



## An update on the genus *Longidorus*, *Paralongidorus* and *Xiphinema* (Family Longidoridae) in Portugal

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### Abstract

The tribe Longidorini within the subfamily Longidorinae (*Longidorus* spp. and *Paralongidorus* spp.) and the subfamily Xiphineminae (*Xiphinema* spp.) are two large nematode groups with about 260 and 230 known species, respectively. They are globally two important groups of ectoparasitic nematodes considered to be major pests because of their activity as vectors of important plant nepovirus, with some species included in the list of quarantine pathogenic organisms in many European countries. Knowledge of the biodiversity and occurrence of this nematode group is a prerequisite for the establishment of sound management strategies and control measures. According to data collected from the databases (such as EPPO, FSTA, and Web of Science) and published in specialised literature, a total of 15 *Longidorus*, 1 *Paralongidorus* and 40 *Xiphinema* species have been recorded as present in Portugal. However, the taxonomic status of some species is controversial, and thus needs to be clarified. A comprehensive review for unravelling the biodiversity and occurrence of nematode species of the genus *Longidorus*, *Paralongidorus* and *Xiphinema* in Portugal is herein provided. This review includes an updated checklist of species with information on the localities, host plants and geographical distribution. Additionally, maps on the species distributions of Longidorinae and Xiphineminae nematodes present in Continental Portugal and the Portuguese Macaronesian islands are provided, as well as unpublished data on authors and comments on the current taxonomic status. Finally, new insights and directions for future research on Longidoridae in Portugal are presented.

**Key words:** biodiversity, identification, longidorid, Longidorinae, occurrence, taxonomy, Xiphineminae

### Introduction

The family Longidoridae Thorne, 1935, includes the genera *Longidorus* Micoletzky, 1922, *Paralongidorus* Siddiqi, Hooper & Klan, 1963 (superfamily Longidorinae), and *Xiphinema* Cobb, 1913 (superfamily Xiphineminae) (Decraemer & Robbins 2007). These migratory ectoparasitic nematodes, commonly known as longidorid nematodes, are characterized by a large body (length between 1.5–12.0 mm) and a long stylet (length between 80–290 µm). They comprise a group of phytopathogenic species that damage a wide range of wild and cultivated plants through direct feeding on root cells and transmission of several plant pathogenic viruses (Taylor & Brown 1997; Decraemer & Robbins 2007; Decraemer & Chaves 2012). *Longidorus*, *Paralongidorus* and *Xiphinema* are the three largest of the virus vector nematode genera with about 160, 70 and 260 known species, respectively (Decraemer & Chaves 2012; Archidona-Yuste *et al.* 2016a, b; Esmaeili *et al.* 2016), but only some species (8, 1 and 9, respectively) have been shown to transmit nepoviruses (Taylor & Brown 1997; Decraemer & Robbins 2007; Decraemer & Chaves 2012). To date only five known virus vector nematodes species from Longidoridae have been reported to be implicated in plant virus transmission in Portugal: *Xiphinema index* Thorne & Allen, 1950, *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. rivesi* Dalmasso, 1969, *Longidorus macrosoma* Hooper, 1961, and *Paralongidorus maximus* (Bütschli, 1874) Siddiqi, 1964 (Bravo & Lemos 1997;

Taylor & Brown 1997) (Table S1). In addition, some Longidoridae nematode species are included in the list of quarantine organisms of the European and Mediterranean Plant Protection Organization (EPPO). *Xiphinema index* is one of the most significant plant parasitic nematodes (Jones *et al.* 2013) because it is the vector nematode of *Grapevine fanleaf virus* (GFLV), one of the most destructive viral diseases affecting grapevine (Andret-Link *et al.* 2004). Taxonomic studies on both Longidorinae and Xiphineminae nematodes, including identification of species, enable us to catalogue and explain the biodiversity of these groups, which is important because of their implications in the natural ecological processes in the soil as well as pest management and the establishing of phytosanitary measures. Historically, nematologists have relied only on morphological and morphometric characters to identify species within the subfamily Longidorinae and Xiphineminae (Lamberti *et al.* 2000; Coomans *et al.* 2001; Decraemer & Chaves 2012). They are characterized by a greatly conserved morphology, with similar anatomical characteristics and high inter- and intra-specific morphometric variability, making species delimitation a very complex and time-consuming task (Coomans *et al.* 2001; Decraemer & Chaves 2012). Also, the large number of anatomical similarities within these taxonomic groups make it difficult to agree on the number of valid species (Lamberti *et al.* 2000; Coomans *et al.* 2001; Decraemer & Chaves 2012). In fact, the use of polytomous keys is recommended for identification at species level (Brown & Halbrecht 1997; Chen *et al.* 1997; Escuer & Arias 1997; Lamberti *et al.* 2000). Despite this, the taxonomy within the genus *Longidorus*, *Paralongidorus* and *Xiphinema* is controversial, with a large number of putative species continuously being the subject of debate (Decraemer & Chaves 2012; Archidona-Yuste *et al.* 2016a, b; Esmaili *et al.* 2016). Currently, molecular techniques are used as supplementary tools to help in the differentiation at species level (Gutiérrez-Gutiérrez *et al.* 2012, 2013a,b; Tzortzakakis *et al.* 2014; Esmaili *et al.* 2016; Handoo *et al.* 2016). An integrative approach based upon the combination of classical morphological taxonomy and molecular barcoding of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) genes is a useful strategy for identification at species level within the family Longidoridae. In addition the identification of DNA fragments has the potential of being used as species-specific molecular markers (Gutiérrez-Gutiérrez *et al.* 2013a, b; Chizhov *et al.* 2014; Tzortzakakis *et al.* 2014; Subbotin *et al.* 2014; Archidona-Yuste *et al.* 2016a; Lazarova *et al.* 2016).

The discovery of the transmission of GFLV by *X. index* (Hewitt *et al.* 1958) and subsequent studies in Portugal (Sequeira & Dias 1964) stimulated interest on the virus vector nematodes and their associated viruses as well as the interactions within different crops (Brown & Taylor 1987; Bravo & Lemos 1997; Brown & Trudgill 1997; Taylor & Brown 1997). Therefore, these plant nematode groups have been recorded throughout the last six decades (Table S1), starting with the works of Lima (Lima 1962, 1965). Unfortunately, this research area has not been studied during the past fifteen years in Portugal, and lacks updated information on the current biodiversity and distribution patterns of these nematodes. In addition, the taxonomic status of some Longidoridae nematodes reported in Portugal is currently considered to be controversial, with some cases of putative species with dubious identifications and other species currently considered to be synonymous and needing re-evaluation.

To date, in Portugal, most of the taxonomic studies on the Longidorinae and Xiphineminae nematodes has been based on morphological and morphometric analysis (Brown & Taylor 1987; Bravo & Lemos 1997; Taylor & Brown 1997; Peña-Santiago *et al.* 2006) (Table S1), and there is little molecular data on longidorid species (He *et al.* 2005; Molinari *et al.* 1997, 2006; Lazarova *et al.* 2006; Chizhov *et al.* 2014). Most nematode species belonging to the genera *Longidorus*, *Paralongidorus* and *Xiphinema* are often found infesting a wide range of forest and cultivated plants in Portugal (Table S1); however, most of them are associated with fruit tree plantations and vineyards (18 *Xiphinema* spp. and 6 *Longidorus* spp.) (Table S1). In Portugal, the biodiversity of these species was reviewed almost 20 years ago (Bravo & Lemos 1997). A large number of reports with new records and descriptions of species have been published (Bravo & Roca 1995, 1998; Roca & Bravo 1996, 1997, 1998; Bravo *et al.* 2001; Gutiérrez-Gutiérrez *et al.* 2015), increasing the number of species occurring in Portugal to a total of 56 (Figures 1–5; Table S1). An inventory of soil and freshwater nematodes from the Iberian Peninsula was published by Peña-Santiago *et al.* (2006) and the diversity of nematode species in the Macaronesian region was reviewed in detail by Vieira *et al.* (2008) and Borges (2010). Several comparative studies on the biodiversity and biogeographic distribution of species belong to the genus *Longidorus*, *Paralongidorus* and *Xiphinema* in the Mediterranean Basin and Central Europe (Navas *et al.* 1990, 1993) and worldwide (Coomans *et al.* 2001) was reported. In general, the biodiversity of plant nematodes of Longidoridae in the Iberian Peninsula appears to be one of the highest in the world (Taylor & Brown 1997; Coomans *et al.* 2001; Peña-Santiago *et al.* 2006; Archidona-Yuste *et al.* 2016a, b), although it appears to be higher in Xiphineminae than in Longidorinae (Bravo & Lemos 1997; Peña-Santiago *et al.*

2006). Many of them are considered as to be species endemic to the Iberian Peninsula (Table S1) and Gutiérrez-Gutiérrez *et al.* (2011) hypothesized that the Iberian Peninsula could be considered as a possible centre of origin for some *Xiphinema* species because of the high number of endemic species and their phylogenetic grouping.

The aim of this comprehensive review is to clarify the biodiversity, and taxonomic positions of species belonging to the genera *Longidorus*, *Paralongidorus* and *Xiphinema* from the family Longidoridae present in Portugal. Information from databases, specialised literature and unpublished data of the authors is provided. An updated checklist of species with notes on the taxonomic status and maps of the diversity and the geographical distribution of species in Portugal is presented. Insights and directions for future research on plant nematodes of Longidoridae in Portugal are also presented.

