2004-016: Draft Annex to ISPM 27– Bursaphelenchus xylophilus

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
1.	*G*	Editorial		Some Junenile stages (i;e. Jx) have not been written properly as it was not possible to format them correctly The Roman numbers will appear bigger in some paragraphs.	European Union
2.	*G*	Substantive	I support the document as it is and I have no comments		New Zealand, Guyana, Congo, Mexico
3.	*G*	Substantive	Footnotes related to the use of commercial brands should be included in this draft DP.	The following paragraphs mention commercial brands: 96, 100, 110, 113, 121, 127, 144, 145, 150, 157, 158, 169, 179 and 180. The same number of footnote should be associated to the brands mentioned in these paragraphs. The footnote would read as follows: "The use of the brands Qiagen, Stratagene,.....in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not consitute and endorsment by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results"	Uruguay, Argentina, Chile, Paraguay
4.	*G*	Technical		Terminology has been aligned with the one used in the EPPO Technical Document No. 1056 (rev. 4): Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology (EPPO, 2013b). No all changes have been highlighted as terminology changes as there were many. Editorial Throughout the document ‘and’ is preceded by a coma this should be checked and the coma deleted.	European Union
5.	*6*	Substantive	To label the literature(s) at the end of paragraph.The pine wood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, is the causal agent of pine wilt disease. PWN is believed to be native	To make enquiries conveniently.	China

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			to North America, where it is widely distributed in Canada and the United States (Ryss *et al.*, 2005) and apparently of limited distribution in Mexico (Dwinell, 1993). North American pine species are resistant or at least tolerant to PWN, but exotic species planted in North America, especially in the warmer southern areas of the United States, are killed when attacked by the nematode.		
6.	*7*	Editorial	*B. xylophilus* was carried to Japan at the beginning of the twentieth century, presumably on timber exported from North America, and it became one of the the most damaging forest pests in the country, where it still causes remarkable losses of pine trees (*Pinus densiflora, P. thunbergii*, *P. luchuensis*) today. *B. xylophilus* was also introduced to China, Korea and Taiwan; it was found there in the mid- to late 1980s. In 1999, *B. xylophilus* was found in Europe (Portugal) on *P. pinaster,* which is killed by the nematode within a few months after infestation (Mota *et al.*, 1999; Fonseca *et al.,* 2012). In 2008, PWN was found in Spain (Abelleira *et al*., 2011).	Needs to be plural	Australia
7.	*7*	Substantive	1.Delete “Taiwan”. 2.Change “PWN” to *“B.xylophilus”.B. xylophilus* was carried to Japan at the beginning of the twentieth century, presumably on timber exported from North America, and it became one of the the most damaging forest pest in the country, where it still causes remarkable losses of pine trees (*Pinus densiflora, P. thunbergii*, *P. luchuensis*) today. *B. xylophilus* was also introduced to China, Korea and Taiwan; it was found there in the mid- to late 1980s. In 1999, *B. xylophilus* was found in Europe (Portugal) on *P. pinaster,* which is killed by the nematode within a few months after infestation (Mota *et al.*, 1999; Fonseca *et al.,* 2012). In 2008, PWN was found in Spain (Abelleira *et al*., 2011).	1.Taiwan belongs to China. 2.There are two types of Bursaphelenchus xylophilus abbreviation, we suggest to use “B.xylophilus”only.	China
8.	*7*	Technical	*B. xylophilus* was carried to Japan at the beginning of the twentieth century, presumably on timber exported from North America, and it became one of the the most damaging forest pest in the country, where it still causes remarkable losses of pine trees (*Pinus*	More precise (for both changes). Regarding the sentence added including 2014 : This is an important recent information worth including in the protocol. Inácio, M.L., Nóbrega, F., Vieira, P., Bonifácio, L., Naves, P., Sousa, E., Mota, M. (2014), First detection	European Union

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			densiflora, P. thunbergii, *P. luchuensis*) today. *B. xylophilus* was also introduced to China, Korea and Taiwan; it was found there in the mid- to late 1980s. In 1999, *B. xylophilus* was found for the first time in Europe (Portugal) on *P. pinaster,* which is killed by the nematode within a few months after infestation (Mota *et al.*, 1999; Fonseca *et al.,* 2012). B. *xylophilus* was also detected on *Pinus nigra*, I nacio *et al. 2014.* In 2008, PWN was found for the first time in Spain (Abelleira *et al*., 2011).	of Bursaphelenchus xylophilus associated with Pinus nigra in Portugal and in Europe. Forest Pathology. doi: 10.1111/efp.12162	
9.	*8*	Substantive	*B. xylophilus* is transmitted from tree to tree by wood-inhabiting beetles of the genus *Monochamus* (Coleoptera: Cerambycidae) (Linit, 1990; Evans *et al*., 1996). The nematodes ~~enter~~ are carried onto the bodies of the insects shortly after the latter emerge from pupation and just before they bore out of the host tree (Wingfield, 1987). The beetles fly to the crown of healthy trees and feed on the young shoots and leaves (maturation feeding). They then mate and the females search for a weakened tree or one that has died recently, or for trunks or bigger branches (including felling debris), depending on the *Monochamus* species, where they lay their eggs through the bark. The beetle larvae that hatch from the eggs feed in the cambial tissues just below the bark for several months. On reaching maturity, they bore deeper into the wood to pupate, and thus their life cycle is completed. *B. xylophilus* takes advantage of this life cycle to obtain transport to new host trees (Wingfield, 1987). The introduction into the new tree may take place during oviposition by the beetle (this appears to be the only means of transmission for *B. xylophilus* and the several other species of *Bursaphelenchus* that colonize dead trees) (Edwards and Linit, 1992). *B. xylophilus*, however, seems to be unique among these species in that it can also be transmitted to a new tree during maturation feeding by beetles, and the development of pine wilt disease can occur as a consequence of transmission through the young shoots (Wingfield,	When the young adult beetle emerges, it brushes against the perithecial necks of fungi, picking up the nematodes, which settle below the elytra and, in particular, in the tracheae. The nematodes are mechanically transmitted through this process.	Singapore

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			1987).		
10.	*8*	Technical	*B. xylophilus* is transmitted from tree to tree by wood-inhabiting beetles of the genus *Monochamus* (Coleoptera: Cerambycidae) (Linit, 1990; Evans *et al*., 1996). The nematodes enter the bodies of the insects shortly after the latter emerge from pupation and just before they bore out of the host tree (Wingfield, 1987). The beetles fly to the crown of healthy trees and feed on the young shoots and leaves (maturation feeding). They then mate and the females search for a weakened tree or one that has died recently, or for trunks or bigger branches (including felling debris), depending on the *Monochamus* species, where they lay their eggs through the bark. The beetle larvae that hatch from the eggs feed in the cambial tissues just below the bark for several months. On reaching maturity, they bore deeper into the wood to pupate, and thus their life cycle is completed. *B. xylophilus* takes advantage of this life cycle to obtain transport to new host trees (Wingfield, 1987). The introduction into the new tree may take place during oviposition by the beetle (this appears to be the only means of transmission for ~~*B. xylophilus* and the~~ several ~~other~~ species of *Bursaphelenchus* that colonize dead trees) (Edwards and Linit, 1992). *B. xylophilus*, however, seems to be unique among these species in that it can also be transmitted to a new tree during maturation feeding by beetles, and the development of pine wilt disease can occur as a consequence of transmission through the young shoots (Wingfield, 1987).	Regarding the sentence "this appears to be the only means of transmission for several species of Bursaphelenchus that colonize dead trees" : As written it contradicts the next one. The current text states that B xylophilus only means of transmission is during oviposition but then continues by stating that transmission is also possible by maturation feeding.	European Union
11.	*8*	Technical	*B. xylophilus* is transmitted from tree to tree by wood-inhabiting beetles of the genus *Monochamus* (Coleoptera: Cerambycidae) more information needed on biology/, morphology/ ecology/ survival of beetle that carries the nematode , (Linit, 1990; Evans *et al*., 1996). The nematodes enter the bodies of the insects shortly after the latter emerge from pupation and just before they bore out of the host tree (Wingfield, 1987). The beetles fly to the crown of healthy trees and feed on the young	1. Detection of the two is necessarry in trade 2. The paragraph would answer the question of whether this would pose a significant risk since the nematode would lack a vector	Kenya

