr = guiding ring; odt = odontostyle; psZ = pseudo-Z organ; rodt = replacement odontostyle; spi = spine; v = vulva. Scale bars: A, G- N = 50µm; B-F, H-M and O-R = 20µm.

FIGURE 8 Phylogenetic relationships of *Longidorus tabernensis* sp. nov. within the genus *Longidorus*. Bayesian 50% majority rule consensus trees as inferred from D2-D3 expansion segments of *28S* rRNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters, and each colour was associated to each species of the complex.

FIGURE 9 Phylogenetic relationships of *Xiphinema subbaetense* sp. nov. within the genus *Xiphinema*. Bayesian 50% majority rule consensus trees as inferred from D2-D3 expansion segments of *28S* rRNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters, and each colour was associated to each species of the complex.

FIGURE 10 Phylogenetic relationships of *Longidorus tabernensis* sp. nov. within the genus *Longidorus*. Bayesian 50% majority rule consensus trees as inferred from *18S* rRNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters.

FIGURE 11 Phylogenetic relationships of *Xiphinema subbaetense* sp. nov. within the genus *Xiphinema*. Bayesian 50% majority rule consensus trees as inferred from *18S* rRNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters.

FIGURE 12 Phylogenetic relationships of *Longidorus tabernensis* sp. nov. within the genus *Longidorus*. Bayesian 50% majority rule consensus trees as inferred from *coxI* mtDNA sequences alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters, and each colour was associated to each species of the complex.

FIGURE 13 Phylogenetic relationships of *Xiphinema subbaetense* sp. nov. within the genus *Xiphinema*. Bayesian 50% majority rule consensus trees as inferred from *coxI* mtDNA sequences

alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. Newly obtained sequences in this study are in bold letters, and each colour was associated to each species of the complex.

TABLE 1 Taxa sampled for *Longidorus* and *Xiphinema* species and sequences used in this study for molecular characterization and haploweb analyses.

Sample code	Nematode Species Locality, province	Host	*28S* haplotype	*coxI* haplotype	GenBank accession numbers *28S*	*coxI*	*ITS1*	*18S*
***Longidorus iliturgiensis*-complex**								
***Longidorus tabernensis* sp. nov.**								
AZ03	Tabernas, Almería	Yellow broom	tab1	co-tab1	**MK941194**	**MK937587**	**MK941256**	**MK941261**
AZ28	Tabernas, Almería	Yellow broom	tab1	co-tab1	**MK941195**	**MK937588**	**MK941257**	-
CA83	Tabernas, Almería	Yellow broom	tab1	co-tab1	**MK941196**	**MT040266**	-	-
CA84	Tabernas, Almería	Yellow broom	tab1	co-tab1	**MK941197**	**MT040267**	-	-
CA85	Tabernas, Almería	Yellow broom	-	co-tab1	-	**MT040268**	-	-
CA86	Tabernas, Almería	Yellow broom	-	co-tab1	-	**MT040269**	-	-
AQ98	Tabernas, Almería	Yellow broom	-	co-tab1	-	**MT040270**	-	-
Longidorus iliturgiensis								
ALANU	Andújar, Jaén	Black alder	ili1	co-ili1	**MH430012**	**MH454065**	**MH429987**	**MH430002**
DD52	Andújar, Jaén	Black alder	ili2	co-ili1	**MH430013**	**MT040271**	-	**MH430003**
DD54	Andújar, Jaén	Black alder	-	co-ili1	-	**MT040272**	-	-
DD55	Andújar, Jaén	Black alder	-	co-ili1	-	**MT040273**	-	-
DD56	Andújar, Jaén	Black alder	-	co-ili1	-	**MT040274**	-	-
DD53	Andújar, Jaén	Black alder	-	co-ili2	-	**MT040275**	-	-
Longidorus indalus								
ST41	Las Tres Villas, Almería	Cultivate olive	ind1	co-ind1	KT308852	**KY816675**	KT308878	KT308894
AR46	Agua Amarga, Almería	Wild olive	ind1	co-ind1	KT308853	**MT040276**	KT308879	KT308895
ST042	Las Tres Villas, Almería	Cultivate olive	ind2	co-ind1	KT308854	**MT040277**	-	-
DD61	Sorbas, Almería	Wild olive	-	co-ind1	-	**MT040278**	-	-
DD62	Sorbas, Almería	Wild olive	-	co-ind1	-	**MT040279**	-	-
***Xiphinema hispanum*-complex**								
***Xiphinema subbaetense* sp. nov.**								
APP-P60	Valdepeñas, Jaén	Asphodel	sub1	co-sub1	**MT039104**	**MT040280**	**MT026293**	-
APP-P61	Valdepeñas, Jaén	Asphodel	sub2	co-sub2	**MT039105**	**MT040281**	**MT026294**	-

