



2004-025: Draft Annex to ISPM 27– *Xiphinema americanum sensu lato*

Comm no.	Para no.	Comment type	Comment	Explanation	Country
1.	G	Substantive	I support the document as it is and I have no comments		New Zealand, Guyana, Congo, Australia, Mexico
2.	G	Substantive	<a href="#">1.Add the original measurements, description of morphological characteristics, line drawing and micro-photographs of the 56 species in the X. american group. 2.Add dichotomous key to species of Xiphinema americanum sensu lato with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries.</a>	1. There is no appropriate molecular method for identification of the X. american group at present, and the identification still relies on traditional morphological method. 2. It is useful for morphological identification.	China
3.	6	Substantive	The group known as <i>Xiphinema americanum sensu lato</i> (s.l.) is considered to comprise of 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 their presence have included all species in this group 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.	Taking into account the continues taxonomic debate about the number of species in the group, and the fact that most members of the group are difficult to distinguish morphologically and biochemically, and as certain putative species have been shown to transmit a range of economically important viruses, And considering that the importance of the group overall is due to the ability of some species to transmit economically important nepoviruses, Even with this draft diagnostic protocol and the existing pressure among trading partners for more clarity on identification to be provided by researchers in an attempt to ease restrictions on trade, We believe that countries, that have not recorded their presence, still have the necessary basis to include all species in this group on their quarantine lists.	Bahrain
4.	7	Substantive	Investigations into the identity of <i>X. americanum</i> 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 <i>et al.</i> , 1983). Despite several	Taking into account the continues taxonomic debate about the number of species in the group, and the fact that most members of the group are difficult to distinguish morphologically and biochemically, and as certain putative species have been shown to transmit a range of economically important viruses, And considering that the importance of the group overall is due to the ability of some species to transmit	Bahrain

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			morphological and molecular studies on <i>X. americanum s.l.</i> , there continues to be taxonomic debate about the number of species in the group (Coomans <i>et al.</i> , 2001). This protocol presents a considered approach to the identification of, and hence pest information for, <i>X. americanum s.l.</i>	economically important nepoviruses, Even with this draft diagnostic protocol and the existing pressure among trading partners for more clarity on identification to be provided by researchers in an attempt to ease restrictions on trade, We believe that countries, that have not recorded their presence, still have the necessary basis to include all species in this group on their quarantine lists	
5.	8	Technical	Nematodes belonging to <i>X. americanum s.l.</i> occur <del>widely</del> in Africa, 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.	it is found only in 3 countries. Two according to CABI and one according to EPPO	Kenya
6.	9	Editorial	In the absence of virus infection, the aerial parts of plants grown in soil infested with <i>X. americanum s.l.</i> 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 <i>X. americanum sensu stricto</i> (s.s.) appears to be economically important in several states (CABI, 2013). However, the	Space missing	European Union

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			importance of the group overall is due to the ability of some species to transmit economically important nepoviruses.		
7.	10	Substantive	<u>Add the detailed descriptions of virus vectors of <i>X. americanum</i> group. It is better to give a table show all the vector species, their transmitted virus, host, distribution, reference and so on. Also, please make sure if this species are virus vectors: <i>X. brevicollum</i>.</u> Brown <i>et al.</i> (1994) reported that <i>X. americanum</i> s.s., <i>X. californicum</i> and <i>X. rivesi</i> transmitted <i>Cherry rasp leaf virus</i> (CRLV) ( <i>Cheravirus</i> ), <i>Tobacco ringspot virus</i> (TRSV) ( <i>Nepovirus</i> ) and <i>Tomato ringspot virus</i> (ToRSV) ( <i>Nepovirus</i> ) 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. <i>X. bricolense</i> transmitted only the two serologically distinguishable strains of ToRSV but were more efficient vectors of the peach stem pitting (PSP) strain than the prune line (PBL) strain of the virus. <i>X. tarjanense</i> and <i>X. intermedium</i> are both reported to vector TRSV and ToRSV, and <i>X. inaequale</i> has recently been shown to vector ToRSV (Verma <i>et al.</i> , 2003).	Namotodes as virus vectors make more economically sense.	China
8.	19	Substantive	<u>Label literatures after Oostenbrink or other elutriation methods.</u> <i>Xiphinema</i> spp., as with most ectoparasitic plant-parasitic nematodes, can be detected only by extraction from soil or growing media. Nematode extraction techniques, such as the Flegg modified Cobb technique (Flegg, 1967), Oostenbrink or other elutriation methods, can be used for extraction of longidorid nematodes.	It is helpful to operate properly.	China
9.	19	Substantive	<i>Xiphinema</i> spp., as with most ectoparasitic plant-parasitic nematodes, can be detected <del>only</del> by extraction from soil <del>or</del> growing media <u>or plant's roots</u> . Nematode extraction techniques, such as the Flegg modified Cobb technique (Flegg, 1967), Oostenbrink or other elutriation methods, can be used for extraction of	There is a possibility that the ectoparasitic plant-parasitic nematodes are also found from a plant's roots.	Japan

