Bulletin OEPP/EPPO Bulletin (2017) **47** (2), 198–210 ISSN 0250-8052. DOI: 10.1111/epp.12382

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/95 (2)

Diagnostics
Diagnostic

PM 7/95 (2): *Xiphinema americanum sensu lato*

Specific scope

This Standard describes a diagnostic protocol for *Xiphinema americanum sensu lato*[1].

This Standard should be used in conjunction with PM 7/76 *Use of EPPO diagnostic protocols*.

Terms used are those in the EPPO Pictorial Glossary of Morphological Terms in Nematology[2].

Specific approval and amendment

Approved in 2009-09.
Revised in 2017-01.
This revision was prepared on the basis of the IPPC Diagnostic Protocol adopted in 2016 (Annex 11 to ISPM 27, IPPC, 2016). This EPPO Diagnostic Standard differs in terms of format but it is consistent with the content of the IPPC Standard. However, since the adoption of the IPPC Protocol 5 new species have been added into the group and are included in this version of the EPPO Protocol.

1. Introduction

The group known as *Xiphinema americanum sensu lato* (s.l.) is considered to comprise 61 nominal species. Both morphologically and biochemically, most members of the group are difficult to distinguish and there remains considerable taxonomic debate about the number of species in the group (Coomans *et al.*, 2001). For this reason, much phytosanitary legislation in Europe continues to list all species assigned to *X. americanum* s.l. (non-European populations), which is the subject of this protocol.

Investigations into the identity of *X. americanum* began in 1979 when Lamberti & Bleve-Zacheo studied populations from disparate geographical areas and concluded that there were in fact 25 different species, 15 regarded as new. Subsequently, new studies and standard virus transmission tests were required to confirm the identity of those species that transmitted viruses (Trudgill *et al.*, 1983).

Nematodes belonging to *X. americanum* s.l. occur in Africa and widely in Asia, Central and South America, Europe and North America, but have been found infrequently in Australasia and Oceania. The worldwide distribution of species belonging to the *X. americanum* s.l. is presented in Appendix 1.

In the United States, direct damage by *X. americanum sensu stricto* (s.s.) appears to be economically important in several states (CABI, 2013). However, the importance of the group overall is due to the ability of some species to transmit economically important nepoviruses. Brown *et al.* (1994) reported that *X. americanum* s.s., *Xiphinema californicum* and *Xiphinema rivesi* transmit *Cherry rasp leaf virus* (CRLV) (*Cheravirus*), *Tobacco ringspot virus* (TRSV) (*Nepovirus*) and *Tomato ringspot virus* (ToRSV) (*Nepovirus*) and noted the broad spectrum virus transmission capabilities of these North American populations compared with the relatively narrow specificity of transmission that exists between indigenous European nepoviruses and their vector species. *Xiphinema bricolense* was shown to transmit only the two serologically distinguishable strains of ToRSV but was a more efficient vector of the peach stem pitting (PSP) strain than the prune line (PBL) strain of the virus. *Xiphinema tarjanense* and *Xiphinema intermedium* are both reported to vector TRSV and ToRSV, and *Xiphinema inaequale* has been shown to vector ToRSV (Verma *et al.*, 2003). CRLV, *Peach rosette mosaic virus* (PRMV) (*Nepovirus*), TRSV and ToRSV are listed as recommended for regulation by EPPO. Until recently, no European populations of *X. americanum* s.l. had been shown to transmit these European quarantine viruses; however, Širca *et al.* (2007) reported transmission of TRSV and ToRSV to bait plants by a Slovenian population of *X. rivesi* with no known links to imported consignments. This knowledge reinforces the need for the current requirement to prevent spread of the listed nepoviruses in Europe.

[1]Use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.
[2]http://www.eppo.int/QUARANTINE/diag_activities/EPPO_TD_1056_Glossary.pdf

2. Identity

Name: *Xiphinema americanum* (*sensu lato*)
Type species: *Xiphinema americanum* (*sensu stricto*) Cobb, 1913
Synonyms: *Tylencholaimus americanus* (Cobb, 1913) Micoletzky, 1922 (of *X. americanum sensu stricto*)
Taxonomic position: Dorylaimida, Longidoridae, Xiphinematinae (after Coomans *et al.*, 2001)
EPPO Code: XIPHAM
Phytosanitary categorization: EPPO A1 for *Xiphinema americanum sensu stricto* no. 150, *Xiphinema bricolense* no. 260, *Xiphinema californicum* no. 261 and A2 for *Xiphinema rivesi* no. 262; EU Annex designation: IAI (non-European populations).

3. Detection

These species have a very wide host range of both herbaceous and woody plants in agriculture, horticulture and forestry. As free-living ectoparasites they are found in soil or growing media, and some species can overcome dry periods and survive for years in soil even in the absence of host plants. These species can therefore be moved in trade with soil associated with plants for planting, plant products (such as potato tubers contaminated with soil), bulk soil and any other goods contaminated with soil. Bare rooted plants free from soil are unlikely to present a pathway for entry of these species. When consignments of ornamental plants are sampled for plant-parasitic nematodes, the growing media from the rhizosphere of the plant should be analysed and evidence of possible re-potting before export should be looked for.

In the absence of virus infection, the aerial parts of plants grown in soil infested with *X. americanum* s.l. show no symptoms unless population levels are high, when roots exhibit swellings close to the root tips, and typical symptoms of root damage (such as reduction in vigour or signs similar to those that occur when a plant is under limited water conditions) may be observed.

