



Food and Agriculture  
Organization of the  
United Nations



**International Plant Protection Convention**  
Protecting the world's plant resources from pests

# DP 11: *Xiphinema americanum* *sensu lato*

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# **ISPM 27**

## **Diagnostic protocols for regulated pests**

### **DP 11: *Xiphinema americanum sensu lato***

Adopted 2016; published 2016

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## 1. Pest Information

The group known as *Xiphinema americanum sensu lato (s.l.)* is considered to comprise 56 nominal species (T. Prior, personal communication, 2014). Both morphologically and biochemically, most members of the group are difficult to distinguish. As certain putative species have been shown to transmit a range of economically important viruses, countries that have not recorded the presence of species in this group have included them all on their quarantine lists. However, there has been pressure among trading partners for more clarity on identification to be provided by researchers in an attempt to ease restrictions on trade.

Investigations into the identity of *X. americanum* started in 1979 when Lamberti and 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). Despite several morphological and molecular studies on *X. americanum s.l.*, taxonomic debate about the number of species in the group continues (Coomans *et al.*, 2001). This diagnostic protocol presents a considered approach to the identification of, and pest information for, *X. americanum s.l.*

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 (Hockland and Prior, 2009; CABI, 2013). 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. 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.*, *X. californicum* and *X. 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. *X. 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. *X. tarjanense* and *X. intermedium* are both reported to vector TRSV and ToRSV, and *X. 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 the European and Mediterranean Plant Protection Organization (EPPO). Until recently, no European populations of *X. americanum s.l.* had been shown to transmit these European quarantine viruses, but in 2007 Širca and colleagues reported transmission of TRSV and ToRSV to bait plants by a Slovenian population of *X. rivesi* with no known links to imported consignments. Auger *et al.* (2009) have also recorded Chilean populations of *X. rivesi* as a vector of ToRSV to cucumber. Although none of the South African *X. americanum s.l.* has been shown to

transmit these viruses, CRLV, *Arabid mosaic virus* (ArMV) and *Grapevine fanleaf virus* (GFLV) have all been reported from South Africa (A. Swart, personal communication, 2014).

## 2. Taxonomic Information

<b>Name:</b>	<i>Xiphinema americanum</i> ( <i>sensu lato</i> )
<b>Type species:</b>	<i>Xiphinema americanum</i> ( <i>sensu stricto</i> ) Cobb, 1913
<b>Synonyms:</b>	<i>Tylencholaimus americanus</i> (Cobb, 1913) Micoletzky, 1922 (of <i>X. americanum sensu stricto</i> )
<b>Taxonomic position:</b>	Nematoda, Adenophorea, Dorylaimida, Longidoridae, Xiphinematinae (after Coomans <i>et al.</i> , 2001)
<b>Common names:</b>	American dagger nematode, tobacco ring spot nematode. Other common names in various languages are listed in the CABI Crop Protection Compendium (CABI, 2013).

## 3. Detection

*Xiphinema* spp., 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* spp. may be found following processing of plant material using methods such as modified Baermann processes (EPPO, 2013a).

To extract longidorid nematodes from soil using the Flegg-modified Cobb technique, the following methodology can be followed. A 1 litre beaker is filled with 250 ml water and a soil sample (approximately 200 ml) is added to the water and soaked for approximately 30 min (loamy soil) to 60 min (clay soil); the suspension is stirred two or three times during the soaking period. A 2 mm aperture sieve is placed on a 5 litre plastic bucket and the soil suspension is washed through the sieve into the bucket. The sieve is removed and the bucket is topped up with water, then the solution is agitated by stirring. After 25 s sedimentation time, the supernatant suspension is decanted through a bank of three 150 µm aperture sieves, ensuring that the sediment remains in the bucket. The residue on the sieves is gently washed with a delicate stream of water (such as from a wash bottle) to a clean 1 litre beaker. The bucket containing the soil residue is topped up again with water and swirled thoroughly. After 15 s sedimentation, the supernatant is decanted through the same bank of three 150 µm aperture sieves (again ensuring the sediment remains in the bucket) and the residue is added to that collected previously. The contents of the 1 litre beaker are poured in entirety onto a 90 µm aperture sieve (with a maximum thickness of soil layer about 2–3 mm), and the sieve is placed onto an appropriately sized, supported glass funnel. Water is added from the side until the bottom of the sieve just touches the water. Nematodes are collected after 24–72 h in a glass beaker by opening the spring or screw clip on the funnel stem. The nematodes are examined under a dissecting microscope.

Detailed descriptions of extraction equipment and procedures can be found in the EPPO standard on nematode extraction (EPPO, 2013a).

## 4. Identification

There are, at present, no appropriate polymerase chain reaction (PCR) 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. Reference material for many of the species of *X. americanum s.l.* is in very short supply, and the contact points listed in section 6 should be consulted for assistance with identification.

## 4.1 Preparation of material

As with other species of plant-parasitic nematodes, morphological observation should be carried out on as many adult specimens as possible. There are numerous published methods for fixing and processing nematode specimens for study, most recently summarized in Manzanilla-López and Marbán-Mendoza (2012). Nematodes processed to anhydrous glycerol are recommended for examination as important taxonomic features can be obscured if specimens are not cleared sufficiently.

Temporary microscope slide preparations can be made quickly for instant examination but such slides may remain usable for only several weeks.