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			longidorid nematodes.		
10.	20	Technical	To extract longidorid nematodes 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), stirring 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 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 be 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 content of the litre beaker is poured in its 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 <a href="#">Include means of detection and extracation on planting material</a> .	such as bulbs, tubers, rooted planting materials, rootstocks,	Kenya
11.	23	Substantive	<a href="#">Add molecular approach, such as PCR-RFLP, micro-satellite marker loci and molecular phylogenetic approach. Related references:Yang Wu. 2007. Morphological and Molecular identification of Major Xiphinema species occuring in China. Zhejiang</a>	Currently, molecular identification methods are more important in nematode identification. Such as PCR-RFLP, micro-satellite marker loci and molecular phylogenetic approach. With 28S and ITS sequences analysis (or DNA barcoding methods). Though not all	China

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			<u>University</u> . There are, at present, no appropriate polymerase chain reaction (PCR) protocols for the identification of <i>X. americanum s.l.</i> 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 <i>X. americanum s.l.</i> is in very short supply, and the contact points listed in section 6 should be consulted for assistance.	the X. american group identification problems can be solved with these methods, but at least part of them. So DNA barcode method should be added .	
12.	28	Technical	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. <del>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.</del> Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	Text deleted for consistency with other DP. Commercial brands if mentioned in the DP should be associated to the agreed footnote.	Uruguay, Argentina, Chile
13.	31	Technical	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 TAF fixative ( <u>7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water</u> <del>7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water</del> ) 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).	To ensure consistency in the explanation of TAF fixative in parentheses as the current listing of ingredients of TAF in this paragraph and in paragraph 40 are not the same as that listed in DP of B. xylophilus para 56 i.e. TAF Fixative (10% of 35% formalin, 1% triethanolamine & 89% distilled water). The current listing in para 31 & 40 in this DP is clear but that in B. xylophilus is not clear.	Singapore
14.	71	Substantive	Identification to species level within <i>X. americanum s.l.</i> 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	As it is mentioned, identification to species level within <i>X. americanum s.l.</i> 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	Bahrain

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			populations.	insufficient illustrations for many populations. As yet, no reliable molecular tests to distinguish between members of <i>X. americanum</i> s.l. can be recommended. These facts support countries which included all species in this group on their quarantine lists to continue in its decision.	
15.	72	Substantive	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 ( <i>X. diffusum</i> , <i>X. incognitum</i> , <i>X. parvum</i> , <i>X. pseudoguirani</i> , <i>X. sheri</i> and <i>X. taylori</i> ) to be synonymous with <i>X. brevicolle</i> (Coomans <i>et al.</i> , 2001). As yet, no reliable molecular tests to distinguish between members of <i>X. americanum</i> s.l. can be recommended.	As it is mentioned, identification to species level within <i>X. americanum</i> 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. As yet, no reliable molecular tests to distinguish between members of <i>X. americanum</i> s.l. can be recommended. These facts support countries which included all species in this group on their quarantine lists to continue in its decision.	Bahrain
16.	73	Technical	<a href="#">Please check whether verrucomicrobial bacteria as an important character or not in this paper of Lamberti et al. (2004).</a> Lamberti and Carone (1991) produced the first dichotomous key for the identification of species within <i>X. americanum</i> s.l. in 1991. Lamberti <i>et al.</i> (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 <i>X. americanum</i> 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	In this paper Lamberti <i>et al.</i> (2004), with or without verrucomicrobial bacteria as an important character was not discussed, please check the paper. Sometimes, it is difficult to observe with or without verrucomicrobial bacteria.	China