APP-P62	Valdepeñas, Jaén	Asphodel	heterozygous	co-sub2	**MT039106**	**MT040282**	-	-
APP-P78	Valdepeñas, Jaén	Asphodel	sub2	co-sub2	**MT039107**	**MT040283**	-	**MT039135**
APP-P79	Valdepeñas, Jaén	Asphodel	sub2	co-sub2	**MT039108**	**MT040284**	-	**MT039136**
APP-P80	Valdepeñas, Jaén	Asphodel	sub3	co-sub2	**MT039109**	**MT040285**	-	-
APP-P81	Valdepeñas, Jaén	Asphodel	sub2	co-sub2	**MT039110**	**MT040286**	-	-
APP-P82	Valdepeñas, Jaén	Asphodel	sub1	co-sub2	**MT039111**	**MT040287**	-	-
APP-P83	Valdepeñas, Jaén	Asphodel	sub3	co-sub2	**MT039112**	**MT040288**	-	-
APP-P84	Valdepeñas, Jaén	Asphodel	sub1	co-sub1	**MT039113**	**MT040289**	-	-
APP-P85	Valdepeñas, Jaén	Asphodel	sub3	co-sub2	**MT039114**	**MT040290**	-	-
PPP-P69	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039115**	**MT040291**	**MT026295**	-
PPP-P70	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039116**	**MT040292**	-	-
PPP-P71	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039117**	**MT040293**	-	-
PPP-P72	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039118**	**MT040294**	-	-
PPP-P73	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039119**	**MT040295**	-	-
PPP-P74	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039120**	**MT040296**	-	**MT039137**
PPP-P75	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039121**	**MT040297**	-	**MT039138**
PPP-P76	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039122**	**MT040298**	-	**MT039139**
PPP-P77	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039123**	**MT040299**	-	**MT039140**
PPP-P63	Valdepeñas, Jaén	Pasture	heterozygous	co-sub2	**MT039124**	**MT040300**	-	-
Xiphinema hispanum								
419-0419	Andújar, Jaén	*Cistus albidus*	his4	co-his3	GU725074	KY816614	GU725061	GU725083
419-AP86	Andújar, Jaén	*Cistus albidus*	his1	-	**MT039125**	-	-	-
419-AP87	Andújar, Jaén	*Cistus albidus*	his2	co-his1	**MT039126**	**MT040301**	-	-
419-AP88	Andújar, Jaén	*Cistus albidus*	his2	-	**MT039127**	-	-	-
419-AP89	Andújar, Jaén	*Cistus albidus*	his3	-	**MT039128**	-	-	-
419-AP90	Andújar, Jaén	*Cistus albidus*	his1	-	**MT039129**	-	-	-
AR52-P055	Andújar, Jaén	Wild olive	his2	-	KX244905	-	-	-
AR52-AP91	Andújar, Jaén	Wild olive	his2	co-his2	**MT039130**	**MT040302**		
AR52-AP92	Andújar, Jaén	Wild olive	his2	co-his2	**MT039131**	**MT040303**	-	-
AR52-AP93	Andújar, Jaén	Wild olive	his2	co-his2	**MT039132**	**MT040304**	-	-