Xiphinema species, as with most ectoparasitic plant-parasitic nematodes, can be detected by extraction from soil or growing media. Nematode extraction techniques, such as the Flegg-modified Cobb technique (Flegg, 1967) or Oostenbrink (Oostenbrink, 1960) or other suitable elutriation methods can be used for extraction of longidorid nematodes. Migratory endoparasites may also be present in soil residues adhered to plant roots, bulbs and tubers. Consequently, *Xiphinema* species may be found following processing of plant material using methods such as modified Baermann processes. Detailed descriptions of extraction equipment and procedures can be found in EPPO PM 7/119 (1) *Nematode extraction* (EPPO, 2013a).

4. Identification

4.1 Identification of the genus *Xiphinema*

Diagnosis of the genus *Xiphinema* has been described by Coomans *et al.* (2001). *Xiphinema* (Cobb, 1913) is among the largest genera in the family Longidoridae, which are migratory, polyphagous root ectoparasites. In summary, members of *Xiphinema* have: a body length of 1.2–7.3 mm; habitus straight to spiral; lip region varying from well offset and knob-like to continuous with body contour, and from low to high; amphidial aperture slit-like; stylet composed of needle-like, heavily sclerotized odontostyle with forked base and odontophore with sclerotized basal flanges; guiding apparatus consisting of a folded tube between guide ring and odontophore; dorsal pharyngeal gland nucleus round, larger than those of the ventrosublateral glands and located adjacent to orifice; variable female reproductive system but typically amphidelphic–didelphic; tail shape varying from elongate filiform to short and bluntly rounded; and tail usually similar in shape in both sexes.

4.2 Identification of *X. americanum sensu lato*

Loof & Luc (1990) defined the particular features of *X. americanum* s.l., but the characters were slightly amended by Lamberti *et al.* (2000) and Coomans *et al.* (2001). The following combination of characters distinguishes members of *X. americanum* s.l. from other *Xiphinema* species; however, characters marked with an asterisk (*) are seldom observed in those species considered to be part of a *Xiphinema pachydermum* group based on morphology (this group is described in more detail following the list of characters):

- body length small to medium (length varies from 1.2 to 3.0 mm)
- body shape assumes a more or less open C to spiral shape when heat-relaxed (Fig. 1a)
- lip region rarely continuous, usually demarcated by a shallow depression or deep constriction (Fig. 1b)
- guide ring more anterior and the folded part of the guiding sheath shorter than in other *Xiphinema* species (Fig. 1b)
- odontostyle robust, length rarely exceeds 150 μm
- pharyngeal bulb usually with thick platelet reinforcements of the lumen wall (Fig. 1c); bulb not offset from the rather wide slender part
- nuclei in the pharyngeal bulb: dorsal nucleus is often recorded as further from the dorsal orifice and the subventral nucleus is placed more posteriorly than in other *Xiphinema* species
- *V*% around or behind the middle of the body (*V*% = 42–65)
- female genital branches equally developed but generally short (Fig. 1d); short or very short uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles*