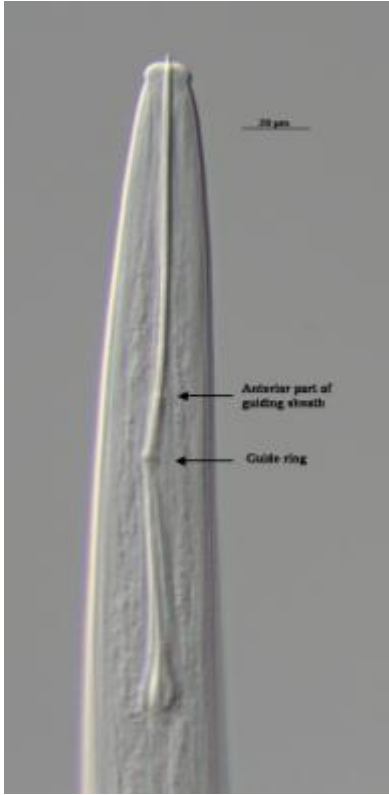

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			<p>qualitative characters. Lamberti <i>et al.</i> (2000) listed species authorities and stated that odontostyle length, ratio <i>c</i> and <i>V</i>% appeared more reliable for examining intra- and inter-population relationships. When ratio <i>c</i> and <i>V</i>% 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 <i>a</i> and tail length and also using subjective characters such as lip region and tail shape. Although ratio <i>c</i>' was considered reliable for identification by Lamberti, other authors (Griesbach and Maggenti, 1990) have found it to be of little significance. The polytomous key (Tables 1 to 4) was revised by Lamberti <i>et al.</i> (2004), with the characters as defined by the author, but unfortunately with few definitions or drawings. There has been confusion regarding the definition of lip region, tail shape and 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).</p>		
17.	78	Technical	<p><b>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.</b></p> <p><a href="#">Guidance on verrucomicrobial bacteria present in the o varies can be found in Coomans</a></p>	<p>As the notion of the verrucomicrobial bacteria is not very easy to understand, we recommend including guidance by citing the following references:                      Coomans, August, Tom T. M. Vandekerckhove and Myriam Claeys. "Transovarial Transmission of Symbionts in <i>Xiphinema Brevicollum</i> (Nematoda: Longidoridae)." <i>Nematology</i> 2, no. 4 (2000): 443-449.                      Vandekerckhove, T. T., A. Willems, M. Gillis and A. Coomans. "Occurrence of Novel Verrucomicrobial Species, Endosymbiotic and Associated with Parthenogenesis in <i>Xiphinema Americanum</i>-Group Species (Nematoda, Longidoridae)." <i>Int J Syst Evol Microbiol</i> 50 Pt 6, (2000): 2197-205.</p>	European Union