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			shoots and leaves (maturation feeding). They then mate and the females search for a weakened tree or one that has died recently, or for trunks or bigger branches (including felling debris), depending on the *Monochamus* species, where they lay their eggs through the bark. The beetle larvae that hatch from the eggs feed in the cambial tissues just below the bark for several months. On reaching maturity, they bore deeper into the wood to pupate, and thus their life cycle is completed. *B. xylophilus* takes advantage of this life cycle to obtain transport to new host trees (Wingfield, 1987). The introduction into the new tree may take place during oviposition by the beetle (this appears to be the only means of transmission for *B. xylophilus* and the several other species of *Bursaphelenchus* that colonize dead trees) (Edwards and Linit, 1992). *B. xylophilus*, however, seems to be unique among these species in that it can also be transmitted to a new tree during maturation feeding by beetles, and the development of pine wilt disease can occur as a consequence of transmission through the young shoots (Wingfield, 1987). provide a paragraph on phytosaniatry risk of imp orting wood with bursaphelenchus xylophilus alone with out the beetle		
12.	*9*	Technical	When *B. xylophilus* is transmitted during oviposition, the nematodes remain relatively close to the site of introduction. But when transmission occurs through the young shoots and when the tree succumbs to pine wilt disease, the nematodes distribute throughout the whole tree, destroying wood tissues such as epithelial cells, parenchyma cells of axial and radial resin canals, cambium and phloem. *B. xylophilus* can also be found in roots, even when the above-ground part of the tree is already dead, dried out or felled. Whether the tree develops pine wilt disease depends on the tree species (in general only *Pinus* spp. of non-American origin are affected), its state of health and the climatic conditions (particularly temperature and water supply). These	Regarding "only Pinus spp. of non-American origin are affected" : There are other species than Pinus spp. in North America which are less tolerant, consequently ‘in general’ should be added.	European Union

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			factors also influence the distribution of nematodes throughout the tree: their distribution can be localized or irregular and this needs to be taken into account in sampling strategies (Schröder *et al*., 2009).		
13.	*10*	Substantive	*B. xylophilus* can also be found in dead trees of *Abies, Chamaecyparis, Cedrus, Larix, Picea* and *Pseudotsuga* and other conifers (except *Thuja* spp.), but none of these genera is known to be affected by pine wilt disease, although pathogenicity tests on seedlings show remarkable reactions including death.	References should be added to substantiate the comment that B. xylophilus can also be found in dead trees of Abies, Chamaecyparis, Cedrus, Larix, Picea and Pseudotsuga and other conifers .	European Union
14.	*11*	Technical	*B. xylophilus* is almost exclusively vectored by *Monochamus* species (Cerambycidae), with the vector species varying among the geographic regions; for example. *M. alternatus* in China and Japan, *M. saltuarius* in Japan, *M. carolinensis* in North America and *M. galloprovincialis* in Portugal. Occasionally other beetles of the family Cerambycidae or other Coleoptera have been found to carry "dauer" ~~larvae~~juveniles of the nematode on their bodies, but there is no evidence that they play a role as vectors in the dissemination of the nematode (Evans *et al.*, 1996).	Larvae should be replaced by juveniles throughout the text. This is the correct terminology.	European Union
15.	*19*	Substantive	1.Subfamily （Parasitaphelenchinae）and genus（ Bursaphelenchus） are suggested to add in taxonomic position.2.Taxonomic position of B. xylophilus in Para. [19] and Para. [49] are inconsistent. Please check and correct**Taxonomic position:** Nematoda, Rhabditida, Tylenchina, Aphelenchoididae (after Hunt, 2008)	1.It is suggested that Subfamily and Genus are listed to distinguish with other taxonomy method. 2.Use the same description in the draft.	China
16.	*21*	Technical	**3. Detection sampling procedure or a guideline should be include**	This would benefit first time handlers	Kenya
17.	*22*	Technical	*B. xylophilus* has six stages of development: egg and L1 (in the egg), three juvenile stages, and adult. Different types of juvenile stages appear under different conditions. Under favourable conditions at 25 °C *B. xylophilus* develops from the egg through four propagative juvenile stages (L1–L4; the first stage L1 occurs within the egg according to Hasegawa and Miwa (2008)) to become adult nematodes within four days	Replace paragraph 22 by the following text: B. xylophilus has six life stages: the egg and four juvenile stages preceding the adult. The first juvenile stage J1 moults to the second juvenile stage (J2) in the egg. J2 hatches from the egg, and there are two more juvenile stages (J3 and J4) preceding the adult. Different types of juvenile stages appear under different conditions. Under favourable conditions at 25 °C B. xylophilus develops from the egg through	European Union

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			(Figure 1).	four propagative juvenile stages (J1–J4) to reach the adult stage within four days (Hasegawa and Miwa 2008) (Figure 1). Clarification of the text and use of proper terminology	
18.	*23*	Technical	Under unfavourable conditions, the ~~L~~J$_{III}$ dispersal stage develops in place of the ~~L~~J3 stage. ~~L~~J$_{III}$ is probably a non-feeding stage. It has lipids accumulated in the intestinal cells (Kondo and Ishibashi 1978) and ~~that~~ can survive unfavourable conditions such as drought, low temperature or lack of nutrition. Normally this stage moults into the ~~L~~J$_{IV}$ dispersal stage (dauer ~~larvae~~juvenile), which is transmitted by vector beetles to new trees. Nevertheless, if the conditions become suitable for nematode development, for example by putting the ~~L~~J$_{III}$ stage on fungal culture plates, the nematodes develop to the ~~L~~J4 propagative juvenile stage (Wingfield *et al*., 1982).	There is no evidence that J3 is a non-feeding stage. In Kondo & Ishibashi (1978) the authors speculate this but there is no scientific evidence. Consequently 'probably' should be added. Some text can be added to explain the assumptions made in Kondo and Ishibashi such as : "It has lipids accumulated in the intestinal cells (Kondo and Ishibashi 1978)" Also, once again, larvae should be replaced by 'juvenile'. L was replaced by J.	European Union
19.	*26*	Substantive	Change "PWN" to "B.xylophilus".If it is not known whether *B. xylophilus* occurs in an area, sampling should be focused on trees near high-risk sites; for example, ports handling imports from countries with known PWN infestation, airports, sawmills, wood processing facilities, places where wood is stored, and areas where forest fires have occurred (*Monochamus* is attracted by forest fires).	There are two types of Bursaphelenchus xylophilus abbreviation, we suggest to use "B.xylophilus"only.	China
20.	*27*	Editorial	To have the best chance of detecting *B. xylophilus* in an area, it is advisable to concentrate sampling on pine trees that are dying or have died recently, both of which may be standing or fallen. Trees and cut waste from a recent felling season that have been colonized after the felling by *Monochamus* beetles may also be used as sampling material. The following symptoms should be searched for: discoloration (e.g. yellowing) of needles, wilting, evidence of insect attack (e.g. the typical flat-headed larvae of *Monochamus* beneath the bark, oval larval galleries ("grub holes"), the round exit holes of adults), blue stain fungal growth in the wood, and lack of oleoresin flow from wounds. The rate of oleoresin	Clarification	European Union

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			flow should be checked while the trees are still green by removing part of the bark from the cambial layer. Healthy trees will cover the wood surface with resin within one hour while no or reduced resin flow will occur in infested trees. However, these symptoms are non-specific for *B. xylophilus* ~~and~~as they may be caused by other pathogens or by physical factors. There is currently no method to visually distinguish between trees that are dying from pine wilt disease and those dying for other reasons. Trees to be sampled preferably should be associated with *Monochamus* attack, either maturation feeding or breeding, but at the least, it should be known that *Monochamus* species occur in the area where samples are to be taken.		
21.	*27*	Technical	To have the best chance of detecting *B. xylophilus* in an area, it is advisable to concentrate sampling on pine trees that are dying or have died recently, both of which may be standing or fallen. Trees and cut waste from a recent felling seasons (i.e. 1-2 year old logging sites) that have been colonized after the felling by *Monochamus* beetles may also be used as sampling material. The following symptoms should be searched for: discoloration (e.g. yellowing) of needles, wilting, evidence of insect attack (e.g. wood shavings on the ground or protruding from cracks in the bark, flat-headed larvae of *Monochamus* beneath the bark, surface galleries beneath the bark with oval entrance holes oriented in the longitudinal direction of the stem, and the round exit holes of adults ~~the typical flat-headed larvae of *Monochamus* beneath the bark, oval larval galleries ("grub holes"), the round exit holes of adults~~), blue stain fungal growth in the wood, and lack of oleoresin flow from wounds. The rate of oleoresin flow should be checked while the trees are still green by removing part of the bark from the cambial layer. Healthy trees will cover the wood surface with resin within one hour while no or reduced resin flow will occur in infested trees. However, these symptoms vary between species of pine and are non-specific for	General comment on this paragraph : Symptoms in forest should be described with more details, and it would be valuable to add pictures. This will be investigated with the Norwegian NPPO and pictures provided to the IPPC Secretariat as soon as they are available. Specific text suggestions : 1. Clarification of the text more specific 2. The entrance holes are oval not the galleries. A proposed change to the text is made to provide more details on the part on symptoms associated with nsect attacks). 3. Symptoms vary between species e.g. P. sylvestris produces less resin flow.	European Union