## Family Longidoridae in Portugal

### Genus *Xiphinema*

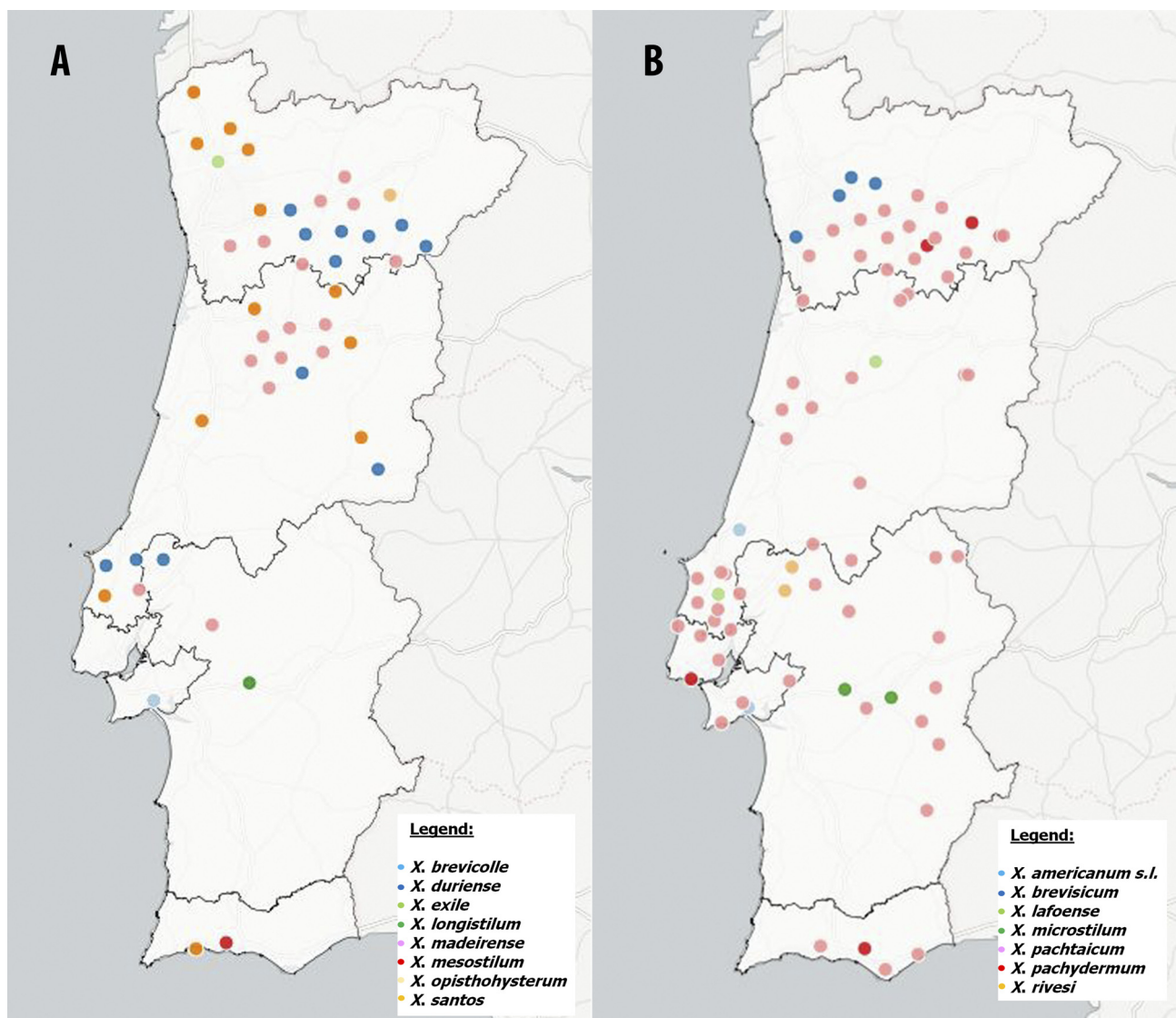
#### *Xiphinema* species are members of the *X. americanum*-group

To our knowledge, the first reference on *Xiphinema* spp. occurring in Portugal dates from 1962 (Lima 1962, 1965). Lima studied in depth a complex of *Xiphinema* species closely related morphologically, which currently comprise the *X. americanum*-group (Lamberti & Bleve-Zacheo 1979). Lima (1968) and Tarjan (1969) were the first researchers to argue that *X. americanum* Cobb, 1913, was not a single species but a species complex containing several species; later this idea of a species complex was strengthened (Lamberti & Bleve-Zacheo 1979). Currently more than 55 valid species are included within this species complex, with some species transmitting important American nepovirus, one of which is *X. americanum sensu stricto* (*s.s.*) (Lamberti *et al.* 2000; Archidona-Yuste *et al.* 2016a). The application of molecular techniques to supplement taxonomic and systematics studies on the genus *Xiphinema* particularly in the *X. americanum* group has revealed that within the *X. americanum*-group some long-assumed single species are in fact cryptic (i.e. genetically distinct but morphologically almost indistinguishable) (Barsi & De Luca 2008; Oliveira *et al.* 2005, 2006; Archidona-Yuste *et al.* 2016a; Lazarova *et al.* 2016). Unfortunately, only a small number of Portuguese populations of this taxonomic group have been molecularly characterized (He *et al.* 2005; Molinari *et al.* 1997, 2006; Lazarova *et al.* 2006) and, as a result, the taxonomic status of some species of the *X. americanum*-group reported in Portugal has been contentious. To date a total of 15 *Xiphinema* species have been reported to occur in Portugal belonging to the *X. americanum*-group (Figures 1, 4, 5; Table S1), most of them being Iberian endemics. In general, this group is characterized morphologically by a short body length (< 3 mm), V (vulva) localized around or behind the middle of the body, and a female reproductive system with two short equally developed genital branches, with short and simplified uteri without uterine differentiation (Lamberti *et al.* 2000; Decraemer & Chaves 2012). Based on hierarchical cluster analysis (HCA) of morphometric data, the Portuguese populations of *X. americanum* group species are grouped in three subgroups: the *X. americanum*-subgroup, the *X. pachtaicum*-subgroup, and the *X. pachydermum*-subgroup that included *X. pachydermum* Sturhan, 1983, and others Portuguese *X. americanum*-group species closely related to *X. pachydermum* (Lamberti *et al.* 1993; Lamberti & Ciancio 1993; Coomans *et al.* 2001). The taxonomic status and validity of some species of these subgroups described in Portugal has been questioned.

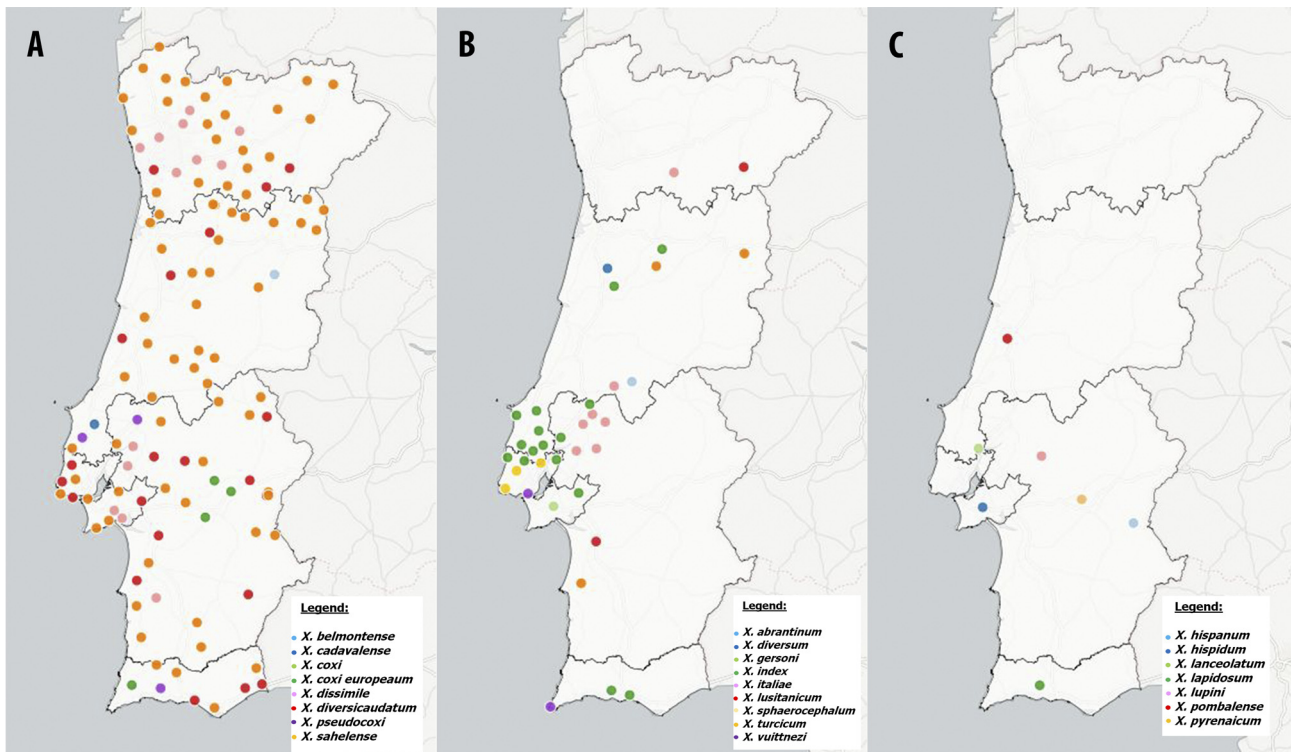
#### *Xiphinema americanum*-subgroup species

A large number of populations belonging to species of the *X. americanum*-subgroup have been reported, including a population from the south-west of Portugal (Setúbal) originally identified firstly as *X. americanum*, and later proposed as *X. brevicolle* Lordello & Da Costa, 1961 (Lima 1968; Luc *et al.* 1998; Lamberti & Bleve-Zacheo 1979) (Figure 1). Sturhan (1973) also reported several populations of this same species in the Azores Islands (S. Miguel, Terceira, S. Jorge, and Faial) (Figure 5; Table S1). Two species closely related morphologically to *X. brevicolle* were identified as *X. incognitum* Lamberti & Bleve-Zacheo, 1979, and *X. diffusum* Lamberti & Bleve-Zacheo, 1979, both species being reported from Madeira Island (Bravo 1989; Lamberti *et al.* 1994) (Table S1). Additionally the second species was also reported in a locality of the west-central continental region (Quinta do

Hilário, Setúbal) (Figure 1; Table S1). However, some authors regard both *X. incognitum* and *X. diffusum* to be synonymous with *X. brevicolle* (Luc *et al.* 1998; Coomans *et al.* 2001). *Xiphinema rivesi* Dalmasso, 1969, and *X. santos* Lamberti, Lemos, Agostinelli & D'Addabbo, 1993 have been reported in Portugal (Lamberti *et al.* 1993, 1994; Bravo & Lemos 1997) (Figures 1, 4, 5; Table S1) and other Mediterranean regions (Lamberti *et al.* 1996, 2000; Taylor & Brown 1997; Decraemer & Chaves 2012); however, only the first species is widespread (Lamberti *et al.* 2000, 2004; Coomans *et al.* 2001; Decraemer & Robbins 2007). Both species belong to the *X. americanum*-subgroup and appear to be morphologically almost identical, but the Iberian populations of both species are clearly separated phylogenetically using molecular ribosomal markers (Gutiérrez-Gutiérrez *et al.* 2011; Archidona-Yuste *et al.* 2016a). *Xiphinema rivesi* is a vector of several important nepovirus (Taylor & Brown 1997; Decraemer & Chaves 2012) but in Portugal its presence has never been associated with viral diseases in plants. *Xiphinema santos* was described the first time by Lamberti *et al.* (1993) from several populations infesting vineyards localized in the Dão wine region and subsequently reported extensively in the continental region as well as Madeira and Azores (Terceira) islands associated with many species of plants (Figures 1, 4, 5; Table S1). Several Macaronesian populations of *X. santos* had previously been identified as *X. intermedium* Lamberti & Bleve-Zacheo, 1979 (Bravo 1983; Bravo & Lemos 1997).