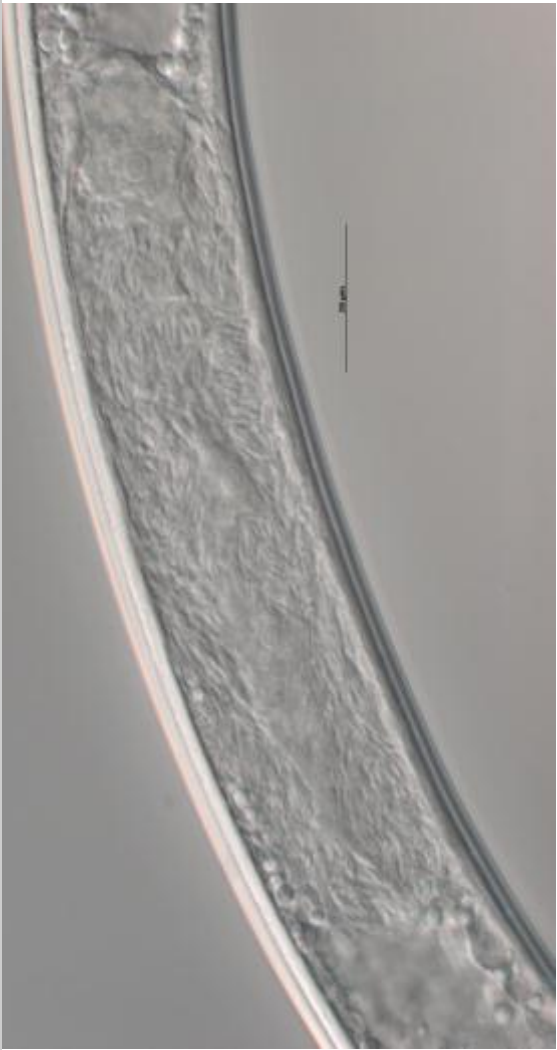
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			<a href="#">et al. (2000) and Vandekerckhove et al. (2000)</a>		
18.	80	Technical	<p><b>A 1</b></p> <p>Females with verrucomicrobial bacteria present in the ovaries, embedded in the epithelial wall cells of the ovaries at the apex, in the multiplication zone and in the distal part of the growing zone, often compressing the developing oocytes (Figure 2(c)) (Tables 2 to 4)</p> <p><b>B 1</b></p> <p>Lip region demarcated by a weak depression or shallow constriction, to almost continuous with the rest of the body (Figure 2(n)–(p))</p> <p><b>C 1</b></p> <p><b>2</b> Tail dorsally convex-conoid, ventrally straight; terminus rounded (Figure 2(t)–(u))</p> <p><b>3</b> Tail broadly convex-conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour (Figure 2(v))</p> <p><b>D 1</b></p> <p><b>2</b> Odontostyle length 71–80 µm</p> <p><b>3</b> Odontostyle length 81–90 µm</p> <p><b>4</b> Odontostyle length 91–100 µm</p> <p><b>5</b> Odontostyle length 101–120 µm</p> <p><b>6</b> Odontostyle length &gt;120 µm</p> <p><b>E 1</b></p> <p><b>2</b> Vulva 51–54%</p> <p><b>3</b> Vulva 55–58%</p> <p><b>4</b> Vulva &gt;58%</p> <p><b>F 1</b></p>	<p>A : The criteria A is the first and key criteria to differentiate between species. But it is difficult to observe. Replacing the ovaries (figure 2(a),(b)) Table 1 and better quality as related pictures would help. C1 : Clarification of the key and more specific links with the pictures.</p> <p>Lip region greatly expanded or separated by a deep constriction (Figure 2(k)–(m))</p> <p>Tail dorsally convex-conoid <a href="#">Figure 2(q)-(r)</a> (conoid <a href="#">(Figure 2(s))</a> in two species), terminus acute to slightly sub-digitate. <del>(Figure 2(q)–(s))</del></p> <p>Odontostyle length ≤70 µm</p> <p>Vulva (V%) ≤50%</p> <p>Value of c' ratio (defined as tail length / body width at</p>	European Union