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			B. xylophilus and may be caused by other pathogens or by physical factors. There is currently no method to visually distinguish between trees that are dying from pine wilt disease and those dying for other reasons. Trees to be sampled preferably should be associated with *Monochamus* attack, either maturation feeding or breeding, but at the least, it should be known that *Monochamus* species occur in the area where samples are to be taken.		
22.	*27*	Technical	To have the best chance of detecting *B. xylophilus* in an area, it is advisable to concentrate sampling on pine trees that are dying or have died recently, both of which may be standing or fallen. Trees and cut waste from a recent felling season that have been colonized after the felling by *Monochamus* beetles may also be used as sampling material. The following symptoms should be searched for: discoloration (e.g. yellowing) of needles, wilting, evidence of insect attack (e.g. the typical flat-headed larvae of *Monochamus* beneath the bark, oval larval galleries ("grub holes"), the round exit holes of adults), blue stain fungal growth in the wood, and lack of oleoresin flow from wounds. The rate of oleoresin flow should be checked while the trees are still green by removing part of the bark from the cambial layer. Healthy trees will cover the wood surface with resin within one hour while no or reduced resin flow will occur in infested trees. However, these symptoms are non-specific for *B. xylophilus* and may be caused by other pathogens or by physical factors. There is currently no method to visually distinguish between trees that are dying from pine wilt disease and those dying for other reasons. Trees to be sampled preferably should be associated with *Monochamus* attack, either maturation feeding or breeding, but at the least, it should be known that *Monochamus* species occur in the area where samples are to be taken. In countries without Monochamus populations, it would be advisable to also trap for Monochamus adults, as this would enhance eradication of the nematode by attempting to remove	[Reason for trapping - Other families of beetles have been shown to carry PWN but none have been shown to transmit it (Forest Pathology 42: 89-99 (2012).]	Australia

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			the vector which has low flight performance and range.		
23.	*29*	Substantive	**3.2 Detection by the use of trap logs and in samples from sawmills and timber yards** Add one paragraph: Detection by traps.	Traps are very useful to attract vector insect of PWN and have been one of important detection methods.	China
24.	*33*	Substantive	All types of coniferous wood, especially solid wood packaging, particularly from countries in which *B. xylophilus* occurs, can be sampled by low-speed drill, borer, saw, axe, hook and so forth. Sampling should be concentrated on pieces with circular grub holes (i.e. the emergence holes of beetles) or those in which flat-headed *Monochamus* larval stages or pupae are detected in oval galleries, which are sometimes blocked with wood particles. In the case of sawn wood, normally no exit holes will be seen, but oval larval galleries may be seen, which are sometimes difficult to detect because they are blocked with shavings. Pieces with fungal growth, especially blue stain fungus, should be sampled. Nevertheless, several interceptions have shown that living *B. xylophilus* can be detected in samples without the above-mentioned indications (EPPO, 2012).	Pupae cannot be detected from the outside. It is more important to concentrate on signs of beetle activity and not on the beetle itself.	European Union
25.	*33*	Technical	All types of coniferous wood, especially solid wood packaging, particularly from countries in which *B. xylophilus* occurs, can be sampled by low-speed drill, borer, saw, axe, hook and so forth. Sampling should be concentrated on pieces with circular grub holes (i.e. the emergence holes of beetles) ~~or those in which flat-headed *Monochamus* larval stages or pupae are detected in oval galleries~~ and oval entrance holes and larval tunnels, which are sometimes blocked with wood particles. Removal of bark when present may help detecting galleries. In the case of sawn wood, normally no exit holes will be seen, but ~~oval~~ larval ~~galleries~~ tunnels may be seen, which are sometimes difficult to detect because they are blocked with shavings. Pieces with fungal growth, especially blue stain fungus, should be sampled. Nevertheless, several interceptions have shown that living *B. xylophilus* can	General comment: Pictures would be useful. This will be investigated with the Norwegian and Polish NPPOs and pictures provided to the IPPC Secretariat as soon as they are available. Specific text suggestions 1. The entrance holes are oval not the galleries. A proposed change to the text is made 2. It should also be mentioned that removal of bark when present may help detecting galleries 3. Same as above, entrance holes are oval not the galleries.	European Union

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			be detected in samples without the above-mentioned indications (EPPO, 2012).		
26.	*36*	Substantive	Change “room temperature” to “room temperature （approximately 25°C）.Living nematodes can be extracted from infested wood using the Baermann funnel technique or modified Baermann funnel technique (Penas *et al*., 2002; EPPO, 2013b). In this method, a glass or plastic funnel with the narrow tube at the base closed by means of a rubber tube and a clamp is filled with water. The sample of small pieces of wood or wood shavings is supported on a sieve with a paper filter or paper towel that is permeable for nematodes, inside to avoid a contamination of the water with wood debris within the mouth of the funnel, so that the wood is immersed in water. The sample is left for 24 to 48 h at room temperature or in an incubator (set at approximately 25 °C), during which time nematodes migrate from the wood into the water and fall to the base of the funnel from where they can be collected by releasing a small quantity of the water (approximately 10 ml) into a small Petri dish or glass.	It is helpful to give the suitable temperature.	China
27.	*36*	Technical	Living nematodes can be extracted from infested wood using the Baermann funnel technique or modified Baermann funnel technique (Penas *et al*., 2002; EPPO, 2013b). In this method, a glass or plastic funnel with the narrow tube at the base closed by means of a rubber tube and a clamp is filled with water. The sample of small pieces of wood or wood shavings is supported on a sieve with a paper filter or paper towel that is permeable for nematodes, inside to avoid a contamination of the water with wood debris within the mouth of the funnel, so that the wood is immersed in water. The sample is left for 24 to 48 h at room temperature or in an incubator (set at approximately 25 °C), during which time nematodes migrate from the wood into the water and fall to the base of the funnel from where they can be collected by releasing a small quantity of the water (approximately 10 ml) into a small Petri dish or glass.	Proposed redrafting of this section to clarify the text. Replace paragraph 36 by ‘Living nematodes can be extracted from infested wood using the Baermann funnel technique or the modified Baermann funnel technique (Penas et al., 2002; EPPO, 2013b). In the Baermann funnel technique, a glass or plastic funnel with the narrow tube at the base closed by means of a rubber tube and a clamp is filled with water. The sample consisting of small pieces of wood or wood shavings is supported on a sieve in the funnel. A paper tissue permeable for nematodes, is placed on the sieve to avoid a contamination of the water with wood debris. The funnel is then filled with water to cover the sample. The sample is left for 24 to 48 h at room temperature or in an incubator (set at approximately 25 °C), during which time nematodes migrate from the wood into the water and fall to the base of the funnel from where they can be collected	European Union