**FIGURE 1.** Geographic distribution of populations from *X. americanum* group species in Continental Portugal: (A) *Xiphinema brevicolle*, *X. duriense*, *X. exile*, *X. longistilum*, *X. madeirense*, *X. mesostilum* and *X. santos*; (B) *X. americanum sensu lato* (s.l.), *X. brevisicum*, *X. lafoense*, *X. microstilum*, *X. pachtaicum*, *X. pachydermum* and *X. rivesi*. This map may be similar but not identical to other published maps of Portugal designed using the mapping software Cartodb. This map is for illustrative purposes only.



**FIGURE 2.** Geographic distribution of populations from *Xiphinema* spp. in Continental Portugal: (A) *Xiphinema belmontense*, *X. cadavalense*, *X. coxi*, *X. coxi europaeum*, *X. dissimile*, *X. diversicaudatum* and *X. pseudocoxi*; (B) *X. abrantinum*, *X. diversum*, *X. gersoni*, *X. index*, *X. vuittenezi*, *X. italiae*, *X. lusitanicum*, *X. sphaerocephalum* and *X. turcicum*; (C) *X. hispanum*, *X. hispidum*, *X. lanceolatum*, *X. lapidosum*, *X. lupini*, *X. pombalense* and *X. pyrenaicum*. This map may be similar but not identical to other published maps of Portugal designed using the mapping software Cartodb. This map is for illustrative purposes only.

### *Xiphinema pachtaicum*-subgroup species

*Xiphinema pachtaicum* (Tulaganov 1938) Kirjanova, 1951, and *X. madeirense* Brown, Faria, Lamberti, Halbrecht, Agostinelli & Jones, 1993, belong to the *X. pachtaicum*-subgroup and they have been both reported in Portugal (Figures 1, 4, 5; Table S1). *Xiphinema pachtaicum* is widespread in the Mediterranean region (Brown & Taylor 1987; Bravo & Lemos 1997; Taylor & Brown 1997; Lamberti *et al.* 2000; Getaneh *et al.* 2015; Archidona-Yuste *et al.* 2016a), whilst the geographical distribution of *X. madeirense* is restricted to Portugal and Spain (Archidona-Yuste *et al.* 2016a). *Xiphinema pachtaicum* was cited in Portugal for the first time by Lima (1962, 1965) and since then a large number of authors have reported this species both in the continental region and the overseas territories including the islands of Madeira and Azores (Azores, Pico, Terceira, S. Miguel and S. Jorge) (Figures 1, 4, 5; Table S1). *Xiphinema madeirense* was described from specimens collected from the rhizosphere of the “Laurisilva” forest (*Laurus nobilis* L.) at Queimadas, Santana (Madeira island); however it has also been reported infesting vineyard soils in the North and Central continental regions and in the Azores islands (Figures 1, 4, 5; Table S1).

### *Xiphinema pachydermum*-subgroup species

The *X. pachydermum*-subgroup is a particular group within the *X. americanum*-group since it is mainly composed of Spanish and Portuguese species characterized by *i*) frequent males; *ii*) females that do not show symbiotic bacteria associated with the ovaries (except in *X. mesostilum*); and *iii*) an oviduct clearly separated from the uterus by a well-developed sphincter muscle and longer uteri (Luc *et al.* 1998; Decraemer & Geraert 2013; Archidona-Yuste *et al.* 2016a). Some researchers proposed *X. pachydermum*-subgroup as a group outside the *X. americanum*-group (Coomans *et al.* 2001; He *et al.* 2005; Gutiérrez-Gutiérrez *et al.* 2012a). A total of nine species belonging to



the *X. pachydermum*-subgroup has been reported in Portugal, included *X. brevisicum* Lamberti, Bravo, Agostinelli & Lemos, *X. duriense* Lamberti, Lemos, Agostinelli & d' Addabo, 1993, *X. exile* Roca, Lamberti, Santos & Abrantes, 1989, *X. lafoense* Roca, Pereira & Lamberti, 1987, *X. longistilum* Lamberti, Bravo, Agostinelli & Lemos, 1994, *X. mesostilum* Lamberti, Bravo, Agostinelli & Lemos, 1994, *X. microstilum* Lamberti, Bravo, Agostinelli & Lemos, 1994, *X. opisthohysterum* Siddiqi, 1961, and *X. pachydermum* (Figures 1, 4, 5; Table S1). Sturhan reported for the first time *X. opisthohysterum* and in 1983 described a new species named *X. pachydermum*, which morphologically resembles *X. pachtaicum* and was found in soil from a farm at Torre de Moncorvo (province of Trás-os-Montes) (Sturhan 1983; Bravo & Lemos 1997). Since then *X. pachydermum* has been reported extensively in the North of Portugal and has also been reported in the Estremadura and Algarve regions (Lamberti *et al.* 1993, 1994; Bravo & Lemos 1997) (Figure 1; Table S1). Two new endemic species, *X. exile* and *X. lafoense*, were described by Roca *et al.* (1987, 1989a). Lamberti *et al.* (1993, 1994) established the occurrence of *X. americanum*-group species in the main grapevine-growing regions of Portugal. Detailed morphometric and morphological studies enabled species discrimination in this group, which is characterized by high morphological similarity and overlapping morphometrics, resulting in the description of five species namely *X. brevisicum*, *X. duriense*, *X. longistilum*, *X. mesostilum* and *X. microstilum*. These authors hypothesized that the population described by Sturhan in 1983 as *X. opisthohysterum* was probably *X. duriense* because of their similar morphology and different geographical localities. Subsequently, Gutiérrez-Gutiérrez *et al.* (2012) and Archidona-Yuste *et al.* (2016a) carried out morphological and molecular studies to establish the species composition of the *X. americanum*-group in Spain, reporting the presence of both *X. opisthohysterum* and *X. duriense*, which were almost identical morphologically but genetically different. Recently, phylogenetic analysis for species delimitation in the *X. americanum*-group complex developed by Archidona-Yuste *et al.* (2016a) as part of a polyphasic study based on morphological and molecular data using the D2-D3 region of 28S gene, showed that the Portuguese and Spanish populations of *X. brevisicum* (GenBank accession number: AY601610, Portugal; KP268966, Spain) clustered together but were clearly separated from other species that are almost identical morphologically belonging to the *X. pachydermum* subgroup, such as *X. duriense* (KP268963, Spain), *X. opisthohysterum* (KP268967, Spain), *X. pachydermum* (AY601608, Portugal) and *X. parapachydermum* Gutiérrez-Gutiérrez, Cantalapiedra-Navarrete, Decraemer, Vovlas, Prior, Palomares Rius, & Castillo, 2012 (KP268970, Spain).

### ***Xiphinema* species that are not members of the *X. americanum*-group**

Among the *Xiphinema* species that do not belong to the *X. americanum*-group are some major European nepovirus vector nematodes (Decraemer & Robbins 2007). There is no morphological feature that enables discrimination between non- virus vector and virus vector nematodes inside this group, which is mainly characterized by *i*) usually large body (length between 1.4-7.3 mm) and odontostyle (length between 100-160 µm); *ii*) from filiform to hemispherical shape tail; *iii*) a female reproductive system with usually two equally developed genital branches; and *iv*) usually with long bipartite uteri with a tubular part/*pars dilatata* uteri to tripartite with uterine differentiation, which is mainly characterized by an outer and internal wall differentiated and sclerotized, respectively, and the presence of some well-developed sclerotized apophyses (the 'Z-organ'), slightly developed outer wall with internal wall weakly sclerotized or absent (the 'pseudo-Z-organ'), and the uterine spines or crystalloid bodies in the uterus sometime agglomerated before the beginning the *pars dilatata* (Brown & Halbrendt 1997; Coomans *et al.* 2001; Decraemer & Chaves 2012). Three of them are recognized as vectors transmitting economically important nepoviruses in Portugal (Brown & Trudgill 1997; Taylor & Brown 1997, Decraemer & Chaves 2012) (Table S1). This group of important phytopathological species has been divided into eight morphospecies groups based on the previously cited characteristics (Brown & Halbrendt 1997; Decraemer & Chaves 2012). A total of 25 known *Xiphinema* species, excluding the *americanum* group, have been reported to occur in Portugal (Figures 2, 4, 5; Table S1); however, some records of species are considered to be dubious identifications (Table S1).

The majority of *Xiphinema* spp. species are included in the morphospecies groups 5-8 described by Brown & Halbrendt (1997). *Xiphinema* species belonging to morphogroup 5, are characterized by two genital branches with uteri containing Z-differentiations plus spines or crystalloid structures and a dorsally convex-conoid tail with digitate terminus resembling the virus vector *X. diversicaudatum* (*X. diversicaudatum*-complex), have been