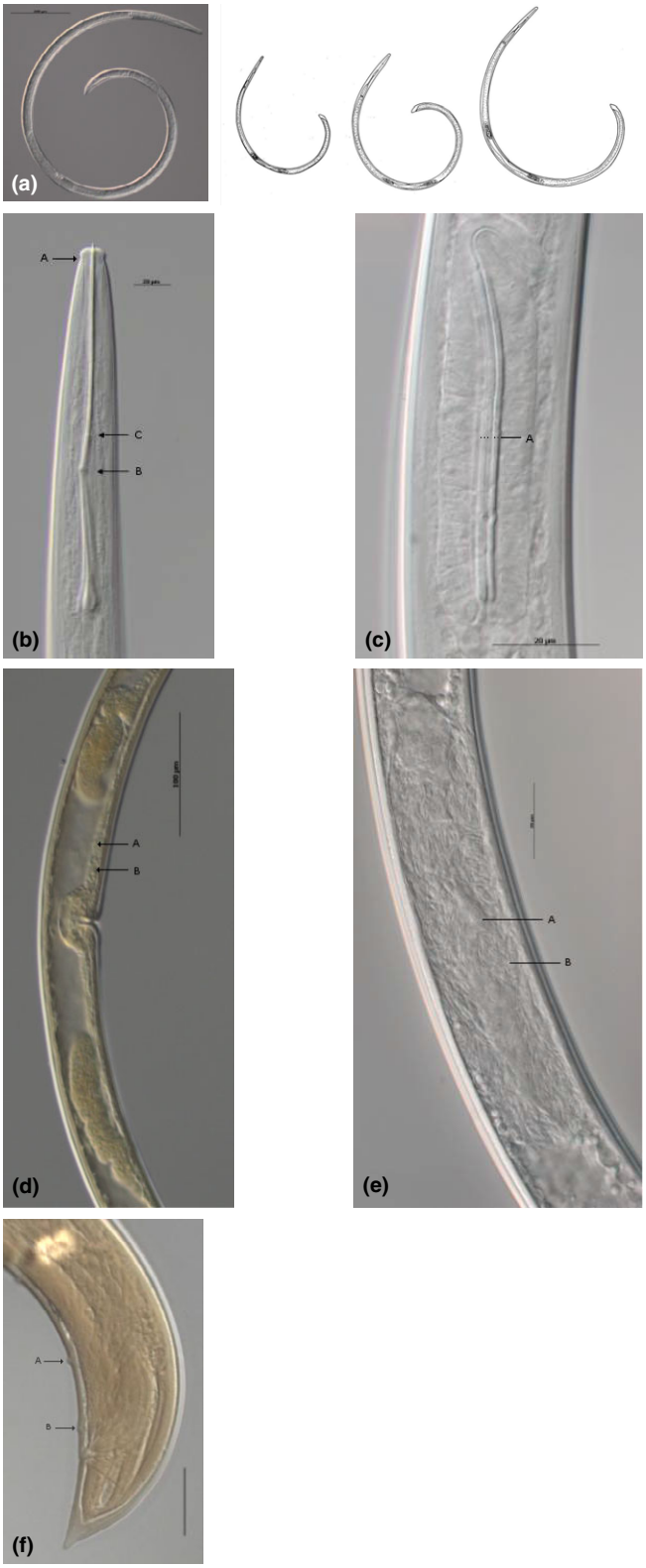

Fig. 1 Diagnostic morphological characters of *Xiphinema americanum sensu lato.* Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawing (a) reproduced from Lamberti *et al.* (1991), courtesy *Nematologia Mediterranea*. (a) Habitus of *X. americanum sensu lato*: (left to right) *X. pachtaicum*, *X. parvum*, *X. pseudoguirani* and *X. taylori* (photomicrograph scale bar: 200 µm). (b) *Xiphinema pachtaicum*, anterior. Lip region demarcated by a constriction (A) and relative position of guide ring (B) and anterior part of guiding sheath (C) (scale bar: 20 µm). (c) *Xiphinema peruvianum,* pharyngeal region. Pharyngeal bulb showing platelet reinforcements of the lumen wall (A) (scale bar: 20 µm). (d) *Xiphinema citricolum*, vulval region. Female genital branches equally developed but relatively short. Uteri without Z-differentiation or spines (A) and usually with weakly developed sphincter muscles (B) (scale bar: 100 µm). (e) *Xiphinema incognitum.* Compact ovaries, comprising rather few and narrow germ cells (A) and typically associated with verrucomicrobial endosymbionts (B) (scale bar: 20 µm). (f) *Xiphinema pachtaicum* male (*X. mediterraneum* allotype) spicular region and posterior ventromedian supplements. Posterior-most (A) lying closer to the precloacal papillae (adanal papillae (B)); within the spicula range (scale bar: 20 µm).