If possible, permanent slides should be prepared for future reference and deposited in nematode reference collections. Methods of preparing permanent slide mounts of nematodes have been described in detail elsewhere (Seinhorst, 1962; Hooper, 1986). The slow evaporation method as described by Hooper (1986) is outlined in section 4.1.2.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

### 4.1.1 Temporary preparations

Place a small drop of water on a glass cavity slide, enough to fill the well. Transfer the nematode specimens to the water and place the slide on a hotplate set at 65 °C. It is vital that the heating should be just sufficient to kill the nematodes, as prolonged heating will result in distortion and deterioration of the specimens. In practice, 10–15 s on a hotplate will be sufficient time for most species, but check the slide at intervals to monitor progress and remove it from the heat only when movement of all the nematodes has ceased.

Select a glass slide, ensure that it is dust free and put it on the side of the microscope stage. Place a small drop of single strength triethanolamine and formalin (TAF) fixative (7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water) or another appropriate fixative in the centre of the slide and position an appropriate amount of paraffin wax shavings around the drop (the wax will help support the coverslip and seal it to the slide).

Transfer the nematodes from the cavity slide to the TAF fixative and ensure they are positioned beneath the meniscus in the centre of the drop and not overlapping one another. The number of specimens able to fit on a slide will vary according to the size of the nematodes.

Carefully clean an appropriately sized coverslip with lens tissue. Lower it gently onto the wax shavings so that contact is made with the drop of TAF fixative. Place the slide on a hotplate and leave it there until the wax has just melted, gently tapping the slide to remove air that may be lodged under the coverslip. Remove from the heat and examine.

There should be a clear area of TAF fixative containing the nematodes in the centre and a complete ring of wax to seal the slide.

Should the seal be broken or the nematodes become embedded in the wax, heat the slide again, carefully remove the coverslip, recover the nematodes and remount them on a new slide. If the wax has spread beyond the coverslip, clear this away with a fine blade.

Seal the coverslip with a ring of clear nail varnish. When the varnish has dried, the specimens are ready for study.

### 4.1.2 Permanent preparations

Place a small drop of water on a glass cavity slide, enough to fill the well. Transfer the nematode specimens to the water and place the slide on a hotplate set at 65 °C. It is vital that the heating should be just sufficient to kill the nematodes, as prolonged heating will result in distortion and deterioration of the specimens. In practice, 10–15 s on a hotplate will be sufficient time for most species, but check the slide at intervals to monitor progress and remove it from the heat only when movement of all the nematodes has ceased.

Transfer the nematodes to an embryo dish or suitable watchglass half full of single strength TAF fixative (see section 4.1.1 for composition). Cover and leave to fix for a minimum of one week.

Transfer the specimens to a watchglass containing a 3% glycerol solution with a trace amount of TAF fixative. Ensure the nematodes are submerged. Place a coverslip over the watchglass and leave overnight.

Move the coverslip slightly so that a small gap is produced to allow evaporation, and leave the watchglass in an incubator (approximately 40 °C) until all the water has evaporated (this will take at least one week). At the same time, leave a small beaker of glycerol in the incubator to ensure it becomes anhydrous.

Using a syringe or dropper, dispense a small drop of the anhydrous glycerol onto the centre of a glass slide and transfer the nematodes to this, arranging them centrally.

Carefully select three coverslip supports, such as glass beads, of similar diameter to that of the nematodes, and place them at intervals in the margin of the glycerol drop, so that they form an even support.

Place small amounts of paraffin wax shavings at regular intervals around the circumference of the glycerol drop.

Heat a coverslip on a hotplate for a few seconds. Clean the coverslip with lens tissue and gently lower it onto the wax, so that contact is just made between coverslip and glycerol.

Place the slide on the hotplate and as soon as the wax has melted and any air bubbles have been expelled by the settling coverslip, remove the slide from the heat and allow the wax to reset.

When the wax is completely hard, remove any excess wax from around the coverslip with a scalpel.

Seal the coverslip with a ring of sealant such as Glyceel or clear nail varnish. Label the slide with an indelible marker, or affix a slide label to it. Include classification, date of slide preparation, host, locality, sample number (if applicable) and method of preservation used.

## 4.2 Identification of the genus *Xiphinema*

Definitions of terminology used in the following sections can be found in EPPO's *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology* (EPPO, 2013b).

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 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 tails usually similar in shape in both sexes.

### 4.3 Identification of *Xiphinema americanum sensu lato*

Loof and 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 *X. pachydermum* group based on morphology (this group is described in more detail following the list of characters):

- body length small to medium (L varies from 1.2 to 3.0 mm)
- body shape assumes a more or less open C to spiral shape when heat-relaxed (Figure 1(a))
- lip region rarely continuous, usually demarcated by a shallow depression or deep constriction (Figure 1(b))
- guide ring more anterior and the folded part of the guiding sheath shorter than in other *Xiphinema* species (Figure 1(b))
- odontostyle robust, length rarely exceeds 150 µm
- pharyngeal bulb usually with thick platelet reinforcements of the lumen wall (Figure 1(c)); 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 (Figure 1(d)); short or very short uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles\*
- compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts (Figures 1(e) and 2(d), (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) (Figure 1(f))
- 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 and Ciancio (1993) distinguished five species subgroups based on hierarchical cluster analysis of morphometrics, among them a *X. pachtaicum* group, which included *X. pachydermum*. *X. pachydermum* and related (mostly Portuguese) species differ from typical *X. americanum s.l.* in females possessing ovaries without associated symbiotic bacteria (except in *X. 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 in males being common in most species (Luc *et al.*, 1998; Coomans *et al.*, 2001; Decraemer and Geraert, 2013). Based solely on morphological characters, the *X. pachydermum* group comprises the following species: *X. brevisicum*, *X. duriense*, *X. exile*, *X. lafoense*, *X. longistilum*, *X. mesostilum*, *X. microstilum*, *X. opisthohysterum*, *X. pachydermum*, *X. parapachydermum* and *X. 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.