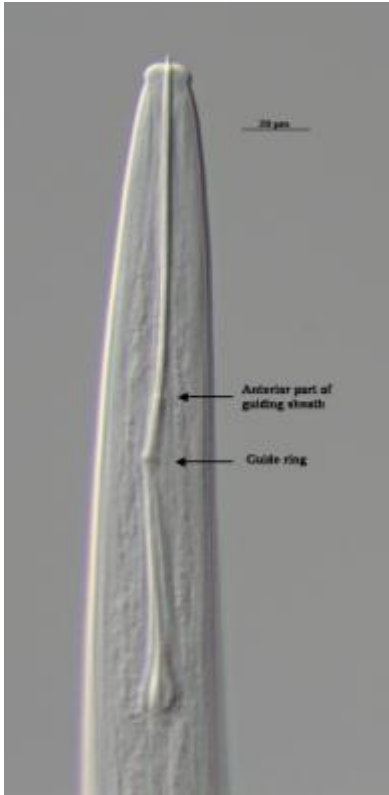



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			<p><b>2</b> Value of <i>c'</i> ratio 1.1–1.4</p> <p><b>3</b> Value of <i>c'</i> ratio 1.5–1.8</p> <p><b>4</b> Value of <i>c'</i> ratio &gt;1.8</p> <p><b>G 1</b></p> <p><b>2</b> Value of <i>c</i> ratio 60–80</p> <p><b>3</b> Value of <i>c</i> ratio &gt;80</p> <p><b>H 1</b></p> <p><b>2</b> Body length 1.5–2.0 mm</p> <p><b>3</b> Body length &gt;2.0 mm</p> <p><b>I 1</b></p> <p><b>2</b> Value of <i>a</i> ratio 61–80</p> <p><b>3</b> Value of <i>a</i> ratio &gt;80</p> <p><b>J 1</b></p> <p><b>2</b> Tail length 27–32 µm</p> <p><b>3</b> Tail length &gt;32 µm</p>	<p>anus) ≤1.0</p> <p>Value of <i>c</i> ratio (defined as body length / tail length) &lt;60</p> <p>Body length &lt;1.5 mm</p> <p>Value of <i>a</i> ratio (defined as body length / greatest body diameter) &lt;60</p> <p>Tail length &lt;27 µm</p>	
19.	143	Technical	<p><b>Cobb, N.A.</b> 1913. New nematode genera found inhabiting freshwater and non-brackish soils. <i>Journal of the Washington Academy of Sciences</i>, 3: 432–444.</p> <p><a href="#">Coomans, August, Tom T. M. Vandekerckhove and Myriam Claeys. "Transovarial Transmission of Symbionts in <i>Xiphinema Brevicollum</i> (Nematoda: Longidoridae)." <i>Nematology</i> 2, no. 4 (2000): 443-449</a></p>	Reference to be added.	European Union
20.	169	Technical	<p><b>Verma, A.K., Khan, M.L. &amp; Handa, A.</b> 2003. Transmission of tomato ringspot virus by <i>Xiphinema inaequale</i> (Khan and Ahmed, 1975) Bajaj and Jairajpuri 1979, associated with <i>gladiolus</i> in Himachal Pradesh. <i>Pest Management and Economic Zoology</i>, 11: 189–192.</p> <p><a href="#">Vandekerckhove, T. T., A. Willems, M. Gillis and A. Coom</a></p>	Reference to be added.	European Union


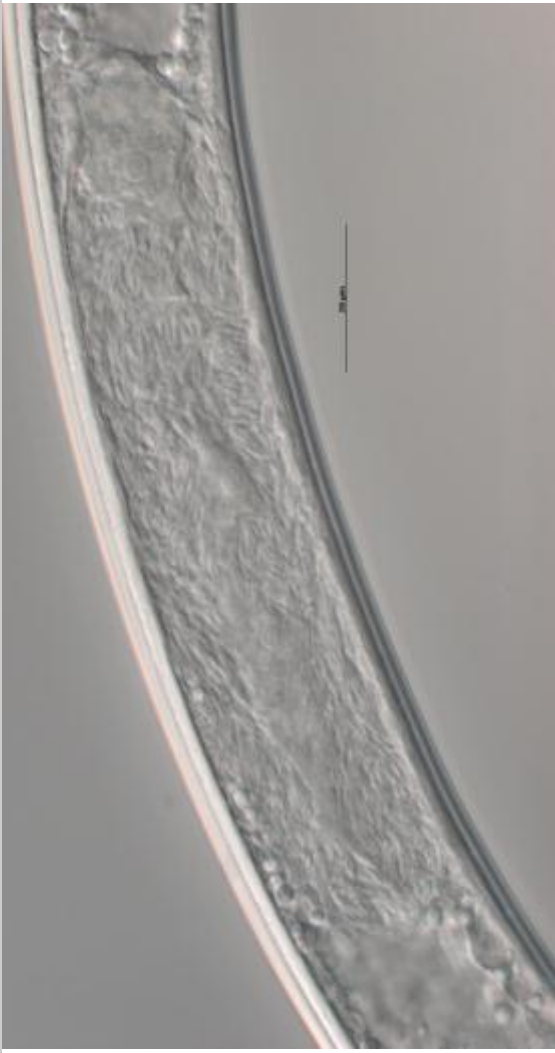
Comm no.	Para no.	Comment type	Comment	Explanation	Country
			<a href="#">ans. "Occurrence of Novel Verrucomicrobial Species, Endosymbiotic and Associated with Parthenogenesis in Xiphinema Americanum-Group Species (Nematoda, Longidoridae). "Int J Syst Evol Microbiol 50 PT 6, (2000): 2197-205.</a>		
21.	176	Substantive	 <p>1b. <i>X. pachtaicum</i>, anterior. Lip region demarcated by a constriction, and relative position of guide ring and anterior part of</p>	 <p>The target feature / criteria to be observed on each of the figure should be pointed with an arrow. This comment also applies to other figures without arrows to highlight the feature to be observed. In addition the text should be made larger.</p> <p>1c. <i>X. peruvianum</i>, pharyngeal region. Pharyngeal bulb showing platelet reinforcements of the lumen wall.</p>	European Union

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			guiding sheath.		