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				by releasing a small quantity of the water (approximately 10 mL) into a small dish.'	
28.	*37*	Technical	The main principle of the Baermann funnel technique is as described above, but several modifications are used in practice (EPPO, 2013b). For instance, wood chips can be directly submerged in water or they can be placed on a cotton wool filter laid in a plastic basket for extraction of nematodes. In addition, each described method can be combined with a mistifier spray apparatus (EPPO, 2013b).	The reference to the EPPO Standard PM 7/119 on Nematode extraction i.e. EPPO, 2013b could be understood as referring to the mystifyer only. I is suggested that a reference should be added earlier in the text as well.	European Union
29.	*39*	Editorial	Nematodes may occur in very low numbers in the sample, so detection might be difficult. It is recommended to allow the nematodes to multiply before extraction. To do this, the moistened wood sample without any bark is sealed ~~without any bark~~ in a plastic bag and incubated at 25 °C for two to three weeks. The nematodes are then extracted with the Baermann funnel technique.	For better clarity.	Singapore
30.	*39*	Technical	Nematodes may occur in very low numbers in the sample, so detection might be difficult. It is recommended to allow the nematodes to multiply before extraction. To do this, the moistened wood sample is sealed without any bark in a plastic bag and incubated at approximately 25 °C for two to three weeks. The nematodes are then extracted with the Baermann funnel technique.	"approximately" should be added before 25°C a variation is acceptable.	European Union
31.	*40*	Technical	The principle of the Baermann funnel technique is based on detecting living nematodes when they exit the wood sample, but within the recommended 24 to 48 h some nematodes die (Baermann, 1917). Nevertheless, one can be sure that those were alive when the extraction was started. This has to be kept in mind when analysing imported wooden material. Some other extraction methods , for example a centrifugation method (not described here; much faster than the Baermann funnel technique), will also extract nematodes that were already dead in the wood (Moens, 2000). The centrifugation method can be used to monitor an area with PWN infestation but not to prove that wood has undergone	Include here also a reference to EPPO standard on nematode extraction (EPPO, 2013b).	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			a successful phytosanitary treatment (EPPO, 2013b).		
32.	*42*	Technical	Beetles of the genus *Monochamus* caught by traps (Pajares *et al*., 2004; Ibeas *et al*., 2007) or trap logs can be assessed for the presence of nematodes. Nematode ~~larvae~~juveniles are usually present as ~~L~~J_{IV} dispersal stage (dauer juveniles) in the tracheae and on the body of the beetles. To isolate the nematodes, the beetles are dissected and crushed in an appropriate dish and kept in water for 24 to 48 h at approximately 24 °C (Sousa *et al.*, 2001; EPPO, 2013b). Dauer juveniles will leave the beetles. J IV dauer juveniles need to be transferred to fungal mats of ~~and can be cultured on~~ *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea)* grown on malt agar (see 4.1.1) ~~for~~to enter the propagative life cycle. ~~f~~Further identification is made on adult nematodes. Alternatively, J IV, ~~or they~~ can be used directly for molecular identification. ~~L_{IV} dauer juveniles do not have a stylet.~~ The Baermann funnel technique may also be used to extract the nematodes from the beetles.	Specific text suggestions 1. Terminology issue explained before 2. Approximately should be added to avoid metrology issues under accreditation 3. Terminology issue explained before 4. The information on the fact that 'juveniles do not have a stylet' is superfluous as the section is about extraction 5. Clarification and simplification of the text 6. Reference section 4.1.1 should be added New text proposed after 'Dauer juveniles will leave the beetles' reads as follows 'JIV dauer juveniles need to be transferred to fungal mats of Botryotinia fuckeliana (anamorph: Botrytis cinerea) grown on malt agar (see 4.1.1.) to enter the propagative life cycle. Further identification is made on adult nematodes. Alternatively, JIV can be used directly for molecular identification. The Baermann funnel technique may also be used to extract the nematodes from the beetles	European Union
33.	*42*	Technical	Change "24 °C" to"25 °C"Beetles of the genus *Monochamus* caught by traps (Pajares *et al*., 2004; Ibeas *et al*., 2007) or trap logs can be assessed for the presence of nematodes. Nematode larvae are usually present as L_{IV} dispersal stage (dauer juveniles) in the tracheae and on the body of the beetles. To isolate the nematodes, the beetles are dissected and crushed in an appropriate dish and kept in water for 24 to 48 h at 24 °C (Sousa *et al.*, 2001; EPPO, 2013b). Dauer juveniles will leave the beetles and can be cultured on *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea)* grown on malt agar for further identification, or they can be used directly for molecular identification. L_{IV} dauer juveniles do not have a stylet. The Baermann funnel technique may also be used to extract the nematodes from the beetles.	Incubation temperature of Bursaphelenchus xylophilus is recommended at 25°C.	China
34.	*46*	Technical	To date, about 110 species of the genus *Bursaphelenchus* have been described (Futai, 2013).	terminology changes (larvae to juvenile)	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			The latest overviews can be found in Ryss *et al.* (2005), Hunt (2008), Braasch *et al.* (2009) and Futai (2013). *B. xylophilus* can be positively identified by either one of two methods: that based on morphological features and that based on molecular biology techniques. Although the number of *Bursaphelenchus* species described in recent years has increased and some of them have similar morphological characters, a determination based on morphology is possible in most cases. However, identification of the mucronate form of *B. xylophilus* based on morphological charactersmay be difficult. Identification based on morphological features requires preparation of good quality microscope slides, access to a high power microscope and considerable experience in nematode taxonomy, especially in the small group of species closely related to *B. xylophilus* (*B. mucronatus mucronatus*, *B. mucronatus kolymensis, B. fraudulentus* and others). Identification methods based on molecular biology require expensive equipment and reagents, but can be applied with less technical experience (and very little nematological training). Adequate experience is, however, needed to ensure that the limited nematode material is not lost during the procedure. While morphological identification is based on adult specimens, molecular identification can be made even if only ~~larval~~juvenile stages or one sex of adults are available, which is an advantage. While DNA-based PCR methods fail to differentiate between dead and living nematodes, new methods based on mRNA can clarify whether the positive detection originates from living nematodes (Leal *et al.*, 2013).		
35.	*47*	Editorial	*B. xylophilus* can be ~~determined~~ identified by an experienced phytopathologist or nematologist using morphological features if the specimens are available as adults and in good condition. However, there may be situations where a combination of morphological features and molecular information is recommended to obtain a higher degree of certainty on the identification; for example, when PWN has been detected in a new	1. Identified is the correct term/ 2. 'Sound ' should be replaced by reliable. 3. A reference to section 4.1.1 should be added.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			area, when PWN has been found by a laboratory for the first time, as quality assurance for compliance with certification schemes, and when PWN is found in consignments during import inspection, especially when the exporting country has been declared to be free from *B. xylophilus*. In addition, *B. xylophilus* can show morphological variations that may make the use of molecular biology techniques necessary, for example, round or mucronate tail tip of females (Figure 5) or the position of the excretory pore. When only a small number of nematodes have been isolated, multiplying them on *B. fuckeliana* before identification is recommended to obtain enough material for a ~~sound~~reliable identification (see 4.1.1).		
36.	*47*	Substantive	*B. xylophilus* can be determined by an experienced ~~phytopathologist or~~ nematologist using morphological features if the specimens are available as adults and in good condition. However, there may be situations where a combination of morphological features and molecular information is recommended to obtain a higher degree of certainty on the identification; for example, when PWN has been detected in a new area, when PWN has been found by a laboratory for the first time, as quality assurance for compliance with certification schemes, and when PWN is found in consignments during import inspection, especially when the exporting country has been declared to be free from *B. xylophilus*. In addition, *B. xylophilus* can show morphological variations that may make the use of molecular biology techniques necessary, for example, round or mucronate tail tip of females (Figure 5) or the position of the excretory pore. When only a small number of nematodes have been isolated, multiplying them on *B. fuckeliana* before identification is recommended to obtain enough material for a sound identification.	Regarding "B. xylophilus can be determinidentified by an experienced phytopathologist or nematologist using morphological features if the specimens are available as adults and in good condition." This sentence is not true in most cases.	European Union
37.	*47*	Substantive	Change “PWN” to *“B.xylophilus”*.*B. xylophilus* can be determined by an experienced phytopathologist or nematologist using morphological features if the	There are two types of Bursaphelenchus xylophilus abbreviation, we suggest to use “B.xylophilus”only.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			specimens are available as adults and in good condition. However, there may be situations where a combination of morphological features and molecular information is recommended to obtain a higher degree of certainty on the identification; for example, when PWN has been detected in a new area, when PWN has been found by a laboratory for the first time, as quality assurance for compliance with certification schemes, and when PWN is found in consignments during import inspection, especially when the exporting country has been declared to be free from *B. xylophilus*. In addition, *B. xylophilus* can show morphological variations that may make the use of molecular biology techniques necessary, for example, round or mucronate tail tip of females (Figure 5) or the position of the excretory pore. When only a small number of nematodes have been isolated, multiplying them on *B. fuckeliana* before identification is recommended to obtain enough material for a sound identification.		
38.	*47*	Technical	*B. xylophilus* can be determined by an experienced phytopathologist or nematologist using morphological features if the specimens are available as male and female adults and in good condition. However, there may be situations where a combination of morphological features and molecular information is recommended to obtain a higher degree of certainty on the identification; for example, when PWN has been detected in a new area, when PWN has been found by a laboratory for the first time, as quality assurance for compliance with certification schemes, and when PWN is found in consignments during import inspection, especially when the exporting country has been declared to be free from *B. xylophilus*. In addition, *B. xylophilus* can show morphological variations that may make the use of molecular biology techniques necessary, for example, round or mucronate tail tip of females (Figure 5) or the position of the excretory pore. When only a small number of nematodes have been isolated, multiplying them on *B. fuckeliana* before identification is recommended to obtain enough	Male and female are needed for identification required as identification criteria refer to both of them (male and female).	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			material for a sound identification.		
39.	*49*	Substantive	Rewrite the contents of the nematode taxonomy in this paragraph.Numerous nematode species may be present in an aqueous extract from coniferous wood, especially if decay of the tissues has begun. Some of these will be saprophagous species where adult nematodes lack the solid mouth stylet that is typical for nematodes of the orders Tylenchida and Aphelenchida. *Bursaphelenchus* spp. belong to the Aphelenchida, which have the dorsal oesophageal gland opening into the median oesophageal bulb, in contrast to the Tylenchida, where the gland opens into the lumen of the oesophagus between the bulb and the stylet (Figure 2). If the extract contains only larvae, morphological identification of *B. xylophilus* will not be possible. In such cases, aphelenchoide species that fall in the range of *B. xylophilus* larval size (see, e.g., Penas *et al.*, 2008) should be separated and either multiplied on a culture plate or used directly for molecular identification.	The description of “Tylenchida and Aphelenchida”in this paragraph is different from “Taxonomic position” in paragraph 19. We suggest use the same taxonomy system.	China
40.	*49*	Technical	Numerous nematode species may be present in an aqueous extract from coniferous wood, especially if decay of the tissues has begun. Some of these will be saprophagous species where adult nematodes lack the ~~solid mouth~~ stylet that is typical for nematodes of the orders Tylenchida, ~~and~~ Aphelenchida and Dorylaimida. *Bursaphelenchus* spp. belong to the Aphelenchida, which have the dorsal ~~oesophageal~~ pharyngal gland opening into the ~~median oesophageal bulb~~metacorpus, in contrast to the Tylenchida, where the gland opens into the lumen of the ~~oesophagus~~ pharynx between the bulb and the stylet (Figure 2). If the extract contains only ~~larvae~~juveniles, morphological identification of *B. xylophilus* will not be possible. In such cases, aphelenchoide species that fall in the range of *B. xylophilus* ~~larval~~juvenile size (see, e.g., Penas *et al.*, 2008) should be separated and either multiplied on a culture plate or used directly for molecular identification.	1. ‘solid mouth stylet’ is not commonly used in nematology. “solid stylets” occur in Trichodoridae which are not present in wood: it is suggested that ‘solid mouth’ should be deleted. 2. Terminology issue explained before. Changes made throughout the paragraph. 3. Stylet are also present in Dorylaimida and they can be present in wood. The addition of Dorylaimida is proposed	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
41.	*50*	Technical	For identification under a light microscope, a magnification of 400× to 1 000× (oil immersion lens) is recommended, differential interference contrast (DIC) may facilitate observation.~~.~~	It is more correct to state that DIC facilitates observation. Change proposed.	European Union
42.	*52*	Substantive	1.Describe more clearly how long the fungi and nematodes be cultured at the definite temperature. 2.Change “room temperature” to “room temperature （approximately 25°C）.It may be necessary to multiply the extracted nematodes to obtain enough material for identification. Most *Bursaphelenchus* species can be cultured on the sporulating form of the fungus *B. fuckeliana*. Some species, especially those belonging to the *sexdentati* group, require culture on the non-sporulating form. Both fungal forms are cultured on 2% malt extract agar (MEA) medium (15 g agar-agar, 15 g malt extract, 750 ml water; pH 7.0). Petri dishes (90 mm diameter) are filled with 25 ml sterilized MEA. Either fungal spores or pieces of agar with fungal growth are transferred to the Petri dishes in a clean bench unit. Incubation of the fungal plates is recommended at room temperature (approximately 20 °C). Nematodes to be reared are transferred in a small droplet using a pipette or other means. Nematode incubation is recommended at 25 °C (based on its biology), which leads to a sufficient reproduction rate.	1.It is helpful to give the suitable temperature. 2.Different countries have different room temperature.	China
43.	*52*	Technical	It may be necessary to multiply the extracted nematodes to obtain enough material for identification. Most *Bursaphelenchus* species can be cultured on the sporulating form of the fungus *B. fuckeliana*. Some species, especially those belonging to the *sexdentati* group, require culture on the non-sporulating form. Both fungal forms are cultured on 2% malt extract agar (MEA) medium (15 g agar-agar, 15 g malt extract, 750 ml water; pH 7.0). Petri dishes (90 mm diameter) are filled with 25 ml sterilized MEA. Either fungal spores or pieces of agar with fungal growth are transferred to the Petri dishes in a clean bench unit. Incubation of the fungal plates is recommended at room temperature (approximately 20 °C). Nematodes to be reared are	For the benefit of first time detection in a country. This will assist in answering the question regarding details of the protocols eg (how long to incubate, how to extract from the media etc	Kenya