#### 4.4 Identification of species within *Xiphinema 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 (56 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 56 species is considered here. Some authorities regard several species (*X. diffusum*, *X. incognitum*, *X. parvum*, *X. pseudoguirani*, *X. sheri* and *X. taylori*) to be synonymous with *X. brevicolle* (Coomans *et al.*, 2001). As yet, no reliable molecular tests to distinguish between members of *X. americanum s.l.* can be recommended.

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 and 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 ratio *c* and V% were used as 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 and Maggenti, 1990) have found it to be of little significance. The polytomous key (Tables 1 to 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 been confusion regarding the definition of the lip region and tail shape as well as the arbitrary division of morphometric data, thus the current morphological characters used to describe species are under review (T. Prior and S. Hockland, personal communication, 2014).

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 of 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*, *X. neoamericanum* and *X. 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.

##### 4.4.1 Polytomous key identification codes

(After Yeates *et al.*, 1997; Coomans *et al.*, 2001; Lamberti *et al.*, 2004; Gozel *et al.*, 2006; Barsi and De Luca, 2008; Gutiérrez-Gutiérrez *et al.*, 2012.)

The polytomous key described in section 4.4.2 uses the following characters with different possible values (coded as 1 to 6) to describe the nematode observed.

##### 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 (Figure 2(a), (b)) (Table 1 and dichotomous key (section 4.4.3)) |
|---|---|---|

- 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 (Figure 2(c)–(e)) (Tables 2 to 4)
- B** 1 Lip region greatly expanded or separated by a deep constriction (Figure 2(l)–(n))
- 2 Lip region demarcated by a weak depression or shallow constriction, to almost continuous with the rest of the body (Figure 2(o)–(q))
- C** 1 Tail dorsally convex-conoid (conoid in two species), terminus acute to slightly sub-digitate (Figure 2(r)–(t))
- 2 Tail dorsally convex-conoid, ventrally straight; terminus rounded (Figure 2(u)–(v))
- 3 Tail broadly convex-conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour (Figure 2(w))
- D** 1 Odontostyle length  $\leq 70$   $\mu\text{m}$
- 2 Odontostyle length 71–80  $\mu\text{m}$
- 3 Odontostyle length 81–90  $\mu\text{m}$
- 4 Odontostyle length 91–100  $\mu\text{m}$
- 5 Odontostyle length 101–120  $\mu\text{m}$
- 6 Odontostyle length  $>120$   $\mu\text{m}$
- E** 1 Vulva (V%)  $\leq 50\%$
- 2 Vulva 51–54%
- 3 Vulva 55–58%
- 4 Vulva  $>58\%$
- F** 1 Value of  $c'$  ratio (defined as tail length / body width at anus)  $\leq 1.0$
- 2 Value of  $c'$  ratio 1.1–1.4
- 3 Value of  $c'$  ratio 1.5–1.8
- 4 Value of  $c'$  ratio  $>1.8$
- G** 1 Value of  $c$  ratio (defined as body length / tail length)  $<60$
- 2 Value of  $c$  ratio 60–80
- 3 Value of  $c$  ratio  $>80$
- 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  $a$  ratio (defined as body length / greatest body diameter)  $<60$
- 2 Value of  $a$  ratio 61–80
- 3 Value of  $a$  ratio  $>80$
- J** 1 Tail length  $<27$   $\mu\text{m}$

- 2 Tail length 27–32 µm  
3 Tail length >32 µm

#### 4.4.2 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

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
<i>exile</i>	1	1	1	1	23	4	12	3	23	2
<i>brevisicum</i>	1	1	1	1	234	4	12	23	23	2
<i>duriense</i>	1	1	1	12	34	34	12	123	23	12
<i>microstilum</i>	1	1	1	12	34	34	23	3	23	2
<i>opisthohysterum</i>	1	1	1	12	4	234	12	12	12	12
<i>parapachydermum</i>	1	1	1	123	34	34	12	123	12	123
<i>pachydermum</i>	1	1	1	23	234	23	23	23	123	12
<i>paratenuiculis</i>	1	1	1	23	34	123	12	23	12	123
<i>mesostilum</i>	1	1	1	34	234	23	23	3	3	12
<i>longistilum</i>	1	1	1	5	23	23	23	3	23	2
<i>lafoense</i>	1	1	2	23	12	2	3	3	3	12

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

An additional dichotomous key for these 11 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; and tail dorsally convex-conoid with terminus acute to slightly sub-digitate

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
<i>lambertii</i>	2	1	1	1	12	34	1	1	1	MD
<i>simile</i> <sup>†</sup>	2	1	1	12	1234	1234	123	23	123	123
<i>parasimile</i> <sup>†</sup>	2	1	1	12	1234	34	12	23	12	123
<i>pachtaicum</i> <sup>‡</sup>	2	1	1	12345	234	1234	123	123	123	123
<i>kosaigudense</i>	2	1	1	2	1	MD	1	1	1	MD
<i>citricolum</i>	2	1	1	23	123	34	12	12	1	23
<i>pacificum</i>	2	1	1	23	23	34	12	23	12	3
<i>tarjanense</i>	2	1	1	234	123	23	12	12	1	123
<i>floridae</i> <sup>¶</sup>	2	1	1	2345	12	12	12	123	1	123
<i>californicum</i>	2	1	1	2345	123	234	123	23	12	123

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
<i>neolongatum</i> <sup>§</sup>	2	1	1	4	23	23	1	12	1	MD
<i>fortuitum</i>	2	1	1	45	123	34	23	3	23	23
<i>madeirense</i>	2	1	1	45	234	34	12	23	123	23
<i>georgianum</i> <sup>¶</sup>	2	1	1	456	123	234	12	23	12	123
<i>incertum</i> <sup>*</sup>	2	12	2	34	23	23	23	23	12	123

MD, missing data.