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			<p><b>1d.</b> <i>X. citricolum</i>, vulval region. Female genital branches equally developed but relatively short. Uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles.</p>	<p><b>1e.</b> <i>X. incognitum</i>. Compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts.</p>	
22.	176	Technical	<p>The morphological features emphasized should be highlighted in the different sub-fig with arrows in the 1c and 1e.</p> 	<p>The legend must explain in more details about the morphological features.</p> 	China
			<p><b>1b.</b> <i>X. pachtaicum</i>, anterior. Lip region demarcated by a</p>		

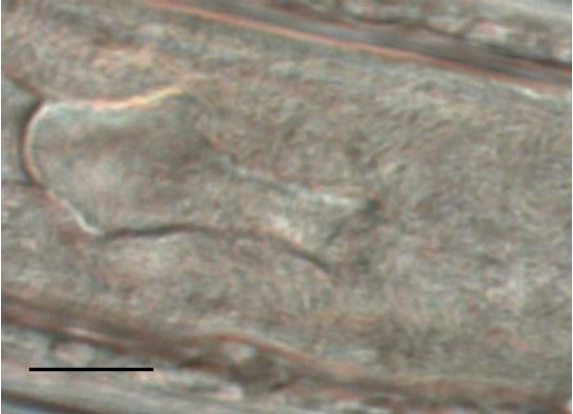
Comm . no.	Para . no.	Comment type	Comment	Explanation	Country
			constriction, and relative position of guide ring and anterior part of guiding sheath.	showing platelet reinforcements of the lumen wall.	

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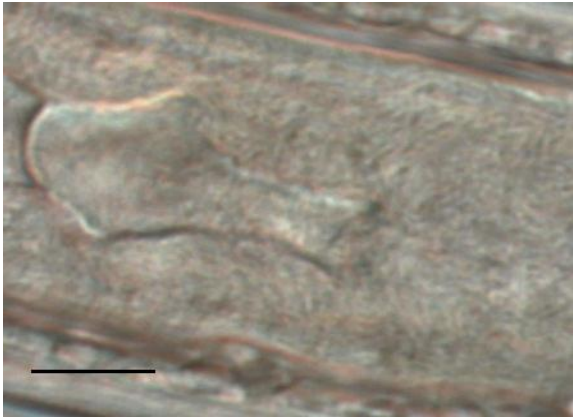


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			<p>1d. <i>X. citricolum</i>, vulval region. Female genital branches equally developed but relatively short. Uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles.</p>	<p>1e. <i>X. incognitum</i>. Compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts.</p>	
23.	177	Technical	<p><u>The morphological features emphasized should be highlighted in the different sub-fig with arrows.</u></p>  <p>1f. <i>X. pachtaicum</i> male (<i>X. mediterraneum</i> allotype) spicule. Posteriormost supplement lying closer to the paired precl</p>	<p>The legend must explain in more details about the morphological features.</p>	China

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24.	180	Editorial	 <p data-bbox="441 1278 1160 1337"><b>2a.</b> Anterior ovary of <i>X. longistilum</i> with no verrucomicrobial bacteria present (scale bar: 20 µm).</p>	<p data-bbox="1068 325 1653 405">Scale bars are randomly disappearing on the screens and prints. This should be taken care during formatting. Picture 2d : Add a reference to this picture in the text.</p>   <p data-bbox="1205 1209 1406 1401"><b>2b.</b> Anterior ovary of <i>X. mesostilum</i> with verrucomicrobial bacteria arranged in parallel strands (scale bar: 20 µm).</p> <p data-bbox="1413 1209 1653 1401"><b>2c.</b> Anterior ovary of <i>X. incognitum</i> with verrucomicrobial bacteria present, compressing the developing oocytes (scale bar: 20 µm).</p>	European Union