AR52-AP94	Andújar, Jaén	Wild olive	his2	co-his2	**MT039133**	**MT040305**	-	-
AR52-AP95	Andújar, Jaén	Wild olive	his2	-	**MT039134**	**-**	-	-
Xiphinema adenohystherum								
SORI	Arevalo, Soria	Holly tree	ade1	-	KC567164	KY816588	GU725063	GU725084
JAO6	La Granjuela, Córdoba	Cultivated olive	ade2	-	KX244898	-	-	-
0431	Bollullos Condado, Huelva	Grapevine	ade2	-	GU725075	-	-	-
AR78	Almodóvar del Rio, Córdoba	Wild olive	ade3	co-ade1	KX244897	KY816591	-	-
ALMAG	Almagro, Ciudad Real	Wild olive	-	co-ade2	-	KY816589	-	-
AR086	Prado del Rey, Cádiz	Wild olive	-	co-ade3	-	KY816590	-	-
IASNB	Jerez de la Frontera, Cádiz	Wild olive	-	co-ade4	-	KY816592	-	-

–, not amplified or not sequenced

Abbreviations: APP = Alto Pandera Population; PPP = Prado Pandera Population; 419 = *X. hispanum* topotype population; AR52 = another *X. hispanum* population

GenBank accession numbers in bold represent sequence data that were generated in this study (96 sequences), other accessions (23 sequences) were from previous studies (Gutiérrez-Gutiérrez et al., 2010; 2013; Archidona-Yuste et al., 2016a; 2016b; 2019; Palomares-Rius et al., 2017).

Morphological and morphometric data of the new species were generated in this study, and those for known species were available from the literature (Gutiérrez-Gutiérrez et al., 2010; 2013; Archidona-Yuste et al., 2016a; 2016b; 2019).

TABLE 2 Eigenvector and SS loadings of factor derived from nematode morphometric characters for *Longidorus iliturgiensis*-complex (*Longidorus tabernensis* sp. nov., *Longidorus indalus, Longidorus iliturgiensis*) and *Xiphinema hispanum*-complex (*Xiphinema subbaetense* sp. nov., *Xiphinema adenohystherum, Xiphinema hispanum*).

	***Longidorus iliturgiensis*-complex**			***Xiphinema hispanum*-complex**		
	Principal components			**Principal components**		
Character[b]	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Body length (L)	-0.039	0.543	-0.004	0.467	-0.012	0.199
a	0.051	0.428	-0.539	0.130	0.167	0.578
c	-0.207	0.558	0.344	-	-	-
c′	0.171	-0.181	-0.665	0.178	0.456	0.265
d	0.439	0.036	0.079	0.441	0.001	-0.380
d′	0.454	-0.014	0.238	0.370	-0.199	-0.315
V	0.105	0.196	-0.118	0.027	-0.506	-0.016
Odt	-0.394	0.097	-0.152	0.454	0.024	0.131
Odph	0.274	0.341	-0.162	0.121	-0.544	0.140
Lip region width	-0.454	-0.097	-0.149	-0.255	-0.384	0.430
Hyaline region length	0.275	0.059	-0.005	0.340	-0.156	0.301
SS loadings	1.93	1.36	1.24	1.81	1.58	1.13
% of total variance	33.92	16.79	14.04	32.90	24.80	12.71
Cumulative % of total variance	33.92	50.71	64.75	32.90	57.71	70.42

[a] Based on 19 female specimens of *Longidorus tabernensis* sp. nov. from a population sample, 36 female specimens of *Longidorus indalus* from seven population samples, 18 female specimens of *Longidorus iliturgiensis* from a population sample, 25 female specimens of *Xiphinema subbaetense* sp. nov. from two population samples, 8 female specimens of *Xiphinema adenohystherum* from a population sample, and 11 female specimens of *Xiphinema hispanum* from a population sample. Values of morphometric variables 1 to 3 (eigenvector > 0.439) are underlined. All populations were molecularly identified and located at southern Spain. The c` ratio was excluded by the multicollinearity test and then, it was not included in the multivariate analysis for the *Xiphinema hispanum*-complex. Odt = odontostyle length; Odph = Odontophore length.

[b] Morphological and diagnostic characters according to Jairajpuri and Ahmad (Jairajpuri & Ahmad, 1992) with some inclusions.