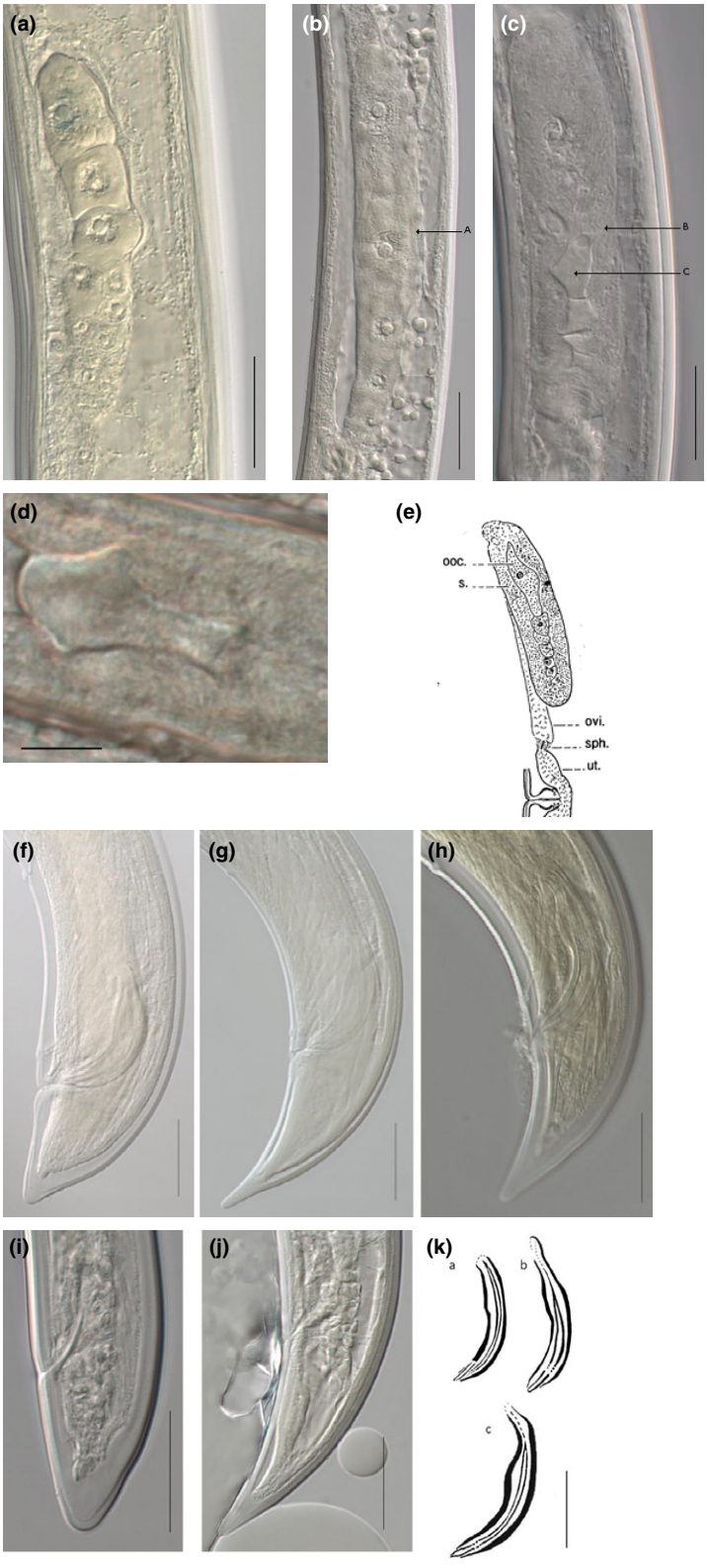

Fig. 2 Diagnostic morphological characters of *Xiphinema americanum sensu lato* for use with identification keys. Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawing (e), after Vandekerckhove *et al.* (2002), courtesy of *Applied and Environmental Microbiology*, and (k), based on Gutiérrez-Gutiérrez *et al.* (2012), courtesy of *European Journal of Plant Pathology*. (a) Anterior ovary of *X. longistilum* with no verrucomicrobial bacteria present (scale bar: 20 µm). (b) Anterior ovary of *X. mesostilum* with verrucomicrobial bacteria arranged in parallel strands (A) (scale bar: 20 µm). (c) Anterior ovary of *X. incognitum* with verrucomicrobial bacteria present (B), compressing the developing oocytes (C) (scale bar: 20 µm). (d) Section of the posterior ovary of *X. incognitum*, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm). (e) Anterior branch of the reproductive system of an *X. americanum* s.l. female: ooc., oocyte; ova., ovary; ovi., oviduct; s., symbiotic bacteria; sph., sphincter; ut., uterus. (f) *Xiphinema lafoense*, male, posterior (scale bar: 20 µm). (g) *Xiphinema exile*, male, posterior (scale bar: 20 µm). (h) *Xiphinema longistilum*, male, posterior (scale bar: 20 µm). (i) *Xiphinema lafoense*, female, tail (scale bar: 20 µm). (j) *Xiphinema exile*, female, tail (scale bar: 20 µm). (k) (a) *Xiphinema pachydermum,* spicule; (b) *X. microstilum,* spicule; (c) *X. paratenuicutis*, spicule (scale bar: 15 µm). (l) *Xiphinema californicum*, lip region (paratype) (scale: 5 µm). (m) *Xiphinema citricolum*, lip region (paratype) (scale bar: 5 µm). (n) *Xiphinema pachtaicum*, lip region (scale bar: 5 µm). (o) *Xiphinema santos*, lip region (paratype) (scale bar: 5 µm). (p) *Xiphinema bricolense*, lip region (paratype) (scale bar: 5 µm). (q) *Xiphinema diffusum*, lip region (paratype) (scale bar: 5 µm). (r) *Xiphinema citricolum*, posterior (scale bar: 10 µm). (s) *Xiphinema santos*, posterior (paratype) (scale bar: 10 µm). (t) *Xiphinema floridae*, posterior (paratype). (scale bar: 10 µm). (u) *Xiphinema utahense*, posterior (paratype) (scale bar: 10 µm). (v) *Xiphinema silvaticum*, posterior (topotype) (scale bar: 10 µm). (w) *Xiphinema bacaniboia*, posterior (paratype) (scale bar: 10 µm).