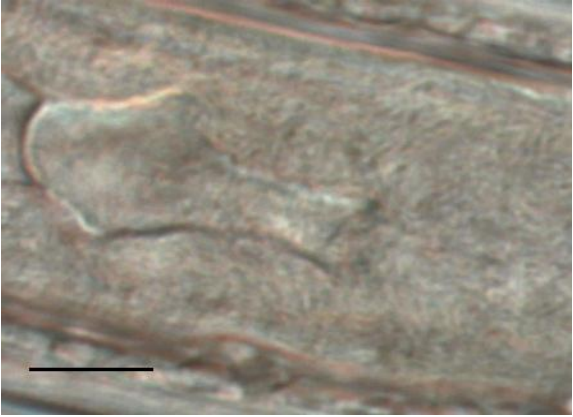
Comm no.	Para no.	Comment type	Comment	Explanation	Country
			 <p data-bbox="439 751 1180 841"><b>2d.</b> Section of the posterior ovary of <i>X. incognitum</i>, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm).</p>		

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25.	180	Technical	 <p data-bbox="450 1241 891 1321"><b>2a.</b> Anterior ovary of <i>X. longistilum</i> with no verrucomicrobial bacteria present (scale bar: 20 µm).</p>	<p data-bbox="1061 325 1653 347">When appropriate mark the bacteria with arrow.</p>  <p data-bbox="904 1241 1189 1377"><b>2b.</b> Anterior ovary of <i>X. mesostilum</i> with verrucomicrobial bacteria arranged in parallel strands (scale bar: 20 µm).</p>	European Union
				 <p data-bbox="1227 1241 1621 1353"><b>2c.</b> Anterior ovary of <i>X. incognitum</i> with verrucomicrobial bacteria present, compressing the developing oocytes (scale bar: 20 µm).</p>	




Comm no.	Para no.	Comment type	Comment	Explanation	Country
			 <p><b>2d.</b> Section of the posterior ovary of <i>X. incognitum</i>, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm).</p>		
26.	180	Technical	<u>The morphological features emphasized should be highlighted in the different sub-fig with arrows.</u>	The legend must explain in more details about the morphological features.	China

Comm no.	Para no.	Comment type	Comment	Explanation	Country
				 	
			<p><b>2a.</b> Anterior ovary of <i>X. longistilum</i> with no verrucomicrobial bacteria present (scale bar: 20 µm).</p>	<p><b>2b.</b> Anterior ovary of <i>X. mesostilum</i> with verrucomicrobial bacteria arranged in parallel strands (scale bar: 20 µm). <b>2c.</b> Anterior ovary of <i>X. incognitum</i> with verrucomicrobial bacteria present, compressing the developing oocytes (scale bar: 20 µm).</p>	

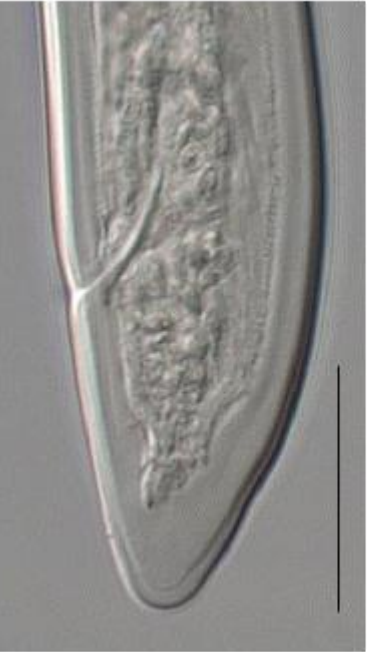







Comm no.	Para no.	Comment type	Comment	Explanation	Country
			 <p data-bbox="439 754 1178 836"><b>2d.</b> Section of the posterior ovary of <i>X. incognitum</i>, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm).</p>		