<sup>†</sup> For detailed comparison of these species, refer to Barsi and Lamberti (2004), Barsi and De Luca (2008), and Lazarova *et al.* (2008).

<sup>‡</sup> *X. pachtaicum* has relatively long uteri compared with those of the other species listed in this table.

<sup>¶</sup> The tail shape of these two species is regularly conoid rather than dorsally convex-conoid (Figure 2(t)).

<sup>§</sup> Considered to be a junior synonym of *X. pachtaicum* by Luc *et al.* (1984).

<sup>\*</sup> Expanded lip region less pronounced in some specimens (Gutiérrez-Gutiérrez *et al.*, 2012). The validity of *X. incertum* was questioned by Barsi and 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; and tail dorsally convex-conoid with terminus acute to slightly sub-digitate

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
<i>pakistanense</i>	2	2	1	1	12	2	1	12	1	123
<i>minor</i>	2	2	1	12	12	3	1	12	1	123
<i>intermedium</i>	2	2	1	12	123	23	1	12	1	32
<i>americanum</i>	2	2	1	123	123	234	1	123	12	123
<i>tenuicutis</i>	2	2	1	2	12	23	12	2	1	123
<i>santos</i>	2	2	1	23	123	1234	12	123	1	123
<i>bricolense</i>	2	2	1	234	12	234	12	23	123	23
<i>peruvianum</i>	2	2	1	234	123	23	12	123	1	123
<i>laevistriatum</i>	2	2	1	234	123	234	12	12	1	123
<i>oxycaudatum</i>	2	2	1	234	123	234	12	123	12	123
<i>franci</i>	2	2	1	34	23	23	1	12	1	123
<i>inaequale</i>	2	2	1	345	12	23	12	23	1	23
<i>rivesi</i>	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; and tail dorsally convex-conoid, ventrally straight, with terminus rounded or broadly convex-conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour

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

A morphological and molecular review of *X. diffusum* and related species is currently in preparation (S.S. Lazarova, personal communication, 2014).

#### 4.4.3 Dichotomous key to species of *Xiphinema 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 with sperm present in uteri or oviduct, body length 1.4–4.4 mm, males common in population.....**3**

– Mature females without sperm present in uteri or oviduct, body length 1.3–2.1 mm, males absent or rare 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*
- 3. Posteriormost ventromedian supplement in the male distinctly anterior to the level of the spicule head (>25 µm) (Figure 2(f), (g)) .....4
- Posteriormost ventromedian supplement in the male at level of or just anterior to level of the spicule head (<20 µm) (Figures 1(f) and 2(h)) .....6
- 4. Female tail dorsally convex-conoid, with a rounded terminus (Figure 2(i))..... *X. lafoense*
- Female tail dorsally convex-conoid, with terminus acute to sub-digitate (Figure 2(j)) .....5
- 5. Male with three ventromedian supplements preceding the cloacal pair..... *X. exile*
- Male with four to five ventromedian supplements preceding the cloacal pair.....*X. brevisicum*
- 6. 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 .....7
- 7. Female odontostyle >100 µm..... *X. longistilum*
- Female odontostyle <100 µm.....8
- 8. Uteri relatively short (45–56 µm).....*X. parapachydermum*
- Uteri longer (≥75 µm) .....9
- 9. Spicule with capitulum simple, not differentiated from lamina, lamina with short ventral expansion (Figure 2(k-a)) ..... *X. pachydermum*
- Spicule with capitulum almost cephalated, demarcation on the dorsal limb, lamina with gradual ventral expansion (Figure 2(k-b))..... *X. microstilum*
- Spicule with capitulum elongated, slight demarcation on the dorsal limb, lamina with prominent ventral expansion (Figure 2(k-c)) ..... *X. paratenuicutis*

## 5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: preserved or slide-mounted specimens, photographs of distinctive taxonomic structure.

For morphological evidence, critical features as outlined in the diagnostic keys should be drawn or photographed while fresh material is available, and relevant measurements should be included.

Good photomicrographs (or scanning videos) of key morphological features are likely to be important for record keeping.

## 6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Nematology Unit, The Food and Environment Research Agency Science (Fera), Sand Hutton, York YO1 1LZ, United Kingdom (Thomas Prior; e-mail: thomas.prior@fera.co.uk; tel.: +44 1904 462206).

Nematology Unit, The Food and Environment Research Agency, Sand Hutton, York YO1 1LZ, United Kingdom (Sue Hockland; e-mail: sue.hockland@plantparasiticnematodes.com).

Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Protection Research Institute (ARC-PPRI), Private Bag X134, Queenswood, 0121 South Africa (Antoinette Swart; e-mail: SwartA@arc.agric.za).

Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia (Sasa Širca; e-mail: sasa.sirca@kis.si).

Laboratorio de Nematología, INTA-Estación Experimental de Balcarce, Casilla de Correo 276, 7620 Balcarce, Argentina (Eliseo Jorge Chaves; e-mail: eliseo\_chaves@yahoo.com.ar).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

## 7. Acknowledgements

The first draft of this protocol was written by Sue Hockland and Thomas Prior (Nematology Unit, Food and Environment Research Agency (Fera), United Kingdom (see preceding section)) Antoinette Swart (Nematology Unit, Biosystematics Division, ARC-PPRI, South Africa (see preceding section)), Eliseo Jorge Chaves (Laboratorio de Nematología, INTA-Estación Experimental de Balcarce, Argentina (see preceding section)) and Sasa Širca (Agricultural Institute of Slovenia, Slovenia (see preceding section)).