TABLE 3 Morphometrics of *Longidorus tabernensis* sp. nov. from Tabernas (Almería, Spain)[a].

Characters-ratios b	Holotype	Paratypes					
		Females	Males	J1	J2	J3	J4
n	1	19	9	2	6	6	7
L (mm)	5.1	5.0 ± 0.4 (4.3-5.5)	4.4 ± 0.35 (4.0-4.9)	0.989, 0.995	1.6 ± 0.2 (1.4-1.8)	2.5 ± 0.1 (2.4-2.7)	3.5 ± 0.2 (3.0-3.7)
a	118.1	125.8 ± 15.5 (107.9-172.9)	142.9 ± 12.3 (123.1-162.5)	48.2, 53.8	68.3 ± 5.4 (61.2-76.5)	86.3 ± 12.6 (73.4-99.6)	107.6 ± 5.0 (97.6-111.6)
b	17.9	17.6 ± 1.6 (14.9-22.4)	13.7 ± 1.2 (12.4-15.2)	6.4, 7.2	7.6 ± 1.6 (6.1-9.9)	9.4 ± 0.6 (8.9-10.1)	14.0 ± 1.6 (12.1-16.4)
c	122.3	106.9 ± 11.4 (89.5-131.5)	94.2 ± 7.7 (85.9-107.8)	25.0, 25.5	39.0 ± 3.5 (33.5-41.8)	56.4 ± 3.9 (52.4-62.2)	73.8 ± 6.3 (67.7-82.7)
c'	1.8	2.1 ± 0.2 (1.8-2.4)	2.2 ± 0.09 (2.1-2.4)	3.0, 2.9	3.0 ± 0.3 (2.7-3.3)	2.7 ± 0.1 (2.5-2.8)	2.5 ± 0.3 (2.1-2.8)
d	2.3	2.3 ± 0.2 (2.1-2.7)	2.4 ± 0.2 (2.2-2.6)	2.0, 2.2	2.2 ± 0.2 (1.9-2.5)	2.3 ± 0.1 (2.2-2.4)	2.3 ± 0.1 (2.2-2.4)
d'	1.4	1.5 ± 0.1 (1.4-1.6)	1.4 ± 0.1 (1.4-1.6)	1.46,1.53	1.5 ± 0.1 (1.4-1.6)	1.5 ± 0.04 (1.5-1.6)	1.5 ± 0.1 (1.4-1.6)
V or T	46.9	47.0 ± 1.2 (45.2-48.9)	32.0 ± 2.9 (28.4-36.5)	-	-	-	-
G1	8.1	8.9 ± 1.1 (6.7-11.4)	-	-	-	-	-
G2	7.7	8.7 ± 0.9 (7.2-10.5)	-	-	-	-	-
Odt	64.0	62.0 ± 1.3 (60.0-64.5)	61.6 ± 1.1 (60.5-63.5)	39.0, 37.0	42.0 ± 1,.7 (40.0-44.5)	47.6 ± 2.2 (44.0-50.0)	53.8 ± 2.1 (51.5-56.0)
Odph	31.5	30.2 ± 2.3 (25.5-34.0)	31. 6± 2.3 (28.5-35.0)	23.5, 24.5	25.8 ± 1.2 (24.5-27.5)	25.5 ± 1.2 (24.5-27.0)	30.3 ± 1.2 (28.5-32.0)
Total stylet	95.5	92.1 ± 2.9 (86.5-96.0)	93.2 ± 2.3 (89.0-95.5)	62.5, 61.5	67.8 ± 2.3 (64.5-69.5)	73.1 ± 2.7 (68.5-75.0)	84.1 ± 1.9 (81.5-86.5)