recorded widely in Portugal since the first report in 1962 (Lima 1962, 1965; Macara 1963; Brown *et al.* 1992; Taylor & Brown 1997) (Table S1). *Xiphinema diversicaudatum* is widespread in temperate region of Europe in cool and humid conditions (Brown & Taylor 1987; Taylor & Brown 1997; Coomans *et al.* 2001; Decraemer & Chaves 2012). This longidorid species has been reported from Continental Portugal and from overseas territories of Madeira and Azores islands) (Brown *et al.* 1992; Taylor & Brown 1997; Vieira *et al.* 2008; Borges 2010; Decraemer & Chaves 2012) (Table S1) (Figures 2, 4, 5). The *X. diversicaudatum* populations reported in Portuguese territories show a high inter- and intra-morphometric variability (Lampreia & Brown 1992), in fact the identification of this species was controversial. The first record of a population of *X. diversicaudatum* was in an apple plantation located in a district of Northern Portugal (Viseu) (Macara 1963; Brown & Taylor 1987; Brown *et al.* 1992; Taylor & Brown 1997); however, its identification was later questioned by Pereira (1984) who studied the occurrence of longidorids in strawberry fields in Portugal and reported the presence of a species closely related to *X. diversicaudatum* but with some biometric and morphological differences. Macara (1994) reported a large list of populations belonging to the *X. diversicaudatum* complex, but unfortunately did not include any detailed morphometric and morphological characterizations. Results of the D2-D3 region of 28S gene obtained by Gutiérrez-Gutiérrez *et al.* (2013) during a morphological and molecular phylogenetic study of subfamily Xiphineminae species belonging to the *X. diversicaudatum* complex suggest that the population of *X. diversicaudatum* (GenBank accession number: AY601624) from northern Portugal, which was not identified on the basis of detailed morphological and morphometric analysis, was a misidentification and should be considered conspecific with *X. coxi europaeum* Sturhan, 1984. Subsequently, the phylogenetic analysis developed by Chizhov *et al.* (2014), during a polyphasic study based on morphological and molecular data where several new Central European *X. diversicaudatum* populations were incorporated, is in agreement with the idea of Gutiérrez-Gutiérrez *et al.* (2013b) regarding a misidentification of the Portuguese population (GenBank accession number: AY601624) which should be considered as *X. coxi europaeum*. During the 1980s two new endemic species belonging to the *X. diversicaudatum* complex were described, *Xiphinema lusitanicum* Sturhan, 1983 and *X. dissimile* Roca, Pereira & Lamberti, 1987 (Sturhan 1983; Roca *et al.* 1987; Roca & Bravo 1997). Subsequently, both species were extensively reported in Portuguese vineyards and forest soils (Macara 1994, Bravo & Lemos 1997, Roca & Bravo 1997, Coomans *et al.* 2001) (Figures 2, 4, 5; Table S1). *Xiphinema coxi* Tarjan 1964, was reported for the first time in Portugal infesting the soil of a conifer tree forest in Estarreja (province of Beira Litoral) (Table S1). Sturhan (1984) established that *X. coxi* was in fact a species subcomplex highly similar morphologically to *X. diversicaudatum*. Unfortunately, these populations of the *X. coxi* subcomplex have never been reviewed, but both *X. pseudocoxi* Sturhan, 1984, and *X. coxi europaeum*, have later been reported in Portugal (Figure 2; Table S1) as well as from other localities of the Iberian Peninsula (Arias *et al.* 1986; Abelleira *et al.* 1996; Gutiérrez-Gutiérrez *et al.* 2013b). Phylogenetic analysis developed by Gutiérrez-Gutiérrez *et al.* (2013b) clearly separated several Iberian populations of *X. coxi europaeum* from other Iberian endemic species with highly similar morphology, such as the Portuguese population of *X. abrantinum* Roca & Pereira, 1991 (GenBank accession number: AY601625) and the Spanish populations of *X. belmontense* Roca & Pereira, 1992 (GenBank accession number: KC567170, KC567171, KC567172). During the 1990s, a large number of records were published with descriptions of new Iberian endemic species belonging to the *X. diversicaudatum* complex, including *X. abrantinum*, *X. belmontense*, *X. cadavalense* Bravo & Roca, 1995 and *X. gersoni* Roca & Bravo, 1993 (Figure 2; Table S1). These species belong to the *X. diversicaudatum* complex are considered Iberian endemics (Figure 2; Table S1) but only *X. belmontense*, *X. cadavalense* and *X. gersoni* have been reported for Spain (Murillo-Navarro *et al.* 2005; Peña-Santiago *et al.* 2006; Gutiérrez-Gutiérrez *et al.* 2013b). Nematode surveys conducted in the forest nurseries of the Forest Administration in the Northern Portugal by Macara (1994) resulted in the description of a new species, *X. amarantum* Macara, 1970, but later it was considered to be a synonym of *X. sahelense* Dalmasso, 1969 (Figures 2, 4, 5; Table S1). Macara (1970) characterized this species by having two genital branches with uteri containing a pseudo-Z-organ and a conoid tail with distinctly digitate terminus resembling the *X. diversicaudatum* species complex. Lampreia & Brown (1992) proposed that the populations described by Macara in 1970 should be a synonymy of *X. diversicaudatum*, since *X. sahelense* did not show Z-differentiations. However, for the description of *X. amarantum*, Macara assigned paratype specimens from several populations, possibly mixing several members of the *X. diversicaudatum* complex species (Table S1). *Xiphinema hispidum* Roca & Bravo, 1994 and *X. lupini* Roca & Pereira, 1993 are two Iberian endemic morphospecies in group 5, phylogenetically and morphologically related to the putative virus vector *X. italiae* (morphospecies group 7) (Gutiérrez-Gutiérrez *et al.* 2011). They were

described originally from the localities of Palmela (province of Estremadura) and Coruche (province of Ribatejo) (Figure 2; Table S1) and have later been reported extensively from cultivated and natural environments of the Southern Iberian Peninsula (Peña-Santiago *et al.* 2006; Gutiérrez-Gutiérrez *et al.* 2011, 2013b). The putative GFLV vector nematode *X. italiae*, one of the most widespread *Xiphinema* species in the Mediterranean area where it has been associated with many plant species (Brown & Taylor 1987; Taylor & Brown 1997; Coomans *et al.* 2001; Decraemer & Chaves 2012), was reported for the first time in the locality of Alpiarça (province of Ribatejo) (Lima 1974) and later found infesting several commercial vineyards in sandy soils in Estremadura, Ribatejo and Douro wine regions (Lima 1974; Bravo & Lemos 1997; Taylor & Brown 1997; Gutiérrez-Gutiérrez *et al.* 2015) (Figure 2; Table S1). Currently, this species is not accepted as a vector nematode because GFLV was transmitted by *X. italiae* under controlled experimental conditions on only one single occasion (Taylor & Brown 1997), thus not fulfilling the postulate proposed by Trudgill *et al.* (1983).

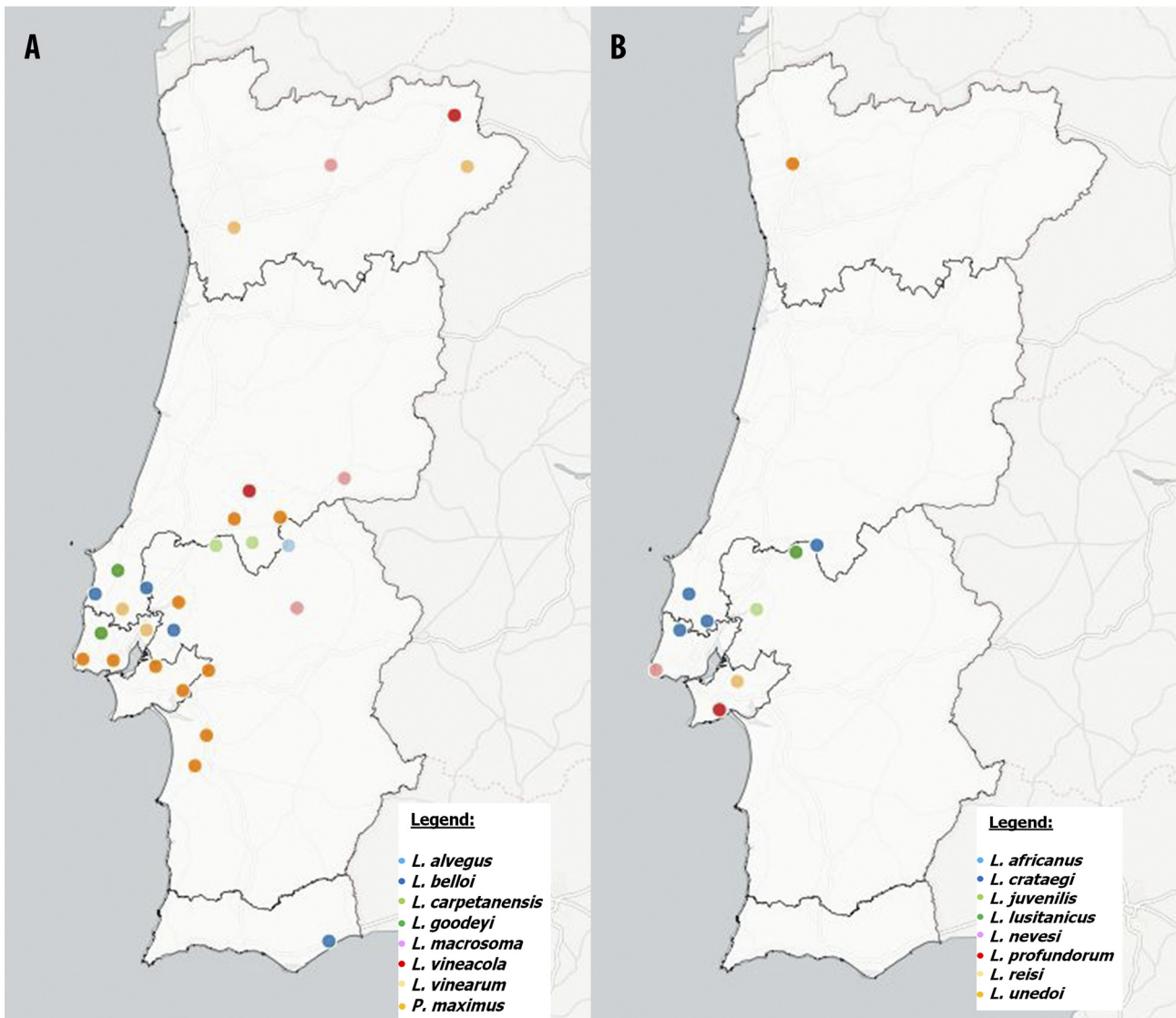
Some species belong to morphogroups 5, 6 and 8 characterized by a dorsally convex-conoid tail with rounded terminus to almost hemispherical, with an inconspicuous terminal bulge resembling *X. pyrenaicum* (*X. pyrenaicum*-complex species) were reported in Portugal (Macara 1994; Roca & Bravo 1993b; Bravo & Lamberti *et al.* 1996; Bravo & Lemos 1997) (Figures 2, 5; Table S1). *Xiphinema turcicum* Luc & Dalmasso, 1963 was mentioned for the first time by Bravo (1983) and later reported in association with forest tree species in several locations from the north to the south of the country (Macara 1994). Some populations identified by Bravo (1983) as *X. turcicum*, were later considered to be two morphologically closely related species previously described in Spain (Lamberti *et al.* 1992; Gutiérrez-Gutiérrez *et al.* 2010), *X. hispanum* Lamberti, Castillo, Gomez-Barcina & Agostinelli, 1992 and *X. sphaerocephalum* Lamberti, Castillo, Gomez-Barcina & Agostinelli, 1992, as well as two new Portuguese endemic species *X. lanceolatum* Roca & Bravo, 1993 and *X. lapidosum* Roca & Bravo, 1993 (Roca & Bravo 1993b) (Figure 2; Table S1).

The other species described in Portugal belong to morphospecies group 8, characterized by two genital branches without Z-differentiations or spines in the uteri and an usually short convex-conoid tail with a digitate ventral or terminal peg resembling the virus vector nematodes *X. index* (Luc *et al.* 1964; Macara *et al.* 1994; Bravo & Lemos 1997; Taylor & Brown 1997; Gutiérrez-Gutiérrez *et al.* 2015) (Figures 2, 5; Table S1). Sequeira & Dias (1964) reported the transmission of GFLV by Portuguese populations of *X. index*, demonstrating that the virus vector nematodes and their associated viruses were causing severe economic losses in Portugal. At that time, the virus vector nematodes were recognized as a priority subject of study in Portugal. Consequently, a large body of literature reported the occurrence and distribution of the virus vector nematode species during the following thirty-five years (Figures 2, 5; Table S1). *Xiphinema index* is widespread in commercial vineyards in the main grapevine-growing areas of the Mediterranean climate regions (Taylor & Brown 1997; Coomans *et al.* 2001; Decraemer & Robbins 2007; Decraemer & Chaves 2012) including the Portuguese vineyards of old grapevine-growing areas (Bravo & Lemos 1997; Gutiérrez-Gutiérrez *et al.* 2015) (Table S1). Bravo (1983) reported the presence of *X. index* in 16 % of the Portuguese vineyards of the Oeste Region (now known as Lisbon wine region). Recently, Gutiérrez-Gutiérrez *et al.* (2015) established that *X. index* is still distributed extensively in vineyards in the same wine area of Portugal with an overall prevalence of 21% (Gutiérrez-Gutiérrez *et al.* 2015). The high prevalence of *X. index* makes this species a severe threat to grapevine production in Portugal since in the Portuguese grapevine-growing regions GFLV has also been detected (Santos 2011).