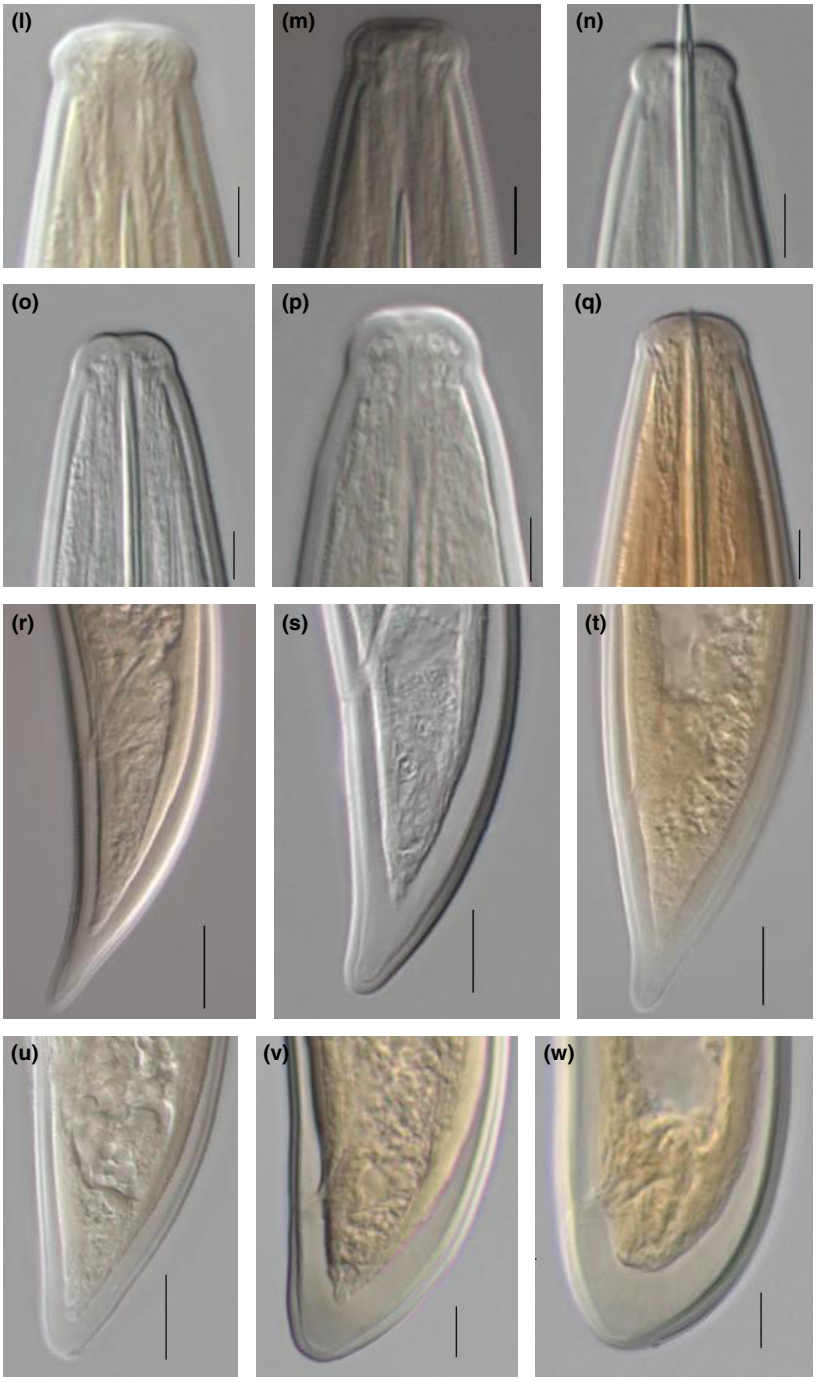

Fig. 2b Continued

- compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts (Figs 1e and 2d,e)*
- tail short, conoid, rounded to slightly digitate, rarely broadly rounded; tail terminus generally pointed or rounded
- males rare, females devoid of sperm*
- male usually with 5–11 ventromedian supplements, with the most posterior lying closer to the paired precloacal papillae (adanal papillae) than in other *Xiphinema* species (i.e. within spicula range) (Fig. 1f)
- three or four juvenile stages.

Detailed descriptions and observations on verrucomicrobial bacteria present in the ovaries of *Xiphinema* can be found in Coomans *et al.* (2000) and Vandekerckhove *et al.* (2000).

Lamberti & Ciancio (1993) distinguished five species subgroups based on hierarchical cluster analysis of

morphometrics, among them a *Xiphinema pachtaicum* group, which included *X. pachydermum*. *Xiphinema pachydermum* and related (mostly Portuguese) species differ from typical *X. americanum* s.l. in that females possess ovaries without associated symbiotic bacteria (except in *Xiphinema mesostilum*, where the bacteria are arranged in parallel strands in the wall of the ovaries), a well-developed sphincter muscle and longer uteri, as well as males being common in most species (Luc *et al.*, 1998; Coomans *et al.*, 2001; Decraemer & Geraert, 2013). Based solely on morphological characters, the *X. pachydermum* group comprises the following species: *Xiphinema brevisicum*, *Xiphinema duriense*, *Xiphinema exile*, *Xiphinema lafoense*, *Xiphinema longistilum*, *X. mesostilum*, *Xiphinema microstilum*, *Xiphinema opisthohysterum*, *X. pachydermum*, *Xiphinema parapachydermum* and *Xiphinema paratenuicutis*. Following recent molecular work (He *et al.*, 2005; Gutiérrez-Gutiérrez *et al.*, 2012), phylogenetic relationships based on sequence comparison of the D2–D3 and internal transcribed spacer (ITS)1 regions partially support the hypothesis that the *X. pachydermum* subgroup is a subgroup outside *X. americanum* s.l.; however, the group does not cluster separately and includes other species such as *X. pachtaicum*. Consequently, the relationships within this subgroup and with other species of *X. americanum* s.l. remain unclear and additional sequences are required for a larger analysis, which may allow the construction of a more complete and precise phylogeny in this group.

4.3 Identification of species within *X. americanum sensu lato*

Identification to species level within *X. americanum* s.l. is of particular importance for phytosanitary regulation because of the risk these nematodes pose as virus vectors, but it is problematic as a result of the general similarity of the morphology of the putative species, the high number of putative species (61 at present), weak differences reported between many species, lack of data on intraspecific morphological and morphometric variability and insufficient illustrations for many populations.

The number of putative species included in this group is constantly under review. The existence of 61 species is considered here. Some authorities regard several species (*Xiphinema diffusum*, *Xiphinema incognitum*, *Xiphinema parvum*, *Xiphinema pseudoguirani*, *Xiphinema sheri* and *Xiphinema taylori*) to be synonymous with *Xiphinema brevicolle* (Coomans *et al.*, 2001). There are, at present, no appropriate molecular protocols for the identification of *X. americanum* s.l. or for the identification of those species that have been acknowledged as virus vectors. Hence there remains the need to rely on morphological identification. Lamberti and Carone produced the first dichotomous key for the identification of species within *X. americanum* s.l. in 1991. Lamberti *et al.* (2000) presented a series of regional polytomous identification keys together with a combined polytomous key to the species occurring worldwide. These keys provided the first comprehensive attempt to resolve the problems with the identification of the *X. americanum* s.l. species. The polytomous key is most useful when some characters are difficult to observe or measure. Luc & Baujard (2001) stated that dichotomous keys can be used to complement a polytomous key in which several species share the same code for one or more characters. In both the dichotomous and polytomous keys, priority was given to quantitative morphological characters to minimize subjective evaluation of qualitative characters. Lamberti *et al.* (2000) listed species authorities and stated that odontostyle length, ratio *c* and *V%* appeared more reliable for examining intra- and inter-population relationships. When *c* and *V* % were used as the principal discriminants, relatively small groups of species were formed, within which demarcation of the individual species could be made using less robust characters such as body length, ratio *a* and tail length and also using subjective characters such as lip region and tail shape. Although ratio *c′* was considered reliable for identification by Lamberti, other authors (e.g. Griesbach & Maggenti, 1990) have found it to be of little significance. The polytomous key (Tables 1–4) was revised by Lamberti *et al.* (2004), with the characters as defined by the author, but unfortunately with few definitions or drawings. There has also been confusion regarding the definition of the lip region and tail shape as well as the arbitrary division of morphometric data.