## 8. References

The present annex refers to international standards for phytosanitary measures (ISPMs). ISPMs are available on the International Phytosanitary Portal (IPP) at <https://www.ippc.int/core-activities/standards-setting/ispms>.

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## 9. Figures

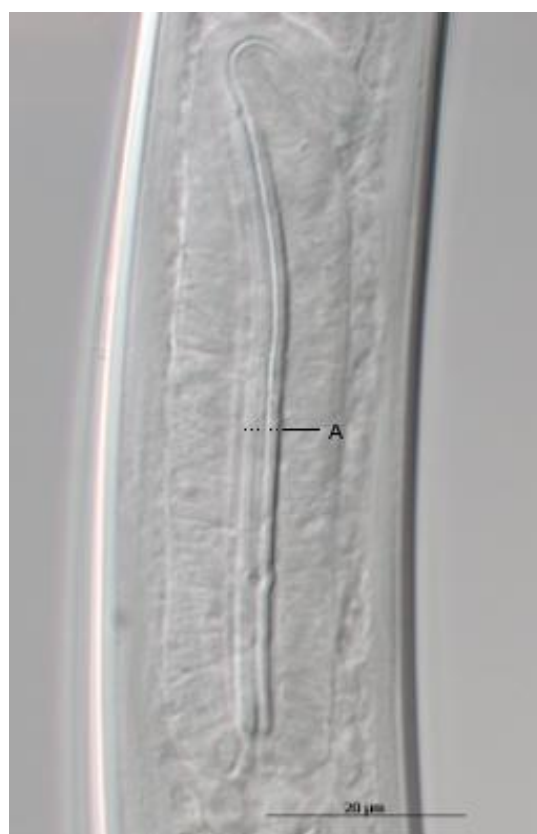
**Figure 1.** Diagnostic morphological characters of *Xiphinema americanum sensu lato* (s.l.). Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawing 1(a), reproduced from Lamberti et al. (1991), courtesy Nematologia Mediterranea.



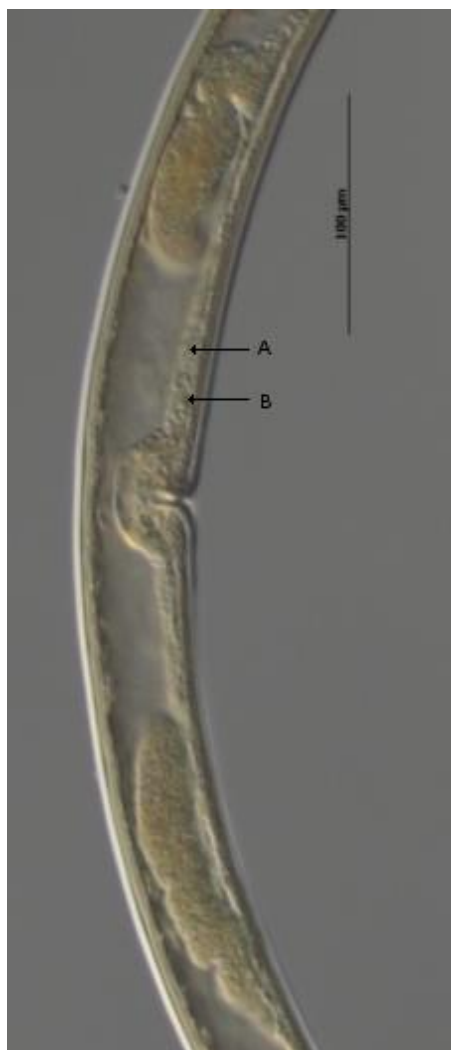
**1a.** Habitus of *X. americanum* s.l.: (left to right) *X. pachtaicum*, *X. parvum*, *X. pseudoguirani* and *X. taylori*.



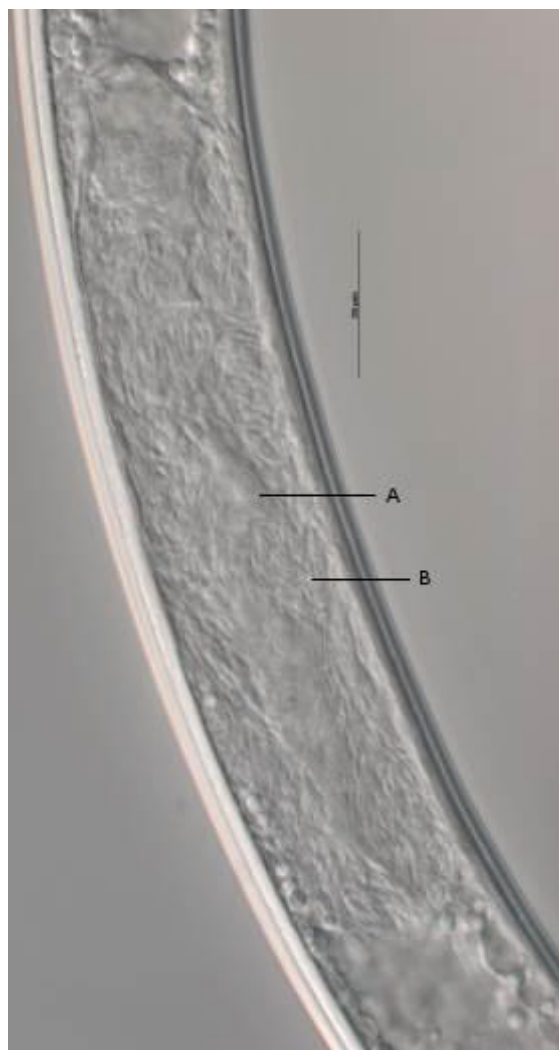
**1b.** *X. pachtaicum*, anterior. Lip region demarcated by a constriction (A) and relative position of guide ring (B) and anterior part of guiding sheath (C).