## Genus *Longidorus*

Among the *Longidorus* species are some of the major European nepovirus vector nematodes (Decraemer & Robbins 2007). There are no morphometric and morphological characteristic that enable to discrimination between non-virus vector and virus vector nematodes in this genus, mainly characterized by a large body (length between 3.0-9.0 mm) and odontostyle (length between 80-160 µm), a lip region continuous or offset by depression or expanded anteriorly with a border rounded or flattened, amphidial fovea usually more or less pouch-like between shallowly or distinctly bilobed symmetrical or asymmetrical, and a tail usually short, which varies between hemispheroid and elongate-conical (Chen *et al.* 1997). The first reference on Longidorinae nematodes (*Longidorus* spp., and *Paralongidorus* spp.) occurring in Portugal dates from 1962 (Macara 1963). This population from Dois Portos (province of Estremadura) was not identified at species level at that time, but thirty years later at the same





**FIGURE 3.** Geographic distribution of populations from *Longidorus* and *Paralongidorus* species in Continental Portugal: (A) *L. alvegus*, *L. belloii*, *L. carpetanensis*, *L. goodeyi*, *L. macrosoma*, *L. vineacola*, *L. vinearum* and *P. maximus*; (B) *L. africanus*, *L. crataegi*, *L. juvenilis*, *L. lusitanicus*, *L. nevesi*, *L. profundorum*, *L. reisi* and *L. unedoi*. This map may be similar but not identical to other published maps of Portugal designed using the mapping software Cartodb. This map is for illustrative purposes only.

locality, infesting the same crop the species *L. vinearum* Bravo & Roca, 1995, was identified (Bravo & Roca 1995). Two new *Longidorus* species named *L. lusitanicus* Macara, 1985 and *L. nevesi* Macara, 1985 were later described (Macara 1985). Both species are considered Iberian endemic species with a large distribution among different forest environments (Brown & Taylor 1987; Navas *et al.* 1990, Archidona-Yuste *et al.* 2016b) (Figure 3; Table S1). The occurrence and distribution in Portugal of two species known as *L. macrosoma* and *L. profundorum* Hooper, 1966 has been reported (Taylor & Brown 1987; Navas *et al.* 1990; Bravo & Lemos 1997; Brown & Taylor 1997) (Figure 3; Table S1). They are phytopathologically important because they are known vectors of Raspberry ringspot virus (RpRSV), and occur in numerous countries in Europe, including the Mediterranean and Iberian countries, although *L. macrosoma* appears to be more frequent in Central Europe (Navas *et al.* 1990; Bravo & Lemos 1997; Taylor & Brown 1997; Nombela *et al.* 1998). Later, two morphologically near identical species were described from Portugal: *L. alvegus* Roca, Pereira & Lamberti, 1989 and *L. reisi* Roca & Bravo, 1993 (Roca *et al.* 1989b; Roca & Bravo 1993). These two *Longidorus* species are considered Iberian endemics (Figure 3; Table S1) but only *L. alvegus* has been reported for Spain (Brown & Taylor 1987; Navas *et al.* 1990; Gutiérrez-Gutiérrez *et al.* 2011; Archidona-Yuste *et al.* 2016b). Roca & Bravo (1996) described a new species named *Longidorus crataegi*

Roca & Bravo, 1996, from several populations found in the rhizosphere of *Crataegus oxycantha* L. in the south-west region of Portugal (Brown & Lemos 1997) (Figure 3; Table S1), and subsequently this species has also been reported from several Spanish localities (Gutiérrez-Gutiérrez *et al.* 2012b; Archidona-Yuste *et al.* 2016b). This Iberian endemic species is characterized by a lip region almost flattened frontally and rounded laterally, continuous with the rest of the body, distinctly asymmetrically bilobed amphidial fovea-pouch, and tail conoid-rounded resembling the *L. vineacola* complex species (Brown *et al.* 1997; Ye *et al.* 2003; Archidona-Yuste *et al.* 2016b). In Portugal, this species complex includes two species, *L. vinearum* and *L. vineacola* Sturhan & Weischer, 1954, which have frequently been found infesting Portuguese vineyards (Bravo & Roca 1995, 1998; Bravo & Lemos 1997; Roca & Bravo 1996; Peña-Santiago *et al.* 2006) (Figure 3; Table S1). *Longidorus vinearum* is an Iberian endemic species (Bravo & Roca 1995, 1998; Archidona-Yuste *et al.* 2016b; Gutiérrez-Gutiérrez, unpublished data), whilst *L. vineacola* has been extensively reported from the Mediterranean Basin and Central Europe (i.e. Belgium, Bulgaria, England, France, Greece, Germany, Israel, Jordan, Moldavia, Netherlands, Portugal, Scotland and Spain), although also have been reported in some regions of Central Asia (Russian) (Boag & Brown 1987; Navas *et al.* 1990; Taylor & Brown 1997; Brown *et al.* 1997; Ye *et al.* 2003; Gutiérrez-Gutiérrez *et al.* 2012b; Archidona-Yuste *et al.* 2016b). Several new records of *Longidorus* species in the Iberian Peninsula were reported (Bravo & Lemos 1997) (Figure 3; Table S1) but no morphological and morphometric analyses were made, except in the descriptions of some *Longidorus* populations (Bravo & Roca 1995, 1998; Roca & Bravo 1996).

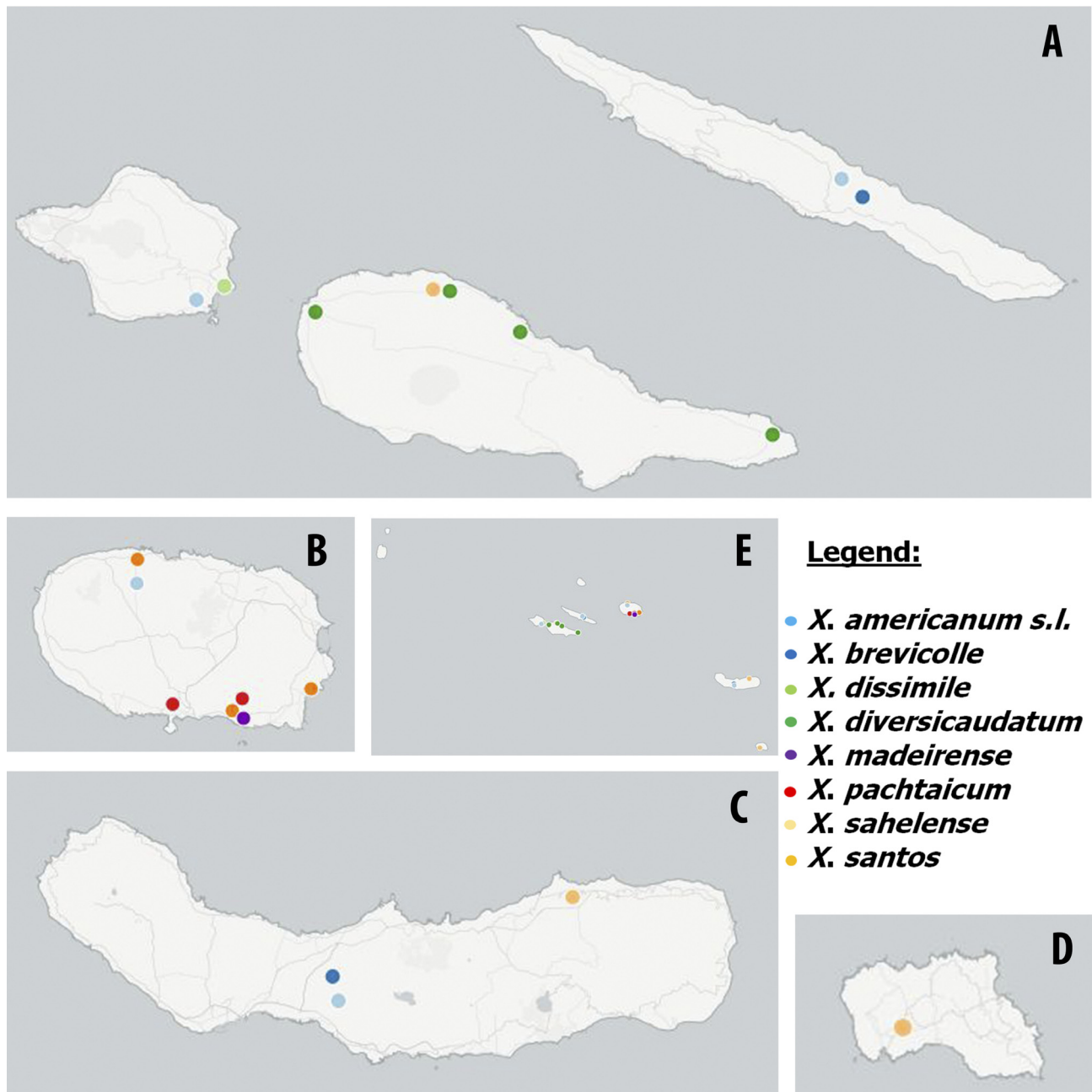
### Genus *Paralongidorus*

Lima in 1968 recorded for the first time the genus *Paralongidorus* as occurring in soils of eucalyptus and cypress-pine forests and other natural woodlands (Macara 1994) (Table S1). Subsequently, this species was collected from soil around roots of seedlings in forest nurseries at several localities (Macara 1982, 1994) and identified for the first time in Portugal as *Siddiqia maxima* (Bütschli, 1874) Khan, Chawla & Saha, 1978 (Macara, 1982); it was subsequently transferred to the genus *Paralongidorus* Siddiqi, Hopper & Khan, 1963 (= *P. maximus* (Bütschli, 1874) Siddiqi, 1964). *Paralongidorus maximus* is the most common and widespread species of the genus and is a vector of RpvRSV (Brown & Taylor 1987; Bravo & Lemos 1997; Taylor & Brown 1997) (Figure 3; Table S1). This species has been detected in some Mediterranean countries and the Iberian Peninsula (Brown & Taylor 1987; Lamberti *et al.* 1983, Taylor & Brown 1997, Lišková & Brown 2003); however, it appears that its geographical distribution is restricted to central European countries where it is commonly found associated with forests and vineyards (Taylor & Brown 1997). Of the seven valid *Paralongidorus* species described in Europe, five of them have been reported to be present in Spain (Palomares-Rius *et al.* 2013) and one of them in Portugal (Bravo & Lemos 1997; Taylor & Brown 1997).