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			transferred in a small droplet using a pipette or other means. Nematode incubation is recommended at 25 °C (based on its biology), which leads to a sufficient reproduction rate.some relevant documents were removed. eg(par52);preparation of specimen. However, these references s hould be included in the document		
44.	*53*	Editorial	***4.41.1.1 Temporary preparations***	Adjust the numbering	European Union
45.	*53*	Editorial	***4.14.1.1 Temporary preparations***	Numbering error	Australia
46.	*53*	Editorial	***4.41.1.1 Temporary preparations***	Corrigendum	Japan
47.	*54*	Technical	Temporary preparations for quick identification or study of features best seen in unfixed specimens are prepared as follows. Living specimens are transferred to a small drop of water on a glass slide. The slide is briefly heated over a spirit flame, checking frequently for nematode movement. Heating should be stopped as soon as the specimens stop twitching. A coverslip is applied and the slide is ready for study. Fixing the cover slide is not recommended as the body of the male nematodes may have to be moved subsequently into the dorso-ventral position to see the bursa. need reference for par 52 and 54	To benefit first time detection country	Kenya
48.	*55*	Editorial	***4.41.1.2 Permanent preparations***	Adjust the numbering	European Union
49.	*55*	Editorial	***4.14.1.2 Permanent preparations***	Numbering error	Australia
50.	*55*	Editorial	***4.41.1.2 Permanent preparations***	Corrigendum	Japan
51.	*56*	Editorial	Permanent preparations for identification under light microscopy are prepared as follows. Living nematodes extracted from plant material are killed by gentle heat, fixed in FAA fixative (35% distilled water, 10% of 40% formalin, 5% glacial acetic acid, 50% of 95% alcohol) (Andrássy, 1984) or TAF fixative (10% of 35% formalin, 1% triethanolamine, 89% distilled water), processed to	more correct	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			anhydrous glycerine (for long-term storage) and mounted on slides in anhydrous glycerine ~~between coverslip slides~~ as described by Seinhorst (1959) and Goodey (1963). A more rapid method (1–1.5 h) to prepare permanent slides was described by Ryss (2003) based on killing the nematodes in hot 4% formalin. Fixation then takes place at different temperatures in a programmable thermal controller, followed by processing to glycerine.		
52.	*56*	Substantive	1.Change“ formalin” to “ formaldehyde solution”. 2.Change “extracted from plant material” to “extracted from plant material and nematode incubation”. 3.Add more information, like what kinds of individuals should be select, how to place nematodes on slides and how to seal coverslips etc.Permanent preparations for identification under light microscopy are prepared as follows. Living nematodes extracted from plant material are killed by gentle heat, fixed in FAA fixative (35% distilled water, 10% of 40% formalin, 5% glacial acetic acid, 50% of 95% alcohol) (Andrássy, 1984) or TAF fixative (10% of 35% formalin, 1% triethanolamine, 89% distilled water), processed to anhydrous glycerine (for long-term storage) and mounted in anhydrous glycerine between coverslip slides as described by Seinhorst (1959) and Goodey (1963). A more rapid method (1–1.5 h) to prepare permanent slides was described by Ryss (2003) based on killing the nematodes in hot 4% formalin. Fixation then takes place at different temperatures in a programmable thermal controller, followed by processing to glycerine.	1.“Formalin”refers to the 35%-40% formaldehyde solution. 2.Some preparations come from nematode incubation. 3.It is helpful to operate properly.	China
53.	*58*	Substantive	The following key, partly derived from Bongers (1989), is used to determine the family of female specimens. The key within the family Parasitaphelenchinae to determine the genus *Bursaphelenchus* was adapted from Hunt (2008). Alternatively a simple key is avaialble in the EPPO Stadnard PM 7/4 (3) *Bursaphelenchus xylophilus* (EPPO, 2013) which has been established by consensus in the EPPO rezgion and is widely used.	Experts in the EPPO region consider that this is not necessary to go via fam Parasitaphelenchinae, when you can go to the B. xylophilus group directly. It is not proposed to change the current keys but it is suggested to add a reference to the EPPO simplified key included in PM 7/4 (3).	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
54.	*58*	Substantive	The following key, partly derived from Bongers (1989), is used to determine the subfamily of female specimens. The key within the subfamily Parasitaphelenchinae to determine the genus *Bursaphelenchus* was adapted from Hunt (2008).	Parasitaphelenchinae is a subfamily of Parasitaphelenchidae.	Japan
55.	*58*	Technical	The following key, partly derived from Bongers (1989), is used to determine the family of female specimens. The key within the family Parasitaphelenchinae to determine the genus *Bursaphelenchus* was adapted from Hunt (2008). Definitions of terminology used in the following sections can be found in EPPO Technical Document No. 1056: Pictorial glossary of morphological terms in nematology (EPPO 2013).	Include a reference to the EPPO pictorial glossary as is done in thedraft IPPC Xiphinema protocol 'Definitions of terminology used in the following sections can be found in EPPO Technical Document No. 1056: Pictorial glossary of morphological terms in nematology (EPPO, 2013)'.	European Union
56.	*58*	Technical	Change"the family Parasitaphelenchinae" to "the Subfamily Parasitaphelenchinae".The following key, partly derived from Bongers (1989), is used to determine the family of female specimens. The key within the family Parasitaphelenchinae to determine the genus *Bursaphelenchus* was adapted from Hunt (2008).	According to "Taxonomic position" in Para.19, Parasitaphelenchinae should be a Subfamily.	China
57.	*59*	Substantive	Key to subfamilies	Parasitaphelenchinae is a subfamily of Parasitaphelenchidae.	Japan
58.	*59*	Technical	4.1.2.1 Key to families or subfamilies using female specimens	provides clarification	Australia
59.	*60*	Editorial	**1** – Nematode with spear or stylet, which can protrude……2 – Nematode without spear or stylet……..NBS[1] **2** – Mouth with tylenchid stylet, oesophagus with median bulb……..3 – Mouth with dorylaimid spear, oesophagus	Some editorial suggestions made (replace 'musculated' by 'muscular' and 'empties' by 'opens'	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			cylindrical or bottle-shaped, without median bulb…….....NBS **3** – Median bulb with valves…….....4 – Median bulb without valves…….....NBS **4** – Procorpus clearly separated from metacorpus (median bulb) by a constriction…….....5 – Procorpus and median bulb shade off into one other, terminal bulb strongly reduced, cuticle conspicuously annulated…….NBS **5** – One gonad (vulva posterior) ……...6 – Two gonads…….....NBS **6** – Lip region without setae……7 – Lip region with		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			setae……..... ..NBS **7** – Median bulb strongly ~~musculated~~ muscular and conspicuously well developed, clearly visible at low magnification, ovoid to rounded rectangular, dorsal oesophageal gland ~~empties~~ opens into lumen of oesophagus within median bulb…… …….....8 – Median bulb normal, dorsal oesophageal gland ~~empties~~ opens into lumen of oesophagus just behind stylet…….....NBS **8** – Oesophageal glands overlap intestine dorsally……...9 – Oesophageal glands within abutting bulb……....NBS **9** – Male tail tip enveloped by small, bursa-like flap of cuticula (only to be seen when nematode is lying in the dorso-ventral position) 10 – No bursa-like flap of		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			cuticula…….....NBS **10** – Stylet knobs usually present, female with anus…….....Parasitaphelenchinae – Stylet knobs usually not present, female without anus…….....NBS		
60.	*60*	Technical	**1** – Nematode with spear or stylet~~, which can protrude~~……2 – Nematode without spear or stylet……...NBS[1] **2** – Mouth with tylenchid stylet, ~~oesophagus~~ pharynx with ~~median bulb~~metacorpus……..3 – Mouth with dorylaimid ~~spear~~stylet, ~~oesophagus~~ pharynx cylindrical or bottle-shaped, without ~~median bulb~~metacorpus……..NBS **3** – ~~Median bulb~~Metacorpus with ~~valves~~metacorpal plates……4 – ~~Median bulb~~Metacorpus without ~~valves~~conspicious metacorpal plates……NBS **4** – Procorpus clearly separated from metacorpus	Terminology issue explained before. Changes made throughout the key. The following part : "Procorpus and median bulb (metacorpus) shade off into one other terminal (basal) bulb" (4), Needs better descriptive terminology : amalgamated ? The following part : "oesophagus (pharynx) just behind stylet" (7), should be in procorpus?	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			~~(median bulb)~~ by a constriction……..5 – Procorpus and ~~median bulb~~metacorpus shade off into one other, ~~terminal~~basal bulb strongly reduced, cuticle conspicuously annulated…….NBS **5** – One gonad (vulva posterior) ……...6 – Two gonads…….NBS **6** – Lip region without setae……7 – Lip region with setae……..NBS **7** – ~~Median bulb~~Metacorpus strongly musculated and conspicuously well developed, clearly visible at low magnification, ovoid to rounded rectangular, dorsal ~~oesophageal~~ pharyngeal gland empties into lumen of ~~oesophagus~~ pharynx within		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			~~median bulb~~metacorpus……..8 – ~~Median bulb~~Metacorpus normal, dorsal ~~oesophageal~~ pharyngeal gland empties into lumen of ~~oesophagus~~ pharynx just behind stylet…….....NBS **8** – ~~Oesophageal~~ Pharyngeal glands overlap intestine dorsally……..9 – ~~Oesophageal~~ Pharyngeal glands within abutting bulb…….....NBS **9** – Male tail tip enveloped by small, bursa-like flap of cuticula (only to be seen when nematode is lying in the dorso-ventral position) 10 – No bursa-like flap of cuticula…….....NBS **10** – Stylet knobs usually present, female with anus…….....Parasitaphelenchinae – Stylet knobs usually not present, female without anus…….....NBS		
61.	*60*	Technical	**1** – Nematode with spear or stylet, which can protrude……2	'Shade off into one another' is not clear. Medium bulb normal is not clear.	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			– Nematode without spear or stylet……...NBS[1] **2** – Mouth with tylenchid stylet, oesophagus with median bulb……..3 – Mouth with dorylaimid spear, oesophagus cylindrical or bottle-shaped, without median bulb…….....NBS **3** – Median bulb with valves…….....4 – Median bulb without valves…….....NBS **4** – Procorpus clearly separated from metacorpus (median bulb) by a constriction……..…….....5 – Procorpus and median bulb not separated by a constriction~~shade off into one other~~, terminal bulb strongly reduced, cuticle conspicuously annulated…….NBS **5** – One gonad (vulva posterior)		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			……...6 – Two gonads……..NBS **6** – Lip region without setae……7 – Lip region with setae……..NBS **7** – Median bulb strongly musculated and conspicuously well developed, clearly visible at low magnification, ovoid to rounded rectangular, dorsal oesophageal gland empties into lumen of oesophagus within median bulb……..8 – Median bulb not as above~~rmal~~, dorsal oesophageal gland empties into lumen of oesophagus just behind stylet……..NBS **8** – Oesophageal glands overlap intestine dorsally……...9 – Oesophageal glands within abutting		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			bulb……....NBS **9** – Male tail tip enveloped by small, bursa-like flap of cuticula (only to be seen when nematode is lying in the dorso-ventral position) 10 – No bursa-like flap of cuticula…….....NBS **10** – Stylet knobs usually present, female with anus…….....Parasitaphelenchinae – Stylet knobs usually not present, female without anus…….....NBS		
62.	*61*	Editorial	4.1.2.2 Subfamily Parasitaphelenchinae	Makes key heading clearer	Australia
63.	*62*	Editorial	- **11** – In most species, L_{III} or L_{IV} dauer juveniles phoretically associated with insects; vulva posterior (usually 60–80% of body length); spicules partially fused or separated; male tail strongly recurved; bursal flap present in most species……*Bur*	Delete vacant first line	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			saphelenchus – L_{IV} dauer juveniles endoparasitic in insect haemocoel; vulva very posterior (80–90% of body length); spicules partially fused; male tail not strongly recurved; bursal flap present……NBS		
64.	*62*	Technical	**11** – In most species, ~~L~~J_{III} or ~~L~~J_{IV} dauer juveniles phoretically associated with insects; vulva posterior (usually 60–80% of body length); spicules partially fused or separated; male tail strongly recurved; bursa~~l flap~~ present in most species……*Bursaphelenchus* – ~~L~~J_{IV} dauer juveniles endoparasitic in insect haemocoel; vulva very posterior (80–90% of body length); spicules partially fused; male tail not strongly recurved; bursa~~l flap~~ present……NBS	Regarding "spicule partially fused or separated" : This is a difficult character for non-specialists and experts from the Panel on diagnostics in nematology considered that this would be difficult for a normal diagnostic laboratory. However no specific proposal for change is made. In the EPPO protocol PM 7/4 (3) a simplified key is included and there is no need to go through this Subfamily Parasitaphelenchinae. Bursal flap is confusing can it be replaced by bursa or more explanation provided? Regarding the J4 dauer juveniles how could someone know in the laboratory if they are endoparasitic in insect haemocoel? This does not seem very helpful for the protocol. Can this be omitted?	European Union
65.	*63*	Editorial	4.1.2.3 Key to species of~~Genus~~ Bursaphelenchus	Makes key heading clearer	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
66.	*64*	Technical	**12** – Vulva with prominent flap; spicules long, slender and semicircular with angular lamina in posterior third, capitulum fattened with small condylus and distinct rostrum, cucullus usually present; lateral field with four lines.....*xylophilus* group – Characters different…….....Not *xylophilus* group	What is a long spicule? What is a slender spicule? What is semicircular spicule? What is angular lamina? More guidance would be appreciated	European Union
67.	*67*	Editorial	**13** – Female tail broadly subcylindrical, with or without mucro (Figures 2 and 5)..... 14 – Female tail conical (Figure 12) or strongly tapering, with or without mucro..... Not *B. xylophilus* **14** – Spicule length <30 µm (measured from condylus to distal end in a straight line) ……..... 15 – Spicule length >30 µm…….. ..... Not *B. xylophilus* **15** – Spicule with long and pointed rostrum, limbs of spicule with an angular curvature (Figures 2(C) and 6) ……..... 16 – Spicule with short and pointed rostrum, limbs of	Formatting should be checked	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			spicule with a rounded curvature…….... Not *B. xylophilus* **16** – Female vulval flap straight, not ending in a deep depression (Figures 2(G) and 8).....17 – Female vulval flap ending in a deep depression (Figure 9(A)) ……..... Not *B. xylophilus* **17** – Female tail with mucro >3 µm (Figure 3(d) and 5(c)) ……..... 18 – Female tail without mucro (Figures 2(H) and 5(a)) and with or without a small projection <2 µm* (Figures 2(I)–(J) and 5(b)) ……..... *B. xylophilus* (round-tailed form) **18** – Excretory pore at or behind median bulb……..... *B. mucronatus* *kolymensis*		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			and *B.* *xylophilus* (mucronated form**) – Excretory pore anterior to median bulb……..... Not *B. xylophilus*		
68.	*67*	Editorial	**13** – Female tail broadly subcylindrical, with or without mucro (Figures 2 and 5)..... 14 – Female tail conical (Figure 12) or strongly tapering, with or without mucro..... Not *B. xylophilus* **14** – Spicule length <30 µm (measured from condylus to distal end in a straight line) ……..... 15 – Spicule length >30 µm…….. ..... Not *B. xylophilus* **15** – Spicule with long and pointed rostrum, limbs of spicule with an angular curvature (Figures 2(C) and 6) ……..... 16 – Spicule with short and pointed rostrum, limbs of	Plural	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			spicule with a rounded curvature…….... Not *B. xylophilus* **16** – Female vulval flap straight, not ending in a deep depression (Figures 2(G) and 8).....17 – Female vulval flap ending in a deep depression (Figure 9(A)) ……..... Not *B. xylophilus* **17** – Female tail with mucro >3 µm (Figures 3(d) and 5(c)) ……..... 18 – Female tail without mucro (Figures 2(H) and 5(a)) and with or without a small projection <2 µm* (Figures 2(I)–(J) and 5(b)) ……..... *B. xylophilus* (round-tailed form) **18** – Excretory pore at or behind median bulb……..... *B. mucronatus* *kolymensis*		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			and *B.* *xylophilus* (mucronated form**) – Excretory pore anterior to median bulb……..... Not *B. xylophilus*		
69.	*67*	Technical	**13** – Female tail broadly subcylindrical, with or without mucro (Figures 2 and 5)..... 14 – Female tail conical (Figure 12) or strongly tapering, with or without mucro..... Not *B. xylophilus* **14** – Spicule length <30 µm (measured from condylus to distal end in a straight line) ……..... 15 – Spicule length >30 µm…….. ..... Not *B. xylophilus* **15** – Spicule with long and pointed rostrum, limbs of spicule with an angular curvature (Figures 2(C) and 6) ……..... 16 – Spicule with short and pointed rostrum, limbs of	Under 14 how can it be measured in a straight line? When is it meant to start and stop. Can a picture be added? Terminology issue explained before. Changes made throughout the key.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			spicule with a rounded curvature…….... Not *B. xylophilus* **16** – Female vulval flap straight, not ending in a deep depression (Figures 2(G) and 8).....17 – Female vulval flap ending in a deep depression (Figure 9(A)) ……..... Not *B. xylophilus* **17** – Female tail with mucro >3 µm (Figure 3(d) and 5(c)) ……..... 18 – Female tail without mucro (Figures 2(H) and 5(a)) and with or without a small projection <2 µm* (Figures 2(I)–(J) and 5(b)) ……..... *B. xylophilus* (round-tailed form) **18** – Excretory pore at or behind ~~median bulb~~metacorpus…….... *B. mucronatus* *kolymensis*		