Replacement Odt	-	-	-	45.0, 44.5	46.7 ± 0.8 (45.5-47.5)	53.5 ± 2.9 (49.5-56.0)	60.9 ± 0.9 (60.0-62.0)
Lip region width	10.0	10.1 ± 0.3 (9.5-10.5)	9.9 ± 0.2 (9.5-10.0)	7.5, 6.5	7.8 ± 0.4 (7.5-8.5)	8.4 ± 0.2 (8.0-8.5)	9.2 ± 0.6 (8.5-10.0)
Oral aperture-guiding ring	22.5	23.7 ± 1.4 (22.0-28.0)	24.2 ± 1.5 (21.5-26.0)	15.0, 14.0	17.3 ± 0.8 (16.5-18.5)	19.3 ± 1.0 (18.0-20.5)	20.9 ± 0.9 (20.0-22.5)
Tail length	42.0	46.7 ± 3.7 (42.0-53.0)	47.3 ± 3.1 (43.5-51.5)	39.0, 39.5	40.7 ± 3.3 (36.5-44.0)	44.3 ± 1.0 (43.5-45.5)	47.8 ± 3.6 (43.0-52.0)
Spicules	-	-	33.4 ± 1.3 (32.0-36.0)	-	-	-	-
Lateral accessory piece	-	-	11.2 ± 0.8 (10.5-12.0)	-	-	-	-
J	9.5	8.9 ± 0.6 (8.0-10.0)	8.6 ± 1.0 (7.0-10.0)	3.5, 4.0	5.8 ± 1.0 (4.5-7.0)	6.4 ± 0.8 (5.5-8.0)	7.1 ± 1.6 (5.5-9.0)

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

[b] a = body length/maximum body width; b = body length/pharyngeal length; c = body length/tail length; c' = tail length/body width at anus; d = anterior to guiding ring/body diameter at lip region; d’ = body diameter at guiding ring/body diameter at lip region; V = (distance from anterior end to vulva/body length) x 100; G1 = (anterior genital branch length/body length) x 100; G2 = (posterior genital branch length/body length) x 100; T= ((distance from cloacal aperture to anterior end of testis/body length) x 100); J = hyaline tail region length; Odt = odontostyle length; Odph = Odontophore length.

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2 **TABLE 4** Morphometrics of *Xiphinema subbaetense* sp. nov. from Asphodel and Pasture at Valdepeñas (Jaén Province) Southern Spain[a].