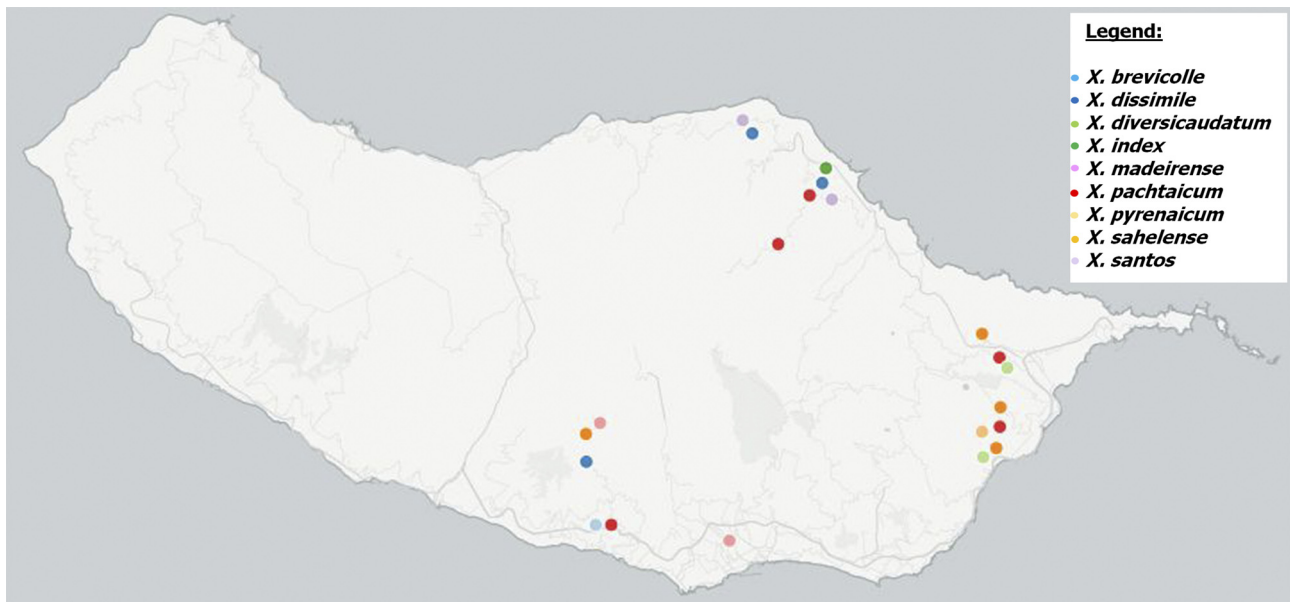
### Conclusions

This review has been undertaken to compile all information available from papers, books and databases, as well as unpublished data of the authors, to include new records and those data that were overlooked, update maps of distribution of species, and clarify the taxonomic position of some species with dubious identifications. Three *Xiphinema* spp. (*X. pombalense* Bravo & Lamberti, 1996, *X. cadavalense* Bravo & Roca, 1995, *X. silvesi* Roca, 1998) and one *Longidorus* spp. (*L. crataegi* Roca & Bravo, 1996) are added to update the list of nematode species from the family Longidoridae in Portugal proposed by Bravo & Lemos in 1997 (Table S1), as well as several new records of other previously reported species such as *L. vineacola*, *L. vinearum*, *X. coxi europaeum*, *X. italiae*, *X. lusitanicum*, *X. pachtaicum*, *X. pseudocoxi*, *X. sahelense* and *X. santos* (Bravo & Roca 1995, 1998; Roca & Bravo 1996, 1997, 1998; Bravo *et al.* 2001; Gutiérrez-Gutiérrez *et al.* 2015; Bravo, unpublished data; Gutiérrez-Gutiérrez, unpublished data). According to the data presented, a total of 56 species have been recorded as present in Portugal; however, in this review we propose that some populations identified as *X. coxi*, *X. diversicaudatum* and *X. sahelense* should be considered as populations with a doubtful identity, requiring further investigation. Longidorid species richness appears to be higher in the Iberian Peninsula (Portugal and Spain) than in the rest of Europe, which could be due to this region possibly being the centre of origin for a large number of longidorid

species because of the high number of endemic species. A total of twenty (four *Longidorus* and sixteen *Xiphinema*) species are tentatively considered as endemic to Portugal since their current distribution is restricted to Portugal; in addition, thirteen other (five *Longidorus* and eight *Xiphinema*) species are regarded as endemic to the Iberian Peninsula. This high biodiversity present in the Portuguese territory confirms the importance of this group of phytopathogenic species in this region.



**FIGURE 4.** Geographic distribution of populations from longidorid species present in Azores Islands: (A) Sao Jorge, Pico and Faial islands; (B) Terceira; (C) Sao Miguel island; (D) Santa Maria island; (E) Azores islands. This map may be similar but not identical to other published maps of Azores Islands and designed using the mapping software Cartodb. This map is for illustrative purposes only.



**FIGURE 5.** Geographic distribution of populations from longidorid species present in Madeira Island. This map may be similar but not identical to other published maps of Madeira Island designed using the mapping software Cartodb. This map is for illustrative purposes only.

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## References

- Abelleira, A., Mansilla, J.P. & Arias, M. (1996) *Xiphinema coxi europaeum* Sturhan asociados con daños en viveros de coníferas en Galicia. *Boletín de Sanidad Vegetal-Plagas*, 22, 551–556.
- Andret-Link, P., Laporte, C., Valat, L., Ritzenthaler, C., Demangeat, G., Vigne, E., Laval, V., Pfeiffer, P., Stussi-Garaud, C. & Fuchs, M. (2004) *Grapevine fanleaf virus*: still a major threat to the grapevine industry. *Journal of Plant Pathology*, 86, 183–95.
- Archidona-Yuste, A., Navas-Cortés, J.A., Cantalapiedra-Navarrete, C., Palomares-Rius, J.E. & Castillo, P. (2016a) Cryptic diversity and species delimitation in the *Xiphinema americanum*-group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular markers. *Zoological Journal of the Linnean Society*, 176, 231–265. <http://dx.doi.org/10.1111/zoj.12316>
- Archidona-Yuste, A., Navas-Cortés, J.A., Cantalapiedra-Navarrete, C., Palomares-Rius, J.E. & Castillo, P. (2016b) Unravelling the biodiversity and molecular phylogeny of needle nematodes of the genus *Longidorus* (Nematoda: Longidoridae) in olive and a description of six new species. *PLoS ONE*, 11, e0147689. <http://dx.doi.org/10.1163/156854108783360212>
- Arias, M., Andres, M.F. & Navas, A. (1986) *Longidorus carpetanensis* sp. n. and *L. unedoi* sp. n. (Nematoda: Longidoridae) from Spain. *Revue de Nématologie*, 9, 101–106.
- Barsi, L. & De Luca, F. (2008) Morphological and molecular characterisation of two putative *Xiphinema americanum* group species, *X. parasimile* and *X. simile* (Nematoda: Dorylaimida) from Serbia. *Nematology*, 10, 15–25. <http://dx.doi.org/10.1163/156854108783360212>
- Boag, B. & Brown, D.J.F. (1987) The occurrence of *Longidorus vineacola* in Scotland with notes on its distribution in Europe.

- Borges, P.A.V. (2010) List of the terrestrial nematodes (Nematoda) from Azores.. In: Borges, P.A.V., Costa, A., Cunha, R., Gabriel, R., Gonçalves, V., Martins, A.F., Melo, I., Parente, M., Raposeiro, P., Rodrigues, P., Santos, R.S., Silva, L., Vieira, P. & Vieira, V. (Eds.), *A list of the terrestrial and marine biota from the Azores*. Principia, Cascais, pp. 157–164.
- Bravo, M.A., Roca, F. & Mota, M.M. (2001) Observations on *Xiphinema coxi europaeum* Sturhan from Portugal and description of the male of *X. lusitanicum* Sturhan (Nematoda: Longidoridae) with multivariate analysis of juveniles. *International Journal of Nematology*, 11, 15–26.
- Bravo, M.A. & Lemos, R.M. (1997) *Longidorus*, *Paralongidorus* and *Xiphinema* in Portugal. In: Santos, M.S. de A., Abrantes, I. O., Brown, D.J.F. & Lemos, R.M. (Eds.), *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Universidade de Coimbra, Coimbra, pp. 421–441.
- Bravo, M.A. & Roca, F. (1995) Observations on *Longidorus africanus* Merny from Portugal with description of *L. vinearum* sp. n. (Nematoda: Longidoridae). *Fundamental and Applied Nematology*, 18, 97–84.
- Bravo, M.A. & Roca, F. (1998) Two *Longidorus* species (Nematoda: Longidoridae) occurring in the rhizosphere of olive trees in Northeastern Portugal. *Agronomia Lusitanica*, 46, 101–121.
- Bravo, M.A. (1983) *Nematodos vectores de viroses da videira*. Encontro de Técnicos de Viticultura. Ministerio de Agricultura Forestal e Alimentação, EAN, Oeiras, pp. 1–6.
- Bravo, M.A. (1989) Especies de nemátodos da familia Longidoridae em Portugal, Madeira e Açores. *Summa Phytopathologica*, 15, 46.
- Brown, D. F., Faria, A., Lamberti, F., Halbrendt, M., Agostinelli, A. & Jones, A.T. (1992) A description of *X. madeirense* sp.n. and the occurrence and virus vector potential of *X. diversicaudatum* (Nematoda: Dorylaimida) from Santana, Madeira. *Nematologia Mediterranea*, 20, 251–259.
- Brown, D.J.F. & Halbrendt, J.M. (1997) Identification of *Xiphinema* species (Nematoda: Dorylaimoidea). In: Santos, M.S.N.A., Abrantes, I.M.O., Brown, D.J.F. & Lemos, R.M. (Eds.), *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, pp. 177–223.
- Brown, D.J.F. & Taylor, C.E. (1987) Comments on the occurrence and geographical distribution of longidorid nematodes in Europe and the Mediterranean region. *Nematologia Mediterranea*, 15, 333–373.
- Brown, D.J.F., Neilson, R., Connolly, T. & Boag, B. (1997) An assessment of morphometric variability between populations of *Longidorus vineacola* Sturhan & Weischer, 1964 (Nematoda: Longidoridae) and morphologically related species. *Systematic Parasitology*, 37, 93–103.  
<http://dx.doi.org/10.1023/A:1005713123568>
- Chen, Q.W., Hooper, D.J., Loof, P.A.A. & Xu, J. (1997) A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology*, 20, 15–28.
- Chizhov, V.N., Pridannikov, M.V., Peneva, V. & Subbotin, S.A. (2014) Morphological and molecular characterisation of the Saratov population of the European dagger nematode, *Xiphinema diversicaudatum* (Nematoda: Dorylaimida), with notes on phylogeography of the species. *Nematology*, 16, 847–862.  
<http://dx.doi.org/10.1163/15685411-00002813>
- Coomans, A., Huys, R., Heyns, J. & Luc, M. (2001) *Character analysis, phylogeny, and biogeography of the genus Xiphinema Cobb, 1913 (Nematoda, Longidoridae)*. Vol. 287. Annales du Musée Royal de l’Afrique Centrale (Zoologie), Tervuren, Belgique, 239 pp.
- Decraemer, W. & Chaves, E. (2012) Chapter 15. Longidoridae and Trichodoridae. In Manzanilla-Lopez, R. & Marban Mendoza, N. (Eds.), *Practical Plant Nematology*. Fundacion Colegio de Posgraduados and Mundi Prensa, Mexico, pp. 579–617.
- Decraemer, W. & Geraert, E. (2013) Ectoparasitic nematodes. In: Perry R. N. & Moens, M. (Eds.), *Plant Nematology*. 2<sup>nd</sup> Edition. CABI, Wallingford, pp. 199–202.  
<http://dx.doi.org/10.1079/9781780641515.0179>
- Decraemer, W. & Robbins, R.T. (2007) The Who, What and Where of Longidoridae and Trichodoridae. *Journal of Nematology*, 39, 295–297.
- EPPO web (2016) European and Mediterranean Plant Protection Organization. Available from: <http://www.eppo.int/> (accessed 19 October 2016)
- Escuer, M. & Arias, M. (1997) *Paralongidorus iberis* sp. n. and *P. monegrensis* sp. n. from Spain with a polytomous key to the species of the genus *Paralongidorus* Siddiqi, Hooper & Khan, 1963 (Nematoda: Longidoridae). *Fundamental and Applied Nematology*, 20, 135–148.
- Esmaili, M., Heydari, R., Archidona-Yuste, A., Castillo, P. & Palomares-Rius, J.E. (2016) A new needle nematode, *Longidorus persicus* n. sp. (Nematoda: Longidoridae), from Kermanshah province, western Iran. *European Journal of Plant Pathology*, 1–15. [published online]  
<http://dx.doi.org/10.1007/s10658-016-0976-9>
- Getaneh, G., Bert, W. & Decraemer, W. (2015) First report, morphological and molecular characterization of *Xiphinema elongatum* and *X. pachtaicum* (Nematoda, Longidoridae) from Ethiopia. *ZooKeys*, 489, 1–13.  
<http://dx.doi.org/10.3897/zookeys.489.8629>
- Gutiérrez-Gutiérrez, C., Bravo, M.A., Santos, M.T., Vieira, P. & Mota, M. (2015) *Integrative approach and molecular barcoding of dagger and needle nematodes infesting grapevine soils in Portugal*. Scientific abstracts from the 6th