The amended key included in this Diagnostic Protocol incorporates all putative species described to date, with updated morphometric data and redefinition of the lip region and tail shape. The key is useful in assigning a provisional identification to species that can then be checked with reference to the original description and finally by an expert. The two *species inquirendae*, *Xiphinema neoamericanum* and *Xiphinema sharmai*, have been omitted from the key. This is because of the poor quality of their original descriptions and the fact that neither species has been unequivocally identified after the publication of their original description. They are considered to have little relevance for phytosanitary regulation.

4.3.1 Polytomous key identification codes

The polytomous key described below uses the following characters with different possible values (coded as 1 to 6) to describe the nematode observed (after Yeates *et al.*, 1997; Coomans *et al.*, 2001; Lamberti *et al.*, 2004; Gozel *et al.*, 2006; Barsi & De Luca, 2008; Gutiérrez-Gutiérrez *et al.*, 2012).

Characters used in the polytomous key and their codes:

A	1	Females without verrucomicrobial bacteria present in the ovaries, or if present, arranged in parallel strands in the wall of the ovaries (Fig. 2a,b) (Table 1 and dichotomous key)
	2	Females with verrucomicrobial bacteria present in the ovaries, embedded in the epithelial wall cells of the ovaries at the apex, in the multiplication zone and in the distal part of the growing zone, often compressing the developing oocytes (Fig. 2c–e) (Tables 2–4)
B	1	Lip region greatly expanded or separated by a deep constriction (Fig. 2l–n)
	2	Lip region demarcated by a weak depression or shallow constriction, to almost continuous with the rest of the body (Fig. 2o–q)
C	1	Tail dorsally convex–conoid (conoid in two species), terminus acute to slightly subdigitate (Fig. 2r–t)
	2	Tail dorsally convex–conoid, ventrally straight; terminus rounded (Fig. 2u,v)
	3	Tail broadly convex–conoid, tapering to a broadly rounded terminus with main
D	1	Odontostyle length ≤70 μm
	2	Odontostyle length 71–80 μm
	3	Odontostyle length 81–90 μm
	4	Odontostyle length 91–100 μm
	5	Odontostyle length 101–120 μm
	6	Odontostyle length >120 μm
E	1	Vulva (*V*%) ≤50%
	2	Vulva 51–54%
	3	Vulva 55–58%
	4	Vulva >58%
F	1	Value of *c′* (defined as ratio of tail length/body width at anus) ≤1.0
	2	Value of *c′* 1.1–1.4
	3	Value of *c′* 1.5–1.8
	4	Value of *c′* >1.8
G	1	Value of *c* (defined as ratio of body length/tail length) <60
	2	Value of *c* 60–80
	3	Value of *c* >80
H	1	Body length <1.5 mm
	2	Body length 1.5–2.0 mm
	3	Body length >2.0 mm
I	1	Value of *a* (defined as ratio of body length/greatest body diameter) <60
	2	Value of *a* 61–80
	3	Value of *a* >80
J	1	Tail length <27 μm
	2	Tail length 27–32 μm
	3	Tail length >32 μm

4.3.2 Polytomous key code to valid species

Table 1. Species of *Xiphinema americanum sensu lato* without verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries

	Identification code									
Species	A	B	C	D	E	F	G	H	I	J
exile	1	1	1	1	23	4	12	3	23	2
brevisicum	1	1	1	1	234	4	12	23	23	2
duriense	1	1	1	12	34	34	12	123	23	12
microstilum	1	1	1	12	34	34	23	3	23	2
opisthohysterum	1	1	1	12	4	234	12	12	12	12
parapachydermum	1	1	1	123	34	34	12	123	12	123
pachydermum	1	1	1	23	234	23	23	23	123	12
paratenuicutis	1	1	1	23	34	123	12	23	12	123
vallense[*]	1	1	1	23	34	23	23	23	12	12
mesostilum	1	1	1	34	234	23	23	3	3	12
longistilum	1	1	1	5	23	23	23	3	23	2
lafoense	1	1	2	23	12	2	3	3	3	12
astaregiense[*]	1	2	2	3	3	12	3	3	2	1

The majority of species included here possess relatively long uteri, clearly differentiated oviducts with the sphincter well developed and not embedded in surrounding cell bodies, and compact ovaries without the presence of symbiotic bacteria (refer to Jairajpuri & Ahmad (1992) and Coomans *et al.* (2001) for descriptions of the female reproductive system); males are common within the population.

[*]New species described since the adoption of the IPPC Protocol (FAO, 2016)

An additional dichotomous key for these 13 species is provided after Table 4.