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			and *B.* *xylophilus* (mucronated form**) – Excretory pore anterior to ~~median bulb~~metacorpus…… Not *B. xylophilus*		
70.	*69*	Editorial	If the position of the excretory pore ~~cannot be observed~~is not discernable, an identification based on morphological characters ~~might~~may result in an incorrect indentification ~~be impossible or leads to doubtful determinations~~. In such instances, a~~nd~~ molecular test~~s~~ should be performed.	For better understanding and more precise wording 1. Replace cannot be observed by is not discernible 2. Replace 'might be impossible or leads to doubtful determinations' by 'may result in an incorrect identification'	European Union
71.	*70*	Technical	*B. xylophilus* has the general characters of the genus *Bursaphelenchus* (Nickle, 1970; Hunt 2008): about 1 mm in length, slender; cephalic region high, offset by a constriction, and with six lips; stylet well developed, usually with small basal thickenings; ~~median bulb~~metacorpus well developed (Figures 2(F) and 4); male tail terminus strongly curved ventrally, conoid, with a small terminal bursa that can be seen in the dorso-ventral position (Figure 7); spicules robust, rose thorn-shaped, usually with a prominent apex and rostrum; gubernaculum absent (Figures 3 and 6); vulva 70–80% of the body length; post-uterine sac well developed (Figure 2(A)).	Terminology issue.	European Union
72.	*71*	Editorial	Most populations of *B. xylophilus* (round-tailed populations) can be distinguished from other *Bursaphelenchus* species by the presence of the following three characters (Figure 3). (1) Males of *B. xylophilus* (Figure 6) have relatively large spicules, evenly arcuate, with a sharply pointed prominent	Corrigendum	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			rostrum and cucullus (disc-like projection) at the distal ends of the spicules. (2) The tail of the females is subcylindrical with a broadly rounded to digitate terminus (Figure 5(a)), normally without a mucro (small projection), but occasionally females of round-tailed populations have a mucro on their tail terminus, which is usually less than 2 µm (Figure 5). (3) The vulva has a long, overlapping anterior lip (Figure 8~~9~~).		
73.	*71*	Substantive	Most populations of *B. xylophilus* (round-tailed populations) can be distinguished from other *Bursaphelenchus* species by the presence of the following three characters (Figure 3). (1) Males of *B. xylophilus* (Figure 6) have relatively large spicules, evenly arcuate, with a sharply pointed prominent rostrum and cucullus (disc-like projection) at the distal ends of the spicules. (2) The tail of the females is subcylindrical with a broadly rounded ~~to digitate~~ terminus (Figure 5(a)), normally without a mucro (small projection), but occasionally females of round-tailed populations have a mucro on their tail terminus, which is usually less than 2 µm (Figure 5b). (3) The vulva has a long, overlapping anterior lip (Figure 9).	Figure 5(a) is therefore in accordance with the second half of the description of key number 17 in paragraph [67]. Figure 5(b) is therefore in accordance with the second half of the description of key number 17 in paragraph [67].	Japan
74.	*73*	Editorial	Characters best seen by scanning electron microscopy are four incisures (Figure 10) in the lateral field, and the number and position of caudal papillae in males (Figure 11): an adanal pair just before the anus, two post-anal pairs just before the origin of the ~~caudal alae~~bursa, and a single median papillae just preanal. These characters sometimes can barely be seen by light microscopy. Figures 10 and 11 are electron micrographs illustrating these two characters as they are cited in section 4.1.3 for grouping *Bursaphelenchus* species in the *xylophilus* group.	Both bursa and caudal alae used in document, replace by Bursa.	European Union
75.	*75*	Substantive	It is better to add data from China.**Table 1.** Measurements (mean, and range in parentheses) of *Bursaphelenchus xylophilus* characters	Literature: Yang Bao-jun, Pan Hong-yang, et al. Pine Wilt Disease. 2003. Chinese Forestry Publishing House.	China
76.	*76*	Editorial	**Males**	Corrigendum	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation						Country
			Character	Author	Nickle *et al.* (1981) (*n* = 5) (United States)[1]	Mamiya and Kiyohara (1972) (*n* = 30) (Japan)[1]	Mota *et al.* (1999) (*n* = 12) (Portugal)[1]	Penas *et al.* (2008) (*n* = 20) (Portugal)[1]	Penas *et al.* (2008) (*n* = 20) (Portugal)[2]	
			Length (L), mm		0.56 (0.52–0.60)	0.730.56 (0.590.52–0.820.60)	1.03 (0.80–1.30)	0.57 (0.45–0.69)	1.04 (0.87–1.17)	
			a (body length / greatest body diameter)		40.8 (35–45)	42.340.8 (3635–4745)	49.4 (44–56)	46.0 (40.2–58.5)	45.7 (41.3–48.9)	
			b (body length / distance from anterior to oesophago-intestinal valve)		9.4 (8.4–10.5)	9.4 (7.68.4–11.310.5)	13.3 (11.1–14.9)	9.6 (8.2–10.7)	13.7 (11.6–15.4)	
			c (body length /tail length)		24.4 (21–29)	26.424.4 (21–3129)	28.0 (24–32)	21.6 (19.1–24.6)	26.8 (23.6–31.4)	
			Stylet, µm		13.3 (12.6–13.8)	14.913.3 (1412.6–1713.8)	12.6 (11–16)	11.0 (10–14)	14.0 (12–15)	
			Spicules, µm		21.2 (18.8–	2721.2 (2518.8–	24 (22–25)	19.3 (16.5–	30.4 (25.0–	