- International Barcode of Life Conference. *Genome*, 58, 163–303.  
<http://dx.doi.org/10.1139/gen-2015-0087>
- Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Decraemer, W., Vovlas, N., Prior, T., Palomares-Rius, J.E. & Castillo, P. (2012) Phylogeny, diversity, and species delimitation in some species of the *Xiphinema americanum*-group complex (Nematoda: Longidoridae), as inferred from nuclear and mitochondrial DNA sequences and morphology. *European Journal of Plant Pathology*, 134, 561–597.  
<http://dx.doi.org/10.1007/s10658-012-0039-9>
- Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Montes-Borrego, M., Palomares-Rius, J.E. & Castillo, P. (2013a) Molecular phylogeny of the nematode genus *Longidorus* (Nematoda: Longidoridae) with description of three new species. *Journal of the Linnean Society*, 167, 473–500.  
<http://dx.doi.org/10.1111/zoj.12019>
- Gutiérrez-Gutiérrez, C., Cantalapiedra-Navarrete, C., Remesal, E., Palomares-Rius, J.E., Navas-Cortés, J.A. & Castillo, P. (2013b) New insight into the identification and molecular phylogeny of dagger nematodes of the genus *Xiphinema* (Nematoda: Longidoridae) with description of two new species. *Journal of the Linnean Society*, 169, 548–579.  
<http://dx.doi.org/10.1111/zoj.12071>
- Gutiérrez-Gutiérrez, C., Palomares-Rius, J.E., 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.  
<http://dx.doi.org/10.1007/s10658-010-9705-y>
- Handoo, Z.A., Carta, L.K., Skantar A.M., Subbotin, S.A. & Fraedrich, S.W. (2016) Molecular and morphological characterization of *Xiphinema chambersi* population from live oak in Jekyll Island, Georgia, with comments on morphometric variations. *Journal of Nematology*, 48, 20–27.
- He, Y., Subbotin, S., Rubtsova, T.V., Lamberti, F., Brown, D.J.F. & Moens, M. (2005) A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). *Nematology*, 7, 111–124.  
<http://dx.doi.org/10.1163/1568541054192108>
- Hewitt, W.B., Raski, D.J. & Goheen, A.C. (1958) Nematode vector of soil-borne fanleaf virus of grapevines. *Phytopathology*, 48, 586–595.
- Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-Lopez, R., Palomares-Rius, J.E., Wesemael, W.M.L. & Perry, R.N. (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14, 946–961.  
<http://dx.doi.org/10.1111/mpp.12057>
- Lamberti, F. & Blevé-Zacheo, T. (1979) Studies on *Xiphinema americanum sensu lato* with descriptions of fifteen new species (Nematoda, Longidoridae). *Nematologia Mediterranea*, 7, 51–106.
- Lamberti, F. & Ciancio, A. (1993) Diversity of *Xiphinema americanum*-group species and hierarchical cluster analysis of morphometrics. *Journal of Nematology*, 25, 332–343.
- Lamberti, F., Agostinelli, A. & Radicci, V. (1996) Longidorid nematodes from northern Egypt. *Nematologia Mediterranea*, 24, 307–339.
- Lamberti, F., Bravo, M.A., Agostinelli, A. & Lemos, R.M. (1994) The *Xiphinema americanum*-group in Portugal with descriptions of four new species (Nematoda, Dorylaimida). *Nematologia Mediterranea*, 22, 189–218.
- Lamberti, F., Castillo, P., Gomez-Barcina, A. & Agostinelli, A. (1992) Description of six new species of *Xiphinema* (Nematoda, Dorylaimida) from the Mediterranean region. *Nematologia Mediterranea*, 20, 125–139.
- Lamberti, F., Choleva, B. & Agostinelli, A. (1983) Longidoridae from Bulgaria (Nematoda, Dorylaimida) with descriptions of three new species of *Longidorus* and two new species of *Xiphinema*. *Nematologia Mediterranea*, 11, 49–72.
- Lamberti, F., Hockland, S., Agostinelli, A., Moens, M. & Brown, D.J.F. (2004) The *Xiphinema americanum* group. 3. Keys to species identification. *Nematologia Mediterranea*, 32, 53–56.
- Lamberti, F., Lemos, R.M., Agostinelli, A. & D’addabo, T. (1993) The *Xiphinema americanum* group in the vineyards of the Dão and Douro regions (Portugal) with description of two new species (Nematoda, Dorylaimida). *Nematologia Mediterranea*, 21, 215–225.
- Lamberti, F., Molinari, S., Moens, M. & Brown, D.J.F. (2000) The *Xiphinema americanum* group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russian Journal of Nematology*, 8, 65–84.
- Lampreia, M.A.B. & Brown, D.L.F. (1992) The *Xiphinema diversicaudatum*-complex in Portugal. *Nematologica*, 38, 418–419.
- Lazarova, S., Peneva, V. & Kumari, S. (2016) Morphological and molecular characterisation, and phylogenetic position of *X. browni* sp. n., *X. penevi* sp. n. and two known species of *Xiphinema americanum*-group (Nematoda, Longidoridae). *ZooKeys*, 574, 1–42.  
<http://dx.doi.org/10.3897/zookeys.574.8037>
- Lazarova, S.S., Malloch, G., Oliveira, C.M.G., Hübschen, J. & Neilson, R. (2006) Ribosomal and Mitochondrial DNA Analyses of *Xiphinema americanum*-group populations. *Journal of Nematology*, 38, 404–410.
- Lemos, R.M., Almeida, M.T.M. de, Santos, M.S.N. de A., Abrantes, I.M.D. de O., Martins, A.I.T. & Reval, J.C.B. de M. (1990) Studies of Portuguese populations of Longidorids and Trichodorids. *Nematologica*, 36, 366.
- Lima, M.B. (1962) *Introdução ao estudo dos nemátodos de Portugal Continental*. Rel. Final Curso Eng. Agrônomo, Instituto Superior de Agronomia, Universidade Técnica, Lisboa, 141 pp.
- Lima, M.B. (1965) *Studies on the species of the genus Xiphinema*. Ph. D. Thesis, University of London, London, 165 pp.