Table 2. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region greatly expanded or separated by a deep constriction; tail dorsally convex–conoid and terminus acute to slightly sub-digitate

	Identification code									
Species	A	B	C	D	E	F	G	H	I	J
lambertii	2	1	1	1	12	34	1	1	1	MD
simile[*]	2	1	1	12	1234	1234	123	23	123	123
parasimile [*]	2	1	1	12	1234	34	12	23	12	123
pachtaicum[†]	2	1	1	12 345	234	1234	123	123	123	123
kosaigudense	2	1	1	2	1	MD	1	1	1	MD
penevi[‡]	2	1	1	2	3	34	12	2	12	12
citricolum	2	1	1	23	123	34	12	12	1	23
pacificum	2	1	1	23	23	34	12	23	12	3
browni[‡]	2	1	1	23	23	34	123	23	123	123
plesiopachtaicum[‡]	2	1	1	23	3	23	2	2	2	12
tarjanense	2	1	1	234	123	23	12	12	1	123
floridae[§]	2	1	1	2345	12	12	12	123	1	123
californicum	2	1	1	2345	123	234	123	23	12	123
neoelongatum[¶]	2	1	1	4	23	23	1	12	1	MD
fortuitum	2	1	1	45	123	34	23	3	23	23
madeirense	2	1	1	45	234	34	12	23	123	23
georgianum[§]	2	1	1	456	123	234	12	23	12	123
incertum[**]	2	12	2	34	23	23	23	23	12	123

MD, Missing data.

[*]For detailed comparison of these species, refer to Barsi & Lamberti (2004), Barsi & De Luca (2008) and Lazarova *et al.* (2008).

[†]*Xiphinema pachtaicum* has relatively long uteri compared with other species listed in this table.

[‡]New species described since the adoption of the IPPC Protocol (FAO, 2016).

[§]The tail shape of these two species is regularly conoid rather than dorsally convex–conoid (Fig. 2(t)).

[¶]Considered to be a junior synonym of *X. pachtaicum* by Luc *et al.* (1984).

[**]Expanded lip region less pronounced in some specimens (Gutiérrez-Gutiérrez *et al.*, 2012). The validity of *X. incertum* was questioned by Barsi & Lamberti (2002).

Table 3. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region demarcated by a weak depression or shallow constriction, to continuous with the rest of the body; tail dorsally convex–conoid and terminus acute to slightly sub-digitate

	Identification code									
Species	A	B	C	D	E	F	G	H	I	J
pakistanense	2	2	1	1	12	2	1	12	1	123
minor	2	2	1	12	12	3	1	12	1	123
intermedium	2	2	1	12	123	23	1	12	1	32
americanum	2	2	1	123	123	234	1	123	12	123
tenuicutis	2	2	1	2	12	23	12	2	1	123
santos	2	2	1	23	123	1234	12	123	1	123
bricolense	2	2	1	234	12	234	12	23	123	23
peruvianum	2	2	1	234	123	23	12	123	1	123
laevistriatum	2	2	1	234	123	234	12	12	1	123
oxycaudatum	2	2	1	234	123	234	12	123	12	123
franci	2	2	1	34	23	23	1	12	1	123
inaequale	2	2	1	345	12	23	12	23	1	23
rivesi	2	2	12	2345	123	1234	12	123	1	123

Table 4. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region demarcated by a weak depression or shallow constriction, to continuous with the rest of the body; tail dorsally convex–conoid, ventrally straight and terminus rounded or broadly convex–conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour

	Identification code									
Species	A	B	C	D	E	F	G	H	I	J
rivesi	2	2	12	2345	123	1234	12	123	1	123
occiduum	2	2	2	1234	123	23	12	23	12	23
thornei	2	2	2	23	12	23	123	23	1	213
diffusum	2	2	2	234	123	12	123	123	1	123
taylori	2	2	2	234	123	12	23	23	1	123
incognitum	2	2	2	34	123	12	123	123	1	123
utahense	2	2	2	34	123	12	12	23	12	123
parvum	2	2	2	34	23	12	12	12	1	12
brevicolle	2	2	2	345	123	12	123	123	1	123
paramanovi	2	2	2	3456	123	2	12	23	1	3
luci	2	2	2	4	12	12	123	2	1	12
sheri	2	2	2	45	23	1	12	2	1	1
parabrevicolle	2	2	2	45	23	1	23	23	1	12
pseudoguirani	2	2	2	45	234	1	3	23	1	12
himalayense	2	2	2	5	2	12	3	3	1	2
waimungui	2	2	2	56	23	12	123	3	12	23
silvaticum	2	2	23	56	23	1	23	23	1	12
bacaniboia	2	2	3	6	23	1	3	3	1	12

A morphological and molecular review of *X. taylori*, including morphologically similar species, is currently in preparation.

4.3.3 Dichotomous key to species of X. americanum sensu lato *without verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries (polytomous key code A1)*

Because of the almost continuous overlap in morphometric characters between species, morphological features have been used as far as is possible. However, the use of male characters could not be avoided.