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			23.0) 3023.0) 24.0) 33.5)		
77.	*76*	Technical	**Males** Character / Author: Nickle *et al.* (1981) (*n* = 5) (United States)[1]; Mamiya and Kiyohara (1972) (*n* = 30) (Japan)[1]; Mota *et al.* (1999) (*n* = 12) (Portugal)[1]; Penas *et al.* (2008) (*n* = 20) (Portugal)[1]; Penas *et al.* (2008) (*n* = 20) (Portugal)[2] Length (L), mm: 0.56 (0.52–0.60); 0.56 (0.52–0.60); 1.03 (0.80–1.30); 0.57 (0.45–0.69); 1.04 (0.87–1.17) a (body length / greatest body diameter): 40.8 (35–45); 40.8 (35–45); 49.4 (44–56); 46.0 (40.2–58.5); 45.7 (41.3–48.9) b (body length / distance from anterior to ~~oesophago~~pharyngo-intestinal valve): 9.4 (8.4–10.5); 9.4 (8.4–10.5); 13.3 (11.1–14.9); 9.6 (8.2–10.7); 13.7 (11.6–15.4) c (body length /tail length): 24.4 (21–29); 24.4 (21–29); 28.0 (24–32); 21.6 (19.1–24.6); 26.8 (23.6–31.4) Stylet, µm: 13.3 (12.6–13.8); 13.3 (12.6–13.8); 12.6 (11–16); 11.0 (10–14); 14.0 (12–15) Spicules, µm: 21.2 (18.8–23.0); 21.2 (18.8–23.0); 24 (22–25); 19.3 (16.5–24.0); 30.4 (25.0–33.5)	Terminology issue.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
78.	*76*	Technical	Check and change the wrong data of “Mamiya and Kiyohara (1972)”. **Males** Character Length (L), mm a (body length / greatest body diameter) b (body length / distance from anterior to oesophago-intes valve) c (body length /tail length) Stylet, µm	The data of “Mamiya and Kiyohara (1972) (n=30)” are wrong.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Spicules, µm: 21.2 (18.8–23.0) | 21.2 (18.8–23.0) | 24 (22–25) | 19.3 (16.5–24.0) | 30.4 (25.0–33.5)		
79.	77	Editorial	**Females**	Corrigendum	Japan