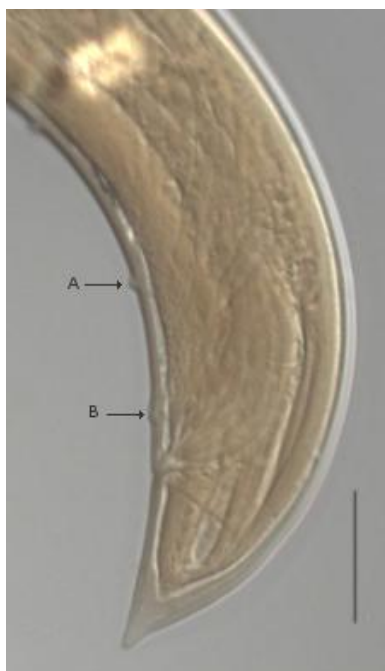
**1c.** *X. peruvianum*, pharyngeal region. Pharyngeal bulb showing platelet reinforcements of the lumen wall (A).



**1d.** *X. 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).



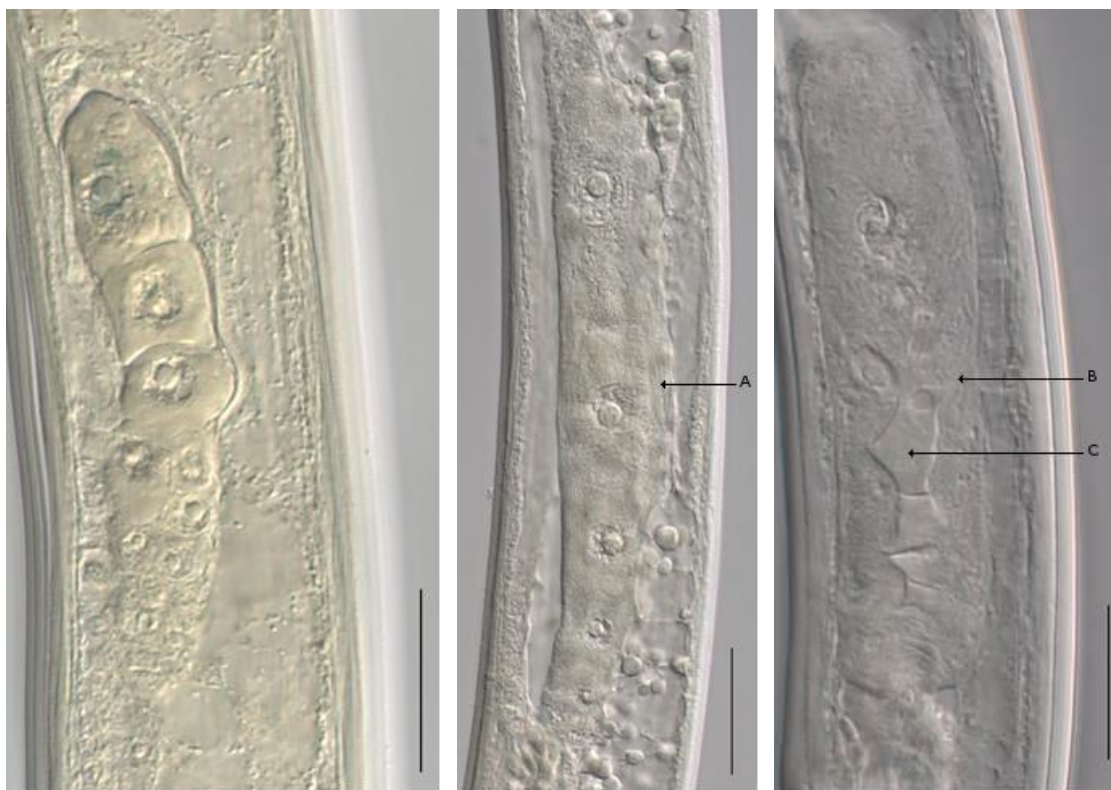
**1e.** *X. incognitum*. Compact ovaries, comprising rather few and narrow germ cells (A), and typically associated with verrucomicrobial endosymbionts (B).



**1f.** *X. pachtaicum* male (*X. mediterraneum* allotype). Spicular region and posterior ventromedian supplements, with posteriormost (A) lying closer to the precloacal papillae (adanal papillae (B)) (within spicula range) (scale bar: 20 µm).

**Figure 2.** Diagnostic morphological characters of *Xiphinema americanum sensu lato* (s.l.) for use with identification keys.

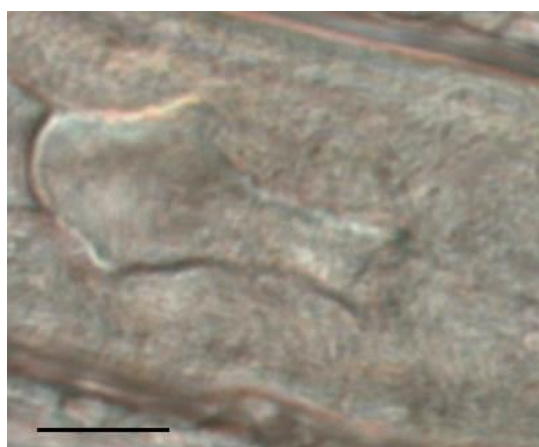
Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawings 2(e), adapted from Vandekerckhove et al. (2002), courtesy Applied and Environmental Microbiology, and 2(k), adapted from Gutiérrez-Gutiérrez et al. (2012), courtesy European Journal of Plant Pathology.