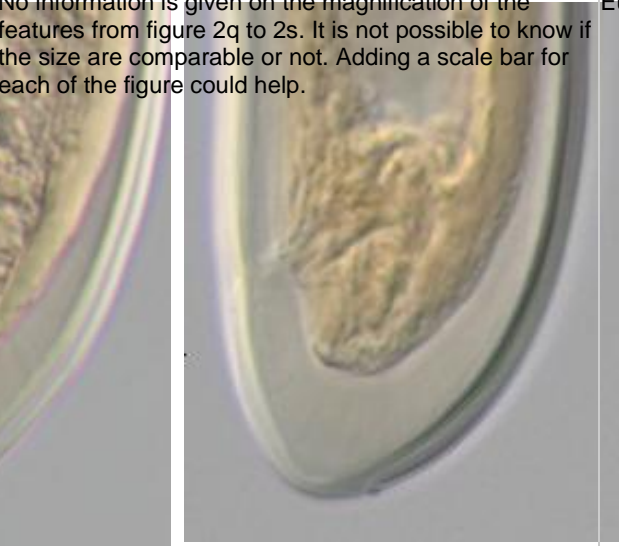


Comm no.	Para no.	Comment type	Comment	Explanation	Country
27.	181	Editorial	 <p data-bbox="441 1098 801 1158"><b>2e.</b> <i>X. lafoense</i>, male, posterior (scale: 20 µm).</p>	<p data-bbox="1068 325 1653 379">Scale bars are randomly disappearing on the screens and prints. This should be taken care during formatting.</p>   <p data-bbox="1173 1098 1653 1158"><b>2g.</b> <i>X. longistilum</i>, male, posterior (scale: 20 µm).</p>	European Union

Comm no.	Para no.	Comment type	Comment	Explanation	Country
28.	181	Technical	 <b>2e.</b> <i>X. lafoense</i> , male, posterior (scale: 20 µm).	The ventral sub-median supplement should be indicated with an arrow.  <b>2f.</b> <i>X. exile</i> , male, posterior (scale: 20 µm).  <b>2g.</b> <i>X. longistilum</i> , male, posterior (scale: 20 µm).	European Union

Comm no.	Para no.	Comment type	Comment	Explanation	Country
29.	182	Editorial	<div style="display: flex; justify-content: space-around;"> <div data-bbox="465 347 831 1002">  <p data-bbox="450 1062 741 1118"><b>2h.</b> <i>X. lafoense</i>, female, tail (scale: 20 µm).</p> </div> <div data-bbox="869 347 1290 1002">  <p data-bbox="835 1062 1189 1118"><b>2i.</b> <i>X. exile</i>, female, tail (scale: 20 µm).</p> </div> </div>	<p data-bbox="1061 325 1653 379">Scale bars are randomly disappearing on the screens and prints. This should be taken care during formatting.</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="1361 395 1480 667">  <p data-bbox="1216 1062 1630 1174"><b>2j.</b> (a) <i>X. pachydermum</i>, spicule; (b) <i>X. microstilum</i>, spicule; (c) <i>X. paratenuicutis</i>, spicule (scale bar: 15 µm).</p> </div> <div data-bbox="1503 395 1621 683">  </div> <div data-bbox="1435 699 1608 1018">  </div> </div>	European Union

Comm no.	Para no.	Comment type	Comment	Explanation	Country
30.	183	Technical	 <b>2k.</b> <i>X. californicum</i> , lip region (paratype).	No information is given on the magnification of the features from figure 2k to 2m. It is not possible to know if the size are comparable or not. Adding a scale bar for each of the figure could help.  <b>2l.</b> <i>X. citricolum</i> , lip region (paratype).	European Union
31.	184	Technical	 <b>2m.</b> <i>X. pachticum</i> , lip region.	No information is given on the magnification of the features from figure 2n to 2p. It is not possible to know if the size are comparable or not. Adding a scale bar for each of the figure could help.  	European Union

Comm no.	Para no.	Comment type	Comment	Explanation	Country	
			<b>2n.</b> <i>X. santos</i> , lip region (paratype).	<b>2o.</b> <i>X. bricolense</i> , lip region (paratype).	<b>2p.</b> <i>X. diffusum</i> , lip region (paratype).	
32.	186	Technical	 <p><b>2t.</b> <i>X. utahense</i>, posterior (paratype).</p>	 <p><b>2u.</b> <i>X. silvaticum</i>, posterior (topotype).</p>	 <p><b>2v.</b> <i>X. bacaniboia</i>, posterior (paratype).</p>	<p>No information is given on the magnification of the features from figure 2q to 2s. It is not possible to know if the size are comparable or not. Adding a scale bar for each of the figure could help.</p> <p>European Union</p>