Host			Paratypes Asphodel (APP population)				Other Population Pasture (PPP population)
Characters-ratios[b]	Holotype	Females	J1	J2	J3	J4	Females
n	1	19	5	5	5	5	11
L (mm)	4.3	4.3 ± 0.2 (4.0-4.7)	1.30 ± 0.07 (1.22-1.41)	1.84 ± 0.11 (1.72-2.00)	2.59 ± 0.15 (2.43-2.75)	3.56 ± 0.21 (3.30-3.75)	4.9± 0.2 (4.6-5.3)
a	56.7	57.2 ± 3.9 (49.0-63.4)	39.9 ± 2.2 (37.2-43.2)	45.0 ± 3.8 (40.5-49.9)	47.4 ± 3.1 (44.4-52.3)	49.5 ± 1.0 (48.5-51.0)	61.1 ± 4.4 (53.3-70.0)
b	8.0	8.2 ± 0.8 (7.1-10.4)	4.5 ± 0.4 (4.1-5.1)	4.7 ± 0.2 (4.4-4.9)	5.3 ± 0.3 (4.9-5.8)	6.8 ± 0.6 (5.7-7.2)	9.2 ± 1.0 (7.8-11.0)
c	119.7	121.9 ± 12.2 (101.9-139.4)	22.1 ± 1.3 (20.0-23.2)	36.3 ± 2.8 (34.1-40.9)	56.9 ± 3.9 (53.2-63.2)	93.6 ± 12.9 (78.7-111.3)	130.4 ± 10.5 (114.2-149.5)
c'	0.8	0.8 ± 0.1 (0.6-0.9)	2.9 ± 0.2 (2.6-3.1)	2.0 ± 0.2 (1.9-2.3)	1.3 ± 0.1 (1.3-1.4)	0.9 ± 0.1 (0.8-1.0)	0.9 ± 0.04 (0.8-0.9)
d	7.4	7.2 ± 0.3 (6.8-7.8)	4.8 ± 0.4 (4.4-5.3)	5.7 ± 0.4 (5.2-6.1)	6.4 ± 0.6 (5.6-7.1)	6.6 ± 0.2 (6.2-6.8)	7.7 ± 0.3 (7.2-8.2)
d'	2.8	2.7 ± 0.1 (2.5-2.9)	2.3 ± 0.2 (2.1-2.6)	2.6 ± 0.2 (2.4-2.8)	2.7 ± 0.3 (2.3-3.1)	2.6 ± 0.1 (2.5-2.8)	2.9 ± 0.1 (2.8-3.2)
V	52.9	51.7 ± 1.6 (48.7-54.3)	-	-	-	-	52.5 ± 0.8 (50.9-53.5)
G1	16.1	12.1 ± 2.7 (9.4-16.1)	-	-	-	-	13.8 ± 1.7 (12.7-15.7)
G2	15.4	13.6 ± 1.3 (12.0-15.4)	-	-	-	-	15.0 ± 1.1 (13.7-15.8)
Odt	135.5	129.1 ± 5.5 (121.5-138.0)	59.2 ± 4.1 (55.5-66.0)	78.9 ± 1.9 (77.0-82.0)	95.8 ± 3.9 (92.0-100.0)	112.6 ± 2.5 (110.5-116.5)	143.2 ± 3.6 (138.0-149.5)
Odph	92.0	88.3 ± 2.7 (82.0-92.0)	47.0 ± 2.8 (44.0-51.0)	56.9 ± 2.3 (54.5-60.5)	66.2 ± 2.8 (61.5-68.0)	80.3 ± 3.5 (77.5-86.0)	91.8 ± 2.5 (89.0-96.5)
Total stylet	227.5	217.5 ± 6.5 (205.5-228.5)	106.2 ± 4.4 (102.0-111.0)	135.8 ± 3.2 (132.5-140.0)	162.0 ± 6.3 (153.5-168.0)	192.9 ± 2.5 (190.0-196.5)	236.4 ± 4.4 (228.5-243.5)
Replacement Odt	-	-	77.2 ± 3.5 (74.0-83.0)	93.9 ± 3.4 (89.0-98.5)	112.4 ± 5.3 (106.5-117.5)	132.0 ± 3.1 (128.0-135.0)	-

Lip region width	16.0	16.4 ± 0.8 (15.5-18.5)	10.1 ± 0.2 (10.0-10.5)	10.7 ± 0.4 (10.5-11.5)	12.7 ± 0.8 (12.0-13.5)	14.8 ± 0.4 (14.5-15.5)	16.2 ± 0.4 (15.5-16.5)
Oral aperture-guiding ring	119.0	118.3 ± 6.5 (106.5-131.5)	48.4 ± 3.8 (45.0-53.0)	60.6 ± 3.7 (54.4-64.5)	81.0 ± 5.1 (75.5-89.0)	97.4 ± 5.3 (90.5-105.0)	125.5 ± 4.1 (119.0-134.0)
Tail length	35.5	35.9 ± 3.2 (30.0-41.5)	59.0 ± 2.2 (55.5-61.0)	50.9 ± 3.6 (45.5-54.4)	45.7 ± 4.8 (38.5-50.5)	38.4 ± 3.4 (33.5-43.0)	38.0 ± 2.9 (34.5-43.0)
J	10.0	11.4 ± 1.7 (8.5-15.0)	15.3 ± 1.8 (13.5-17.5)	16.9 ± 1.9 (14.0-19.0)	13.4 ± 0.7 (12.5-14.5)	10.1 ± 2.1 (7.5-13.0)	13.4 ± 2.2 (11.0-19.0)

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

[b] a = body length/maximum body width; b = body length/pharyngeal length; c = body length/tail length; c' = tail length/body width at anus; d = anterior to guiding ring/body diam. at lip region; d'= body diam. at guiding ring/body diam. at lip region; V = (distance from anterior end to vulva/body length) x 100; J = hyaline tail region length; G1 = (anterior genital branch length/body length) x 100; G2 = (posterior genital branch length/body length) x 100; Odt = odontostyle length; Odph = Odontophore length.