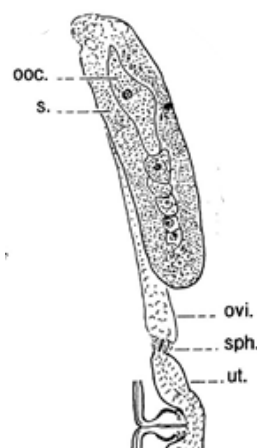
**2a.** Anterior ovary of *X. longistilum* with no verrucomicrobial bacteria present (scale bar: 20 µm).

**2b.** Anterior ovary of *X. mesostilum* with verrucomicrobial bacteria arranged in parallel strands (A) (scale bar: 20 µm).

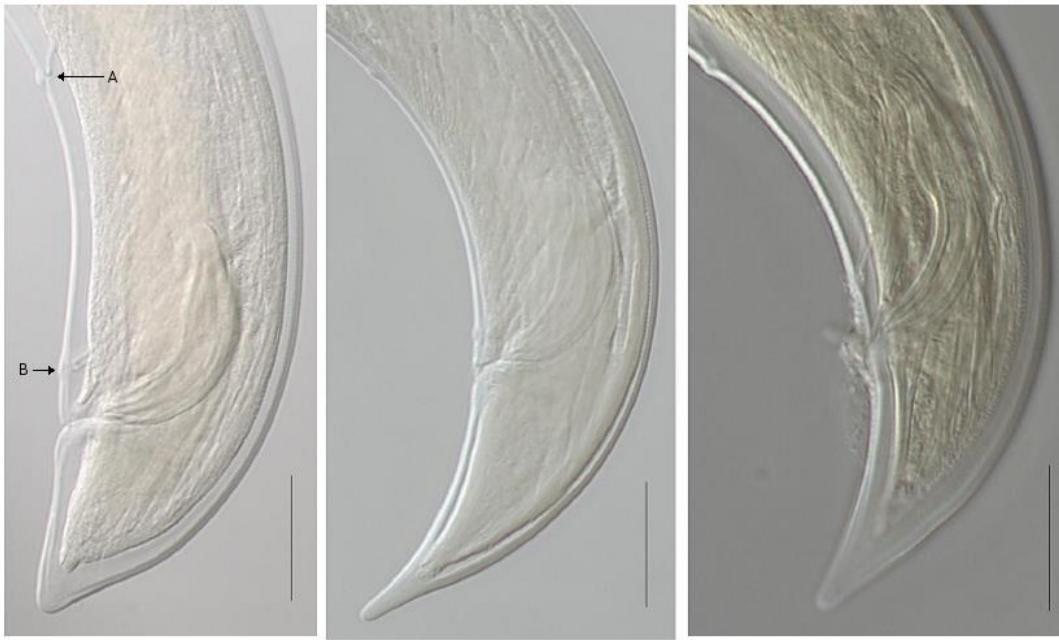
**2c.** Anterior ovary of *X. incognitum* with verrucomicrobial bacteria present (B) compressing the developing oocytes (C) (scale bar: 20 µm).



**2d.** Section of the posterior ovary of *X. incognitum*, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm).



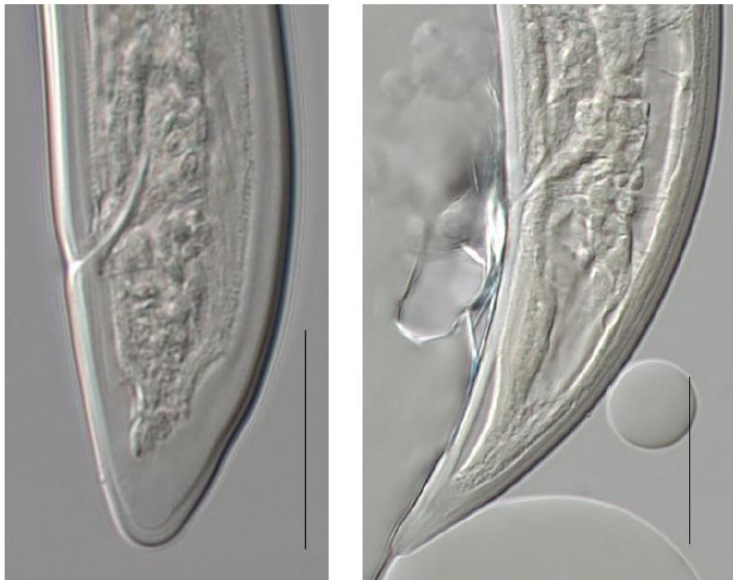
**2e.** Anterior branch of the reproductive system of an *X. americanum* s.l. female.  
ooc., oocyte; ovi., oviduct; s., symbiotic bacteria; sph., sphincter; ut., uterus.



**2f.** *X. lafoense*, male, posterior. Spicular region and posterior ventromedian supplements, with posteriormost (A) lying further from the precloacal papillae (adanal papillae (B)) (not within spicular range) (scale bar: 20  $\mu$ m).