1.	Mature females without sperm present in uteri or oviduct, body length 1.3–2.2 mm, males absent or rare	3
	Mature females with sperm present in uteri or oviduct, body length 1.4–4.4 mm, males common in population	2
2.	Female odontostyle 54–72 µm, guide ring 49–51 µm from oral aperture	*X. opisthohysterum*
	Female odontostyle 68–74 µm, guide ring 53–60 µm from oral aperture	*X. duriense*
	Female odontostyle 73–85.5 µm, guide ring 62–75.5 µm from oral aperture	*X. vallense*[*]
3.	Lip region greatly expanded or separated by a deep constriction (Fig. 2l–n); Tail dorsally convex–conoid, terminus acute to slightly subdigitate (Fig. 2r–t)	4
	Lip region demarcated by a weak depression or shallow constriction (Fig. 2o–q); tail dorsally convex–conoid, ventrally straight; terminus rounded (Fig. 2u,v)	*X. astaregiense*[*]
4.	Posterior-most ventromedian supplement in the male distinctly anterior to the level of the spicule head (>25 µm) (Fig. 2f,g)	5
	Posterior-most ventromedian supplement in the male at the level of or just anterior to the level of the spicule head (<20 µm) (Figs 1f and 2h)	7
5.	Female tail dorsally convex–conoid with a rounded terminus (Fig. 2i)	*X. lafoense*
	Female tail dorsally convex conoid, tail terminus acute to sub-digitate (Fig. 2j)	6
6.	Male with three ventromedian supplements preceding the cloacal pair	*X. exile*
	Male with four to five ventromedian supplements preceding the cloacal pair	*X. brevisicum*
7.	Verrucomicrobial bacteria present and arranged in parallel strands in the wall of the ovaries	*X. mesostilum*
	No verrucomicrobial bacteria present in the wall of the ovaries	8

(continued)

(continued)

8.	Female odontostyle >100 µm	*X. longistilum*
	Female odontostyle <100 µm	9
9.	Uteri relatively short (45–56 µm)	*X. parapachydermum*
	Uteri longer (≥75 µm)	10
10.	Spicule with capitulum simple, not differentiated from lamina, lamina with short ventral expansion (Fig. 2k(a))	*X. pachydermum*
	Spicule with capitulum almost cephalated, demarcation on the dorsal limb, lamina with gradual ventral expansion (Fig. 2k(b))	*X. microstilum*
	Spicule with capitulum elongated, slight demarcation on the dorsal limb, lamina with prominent ventral expansion (Fig. 2k(c))	*X. paratenuicutis*

*New species described since the adoption of the IPPC Protocol (FAO, 2016).

5. Reference material

Reference material for many of the species of *X. americanum* s.l. is difficult to find, especially for those only recorded once. Please consult the authors for the latest advice.

6. Reporting and documentation

Guidance on reporting and documentation is given in EPPO Standard PM 7/77 (1) *Documentation and reporting on a diagnosis.*

7. Performance criteria

When performance criteria are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8. Further information

Further information on this group of organisms can be obtained from T. Prior, Food and Environment Research Agency, Sand Hutton, York YO41 1LZ (GB); S. Širca, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana (SI).

9. Feedback on this Diagnostic Protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share please contact diagnostics@eppo.int .

10. Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

11. Acknowledgements

This protocol was originally drafted by: S. Hockland and T. Prior, Fera, Sand Hutton, York (GB), supported by the UK Defra Plant Health Division; S. Širca Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia. Contributions were made by: A. Swart, Nematology Unit, Biosystematics Division, ARC-PPRI, South Africa; E. J. Chaves, Laboratorio de Nematología, INTA-Estación Experimental de Balcarce, Argentina; W. Decraemer, Royal Belgian Institute of Natural Sciences; D. Hunt, CABI; and F. Lamberti (deceased) and the nematologists who attended the *Xiphinema americanum* workshop at CSL 29 April–1 May 2002 in as part of the EU-funded project *Xiphinema americanum* group virus-vector nematodes: development of a diagnostic protocol SMT4-CT98-2229. It was reviewed by the Panel on Diagnostics in Nematology.

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Appendix 1 – Worldwide distribution of species belonging to *Xiphinema americanum sensu lato*.

Species	Africa	Asia	Australasia/ Oceania	Central and South America	Europe	Former Soviet Union	North America
americanum	✓						✓
astaregiense					✓		
bacaniboia			✓				
brevicolle[*]	✓	✓		✓	✓		
brevisicum					✓		
bricolense							✓
browni					✓		
californicum				✓			✓
citricolum							✓
diffusum	✓	✓		✓	✓		✓
duriense					✓		
floridae							✓
fortuitum					✓		
franci	✓	✓					
georgianum				✓			✓
himalayense		✓					
inaequale		✓		✓			
incertum					✓		
incognitum	✓	✓					
intermedium							✓
kosaigudense		✓					
laevistriatum							✓
lambertii		✓					
longistilum					✓		
luci	✓						
madeirense					✓		
mesostilum					✓		
microstilum					✓		
minor		✓					
neoelongatum		✓					
occiduum							✓
opisthohysterum		✓			✓		
oxycaudatum	✓	✓		✓			
pachtaicum	✓	✓		✓	✓	✓	
pachydermum					✓		
pacificum							✓
pakistanense		✓					
parabrevicolle					✓		
paramanovi						✓	
parapachydermum					✓		
parasimile					✓		
paratenuicutis					✓		
parvum				✓			
penevi	✓						
peruvianum				✓			
plesiopactaicum					✓		
pseudoguirani	✓	✓	✓				
rivesi		✓		✓	✓		✓
santos	✓				✓		
sheri		✓					
silvaticum		✓					
simile	✓				✓		
tarjanense							✓
taylori		✓			✓	✓	
tenuicutis							✓

(continued)

Appendix 1 *(continued)*

Species	Africa	Asia	Australasia/ Oceania	Central and South America	Europe	Former Soviet Union	North America
thornei		✓				✓	✓
utahense							✓
vallense					✓		
waimungui			✓				

✓, Indicates the presence of the pest in the region concerned (further references for the geographical distribution are available from the authors).
*Based on recent morphological studies and molecular data, many European populations previously assigned to *X. brevicolle* have since been confirmed as *X. taylori.* Consequently, the widespread distribution of this species should be reconsidered.