- Lima, M.B. (1968) A numerical approach to the *Xiphinema americanum* complex. In: *Comptes Rendus VIII Symposium International de Nématologie. Antibes, France, 1965*. E.J. Brill, Leiden, pp. 30.
- Lima, M.B. (1974) *Xiphinema italiae* Meyl, 1953 (Nematoda: Dorylaimida) found around roots of grapevine in Portugal. *Agronomia Lusitana*, 35, 273–276.
- Lišková, M. & Brown, D.J.F. (2003) Longidoridae (Nematoda: Dorylaimida) in the Slovak Republic. *Helminthologia*, 40, 165–172.
- Luc, M., Coomans, A., Loof, P.A.A. & Baujard, P. (1998) The *Xiphinema americanum*-group (Nematoda: Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group. *Fundamental and Applied Nematology*, 21, 475–490.
- Luc, M., Lima, M.B., Weischer, B. & Flegg, J.J.M. (1964) *Xiphinema vuittenezi* n. sp. (Nematoda: Dorylaimidae). *Nematologica*, 10, 151–163.  
<http://dx.doi.org/10.1163/187529264X00781>
- Macara, A.M. (1963) Aspectos sobre a importância do nemátodos de interesse agrícola em Portugal e no Ultramar português. *Agros*, 46, 367–384.
- Macara, A.M. (1970) *Xiphinema amarantum* sp. nov. (Nematoda: Dorylaimidae). *Revista Ibérica de Parasitologia*, 30, 649–658.
- Macara, A.M. (1982) First record of *Siddiqia maximus* (Nematoda: Longidoridae) in Portugal. *Garcia de Orta, Serie de Estudos Agronomicos*, 9 (1–2), 309–320.
- Macara, A.M. (1985) Two new species of *Longidorus* (Nematoda, Longidoridae) associated with forest plants in Portugal. *Nematologica*, 31, 410–423.  
<http://dx.doi.org/10.1163/187529285X00508>
- Macara, A.M. (1994) Nematodes associated with forest plants in Portugal (no período de 1987-1992). *Revista de Ciências Agrárias*, 17, 77–126.
- Molinari, S., De Luca, F., Lamberti, F. & De Giorgi, C. (1997) Molecular methods for the identification of longidorid nematodes. *Nematologia Mediterranea*, 25, 55–61.
- Molinari, S., Lamberti, F., Duncan, L.W., Halbrendt, J., Kotcon, J., Abawi, G.S., Robbins, R.T., Nyczepir, A.P., Mchenry, M., Magunacelaya, J.C., Crozzoli, R., Lemos, R.M., Nagy, P., Moens, M. & Brown, D.J.F. (2004) SOD polymorphism in *Xiphinema americanum* group (Nematoda: Longidoridae). *Nematology*, 6, 867–876.  
<http://dx.doi.org/10.1163/1568541044038579>
- Navas, A., Baldwin, J.G., Barrios, L. & Nombela, G. (1993) Phylogeny and biogeography of *Longidorus* (Nematoda: Longidoridae) in Euromediterranea. *Nematologia Mediterranea*, 21, 71–88.
- Navas, A., Fe Andres, M. & Arias, M. (1990) Biogeography of Longidoridae in the Euromediterranean area. *Nematologia Mediterranea*, 18, 103–112.
- Nombela, G., Navas, A. & Bello, A. (1998) Effects of crop rotations of cereals with vetch and fallow on soil nematofauna in central Spain. *Nematologica*, 44, 63–80.  
<http://dx.doi.org/10.1163/005225998X00073>
- Oliveira, C.M.G., Fenton, B., Malloch, G., Brown, D.J.F. & Neilson, R. (2005) Development of species-specific primers for the ectoparasitic nematodes species *X. brevicolle*, *X. diffusum*, *X. elongatum*, *X. ifacolum* and *X. longicaudatum* (Nematoda: Longidoridae) based on ribosomal DNA sequences. *Annals of Applied Biology*, 146, 281–288.  
<http://dx.doi.org/10.1111/j.1744-7348.2005.040031.x>
- Oliveira, C.M.G., Ferraz, L.C.C.B. & Neilson, R. (2006) *Xiphinema krugi*, species complex or complex of cryptic species? *Journal of Nematology*, 38, 418–428.
- Palomares-Rius, J.E., Cantalapedra-Navarrete, C., Gutiérrez- Gutiérrez, C., Liebana, G. & Castillo, P. (2013) Morphological and molecular characterisation of *Paralongidorus plesioepimikis* n. sp. (Nematoda: Longidoridae) from southern Spain. *Nematology*, 15, 363–378.  
<http://dx.doi.org/10.1163/15685411-00002680>
- Peña-Santiago, R., Abolafia, J., Guerrero, P., Liébanas, G. & Peralta, M. (2006) Soil and freshwater nematodes of the Iberian fauna: a synthesis. *Graellsia*, 62, 179–198.  
<http://dx.doi.org/10.3989/graellsia.2006.v62.i2.65>
- Pereira, M.J. & Roca, F. (1992) The occurrence of *Xiphinema pseudocoxi* Sturhan (Nematoda, Longidoridae) in Portugal. *Nematologica*, 38, 428.
- Pereira, M.J. (1980) Situação nematológica dos campos de pés-mães de videira na Direcção Regional do Alentejo. 1º Congresso Português de Fitiatria e Fitofarmacologia, Lisboa, Portugal, II, 137–145.
- Pereira, M.J. (1984) *Prospecção de nemátodos (Xiphinema e Longidorus) em campos de multiplicação de morangueiros*. Ex-Direcção Geral de Protecção da Produção Agrícola, Documento PPA (SVD), Lisboa, N° 12/84, 6 pp.
- Roca, F. & Bravo, M.A. (1998) Description of *Xiphinema silvesi* n. sp. in Portugal (Nematoda: Longidoridae). *Fundamental and Applied Nematology*, 21, 389–392.
- Roca, F. & Bravo, M.A. (1993a) *Longidorus reisi* sp. n. (Nematoda: Longidoridae) from Portugal. *Fundamental and Applied Nematology*, 16, 401–405.
- Roca, F. & Bravo, M.A. (1993b) The occurrence of *Xiphinema sphaerocephalum* Lamberti et al. and *X. hispanum* Lamberti et al. (Nematoda: Longidoridae) in Portugal with descriptions of *X. lanceolatum* sp. n. and *X. lapidosum* sp. n. *Fundamental and Applied Nematology*, 16, 455–465.
- Roca, F. & Bravo, M.A. (1993c) *Xiphinema gersoni* sp. n. (Nematoda: Longidoridae) from Portugal. *Fundamental and Applied*

- Nematology*, 16, 543–547.
- Roca, F. & Bravo, M.A. (1994) *Xiphinema hispidum* sp. n. (Nematoda: Longidoridae) from Portugal. *Fundamental and Applied Nematology*, 17, 79–84
- Roca, F. & Bravo, M.A. (1996) Description of *Longidorus crataegi* sp. n. from Portugal and observations on *L. vineacola* Sturhan & Weischer (Nematoda: Longidoridae). *Fundamental and Applied Nematology*, 19, 529–535
- Roca, F. & Bravo, M.A. (1997) Multivariate analysis of *Xiphinema diversicaudatum* and some related species (Nematoda: Longidoridae). *Fundamental and Applied Nematology*, 20, 357–369.
- Roca, F. & Pereira, M.J. (1991) *Xiphinema abrantinum* sp. n. (Nematoda: Longidoridae) from Portugal. *Revue de Nematologie*, 14, 485–490.
- Roca, F. & Pereira, M.J. (1992) *Xiphinema belmontense* sp. n. (Nematoda: Longidoridae) from Portugal. *Fundamental and Applied Nematology*, 15, 251–255.
- Roca, F. & Pereira, M.J. (1993) *Xiphinema lupini* sp. n. (Nematoda: Longidoridae) from Portugal. *Fundamental and Applied Nematology*, 16, 515–519.
- Roca, F., Lamberti, F., Santos, M.S.N. de A. & Abrantes, I.M. de O. (1989a) *Xiphinema diversum* and *X. exile* (Nematoda, Dorylaimida), two new species from Portugal. *Nematologia Mediterranea*, 16, 213–219.
- Roca, F., Pereira, M.J. & Lamberti, F. (1987) Two new *Xiphinema* species (Nematoda: Longidoridae) from Portugal. *Nematologia Mediterranea*, 15, 323–332.
- Roca, F., Pereira, M.J. & Lamberti, F. (1989b) *Longidorus alvegus* sp. n. (Nematoda, Dorylaimida) from Portugal. *Nematologia Mediterranea*, 17, 1–4.
- Santos, M.T. (2011) Os vírus do urticado e do enrolamento foliar da videira. In: *Agrorrural: contributos científicos*. INCM, Lisboa, pp. 650–658.
- Sequeira, O.A. & Dias, H.F. (1964) Transmissão do vírus do urticado da videira pelo nemátodo *X. index* Thorne et Allen. *Agronomia Lusitana*, 24, 307–316.
- Siddiqi, M.R., Hooper, D.J. & Khan, E. (1963) A new nematode genus *Paralongidorus* (Nematoda : Dorylaimoidea) with description of two new species and observations on *Paralongidorus citri* (Siddiqi, 1959) n. comb. *Nematologica*, 9, 7–14. <http://dx.doi.org/10.1163/187529263X00025>
- Sturhan, D. (1973) Ergebnisse der forschungsreise auf die Azoren, 1969. Internationales Forschungsproject Makaronesischer Raum. II Zur Nematodenfauna der Azoren. *Boletim do Museu Municipal do Funchal*, 27, 18–25.
- Sturhan, D. (1983) Description of two new *Xiphinema* species from Portugal, with notes on *X. pachtaicum* and *X. opisthohysterum* (Nematoda, Longidoridae). *Nematologica*, 29, 270–283. <http://dx.doi.org/10.1163/187529283X00032>
- Sturhan, D. (1984) Untersuchungen über den *Xiphinema coxi*-komplex (Nematoda: Longidoridae). *Nematologica*, 30, 305–323. <http://dx.doi.org/10.1163/187529284X00211>
- Subbotin, S., Rogozhin, E. & Chizhov, V. (2014) Molecular characterisation and diagnostics of some *Longidorus* species (Nematoda: Longidoridae) from Russia and other countries using rRNA genes. *European Journal of Plant Pathology*, 138, 377–390. <http://dx.doi.org/10.1007/s10658-013-0338-9>
- Tarjan, A.C. (1969) Variation within the *Xiphinema americanum* group (Nematoda: Longidoridae). *Nematologica*, 15, 241–252. <http://dx.doi.org/10.1163/187529269X00678>
- Taylor, C.A. & Brown, D.J.F. (1997) Nematode-transmitted viruses. In: Lamberti, F., Taylor, C.E. & Seinhorst, J.W. (Eds.), *Nematode vectors of plant viruses*. CAB International, Wallingford, pp. 142–143.
- Trudgill, D.L., Brown, D.J.F. & McNamara, D.G. (1983) Methods and criteria for assessing the transmission of plant viruses by longidorid nematodes. *Revue de Nématologie*, 6, 133–141.
- Tzortzakakis, E.A., Archidona-Yuste, A., Cantalapedra-Navarrete, C., Nasiou, E., Lazanaki, M.S., Kabourakis, E.M., Palomares-Rius, J.E. & Castillo, P. (2014) Integrative diagnosis and molecular phylogeny of dagger and needle nematodes of olives and grapevines in the island of Crete, Greece, with description of *Xiphinema cretense* n. sp. (Nematoda, Longidoridae). *European Journal of Plant Pathology*, 140, 563–590. <http://dx.doi.org/10.1007/s10658-014-0488-4>
- Vieira, P., Mota, M., Padre, L. & Adão, H. (2008) List of Nematodes (Nematoda). In: Borges, P.A.V.; Abreu, C., Cunha, R., Aguiar, A.M.F., Carvalho, P., Jardim, R., Melo, I., Oliveira, P., Sérgio, C., Serrano, A.R. & Vieira, P. (Eds.), *A list of the terrestrial fungi, flora and fauna of Madeira and Selvagens archipelagos*. Direcção Regional do Ambiente da Madeira and Universidade dos Açores, Funchal and Angra do Heroísmo, Madeira, pp. 221–236.
- Ye, W. & Robbins, R.T. (2003) *Longidorus grandis* n. sp. and *L. paralongicaudatus* n. sp. (Nematoda: Longidoridae), Two parthenogenetic species from Arkansas. *Journal of Nematology*, 35, 375–387.

## Electronic supplementary material

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:  
**TABLE S1.** Occurrence and distribution of *Longidorus*, *Paralongidorus* and *Xiphinema* species in Portugal.