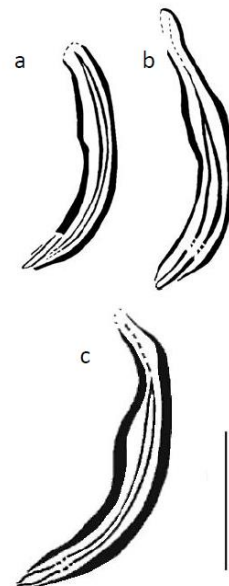
**2g.** *X. exile*, male, posterior (scale bar: 20  $\mu$ m).

**2h.** *X. longistilum*, male, posterior (scale bar: 20  $\mu$ m).



**2i.** *X. lafoense*, female, tail (scale bar: 20  $\mu$ m).

**2j.** *X. exile*, female, tail (scale bar: 20  $\mu$ m).

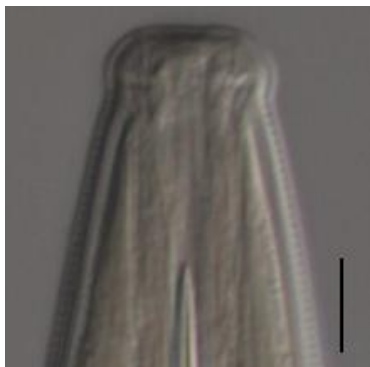


**2k.** (a) *X. pachydermum*, spicule; (b) *X. microstilum*, spicule; and (c) *X. paratenuiculis*, spicule (scale bar: 15  $\mu$ m).

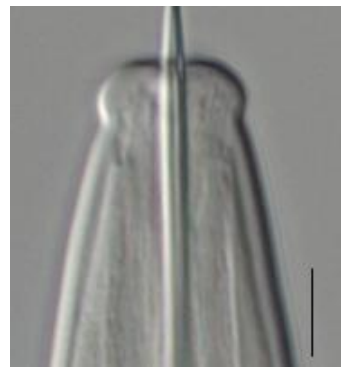




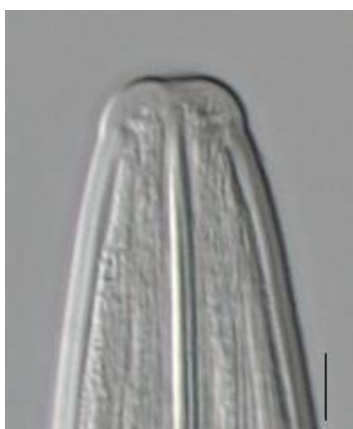
**2l.** *X. californicum*, lip region (paratype) (scale bar: 5 µm).



**2m.** *X. citricolum*, lip region (paratype) (scale bar: 5 µm).



**2n.** *X. pachtaicum*, lip region (scale bar: 5 µm).



**2o.** *X. santos*, lip region (paratype) (scale bar: 5 µm).



**2p.** *X. bricolense*, lip region (paratype) (scale bar: 5 µm).



**2q.** *X. diffusum*, lip region (paratype) (scale bar: 5 µm).



**2r.** *X. citricolum*, posterior (scale bar: 10 µm).



**2s.** *X. santos*, posterior (paratype) (scale bar: 10 µm).



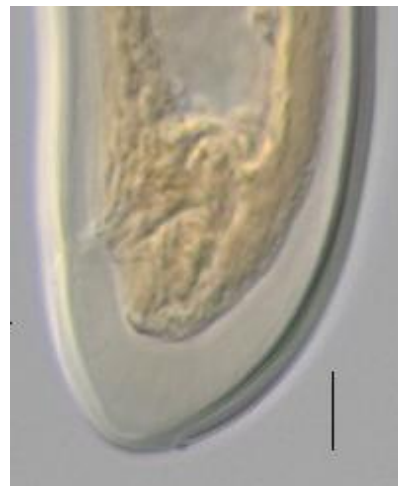
**2t.** *X. floridae*, posterior (paratype) (scale bar: 10 µm).



**2u.** *X. utahense*, posterior (paratype) (scale bar: 10 µm).



**2v.** *X. silvaticum*, posterior (topotype) (scale bar: 10 µm).



**2w.** *X. bacaniboia*, posterior (paratype) (scale bar: 10 µm).

#### Publication history

*This is not an official part of the standard*

2004-11 SC introduced original subject: *Xiphinema americanum* (2004-025).

2005-12 First draft presented to TPDP.

2006-04 CPM-1 (2006) added work programme topic: Nematodes (2006-008).

2014-02 Expert consultation.

2014-10 SC approved for member consultation (2014\_eSC\_Nov\_14).

2015-02 Member consultation .

2015-10 TPDP approved to submit to SC for adoption (eTPDP\_Oct\_01).

2015-11 SC approved for DP notification period (2015\_eSC\_Nov\_11).

2016-01 SC adopted DP on behalf of CPM (with no formal objections received).

**ISPM 27. Annex 11.** *Xiphinema americanum* (2016). Rome, IPPC, FAO.

Publication history last updated: 2016-04



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## IPPC

The International Plant Protection Convention (IPPC) is an international plant health agreement that aims to protect cultivated and wild plants by preventing the introduction and spread of pests. International travel and trade are greater than ever before. As people and commodities move around the world, organisms that present risks to plants travel with them.

### Organization

- ◆ There are over 180 contracting parties to the IPPC.
- ◆ Each contracting party has a national plant protection organization (NPPO) and an Official IPPC contact point.
- ◆ Nine regional plant protection organizations (RPPOs) work to facilitate the implementation of the IPPC in countries.
- ◆ IPPC liaises with relevant international organizations to help build regional and national capacities.
- ◆ The Secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).



### International Plant Protection Convention (IPPC)

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