Character	Author	Nickle *et al.* (1981) (*n* = 5) (United States)[1]	Mamiya and Kiyohara (1972) (*n* = 40~~3 9~~) (Japan)[1]	Mota *et al.* (1999) (*n* = 12) (Portugal)[1]	Penas *et al.* (2008) (*n* = 20) (Portugal)[1]	Penas *et al.* (2008) (*n* = 20) (Portugal)[2]
Length (L), mm		0.52 (0.45–0.61)	0.81 (0.71–1.01)	1.05 (0.89–1.29)	0.58 (0.51–0.66)	1.13 (0.91–1.31)
a (body length / greatest body diameter)		42.6 (37–48)	40.0 (33–46)	50.0 (41–58)	41.9 (32.8–50.6)	45.6 (39.4–50.3)
b (body length / distance from anterior to oesophago-intestinal valve)		9.6 (8.3–10.5)	10.3 (9.4–12.8)	13.8 (12.7–16.4)	10.1 (9.1–11.2)	14.7 (11.6–16.8)
c (body length / tail length)		27.2 (23–31)	26.0 (23–32)	26.6 (22–32)	25.4 (20.2–29.0)	28.1 (21.9–34.4)
Stylet, µm		12.8 (12.6–13.0)	15.9 (14–18)	12.3 (11–15)	11.2 (10.0–12.5)	14.4 (12–16)

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Vulva position (V), % of L: 74.7 (73–78); 72.7 (67–78); 73.3 (70–76); 71.5 (70.1–72.9); 72.6 (70.4–74.5)		
80.	*77*	Technical	**Females** Character / Author / Nickle *et al.* (1981) (*n* = 5) (United States)[1] / Mamiya and Kiyohara (1972) (*n* = 30) (Japan)[1] / Mota *et al.* (1999) (*n* = 12) (Portugal)[1] / Penas *et al.* (2008) (*n* = 20) (Portugal)[1] / Penas *et al.* (2008) (*n* = 20) (Portugal)[2] Length (L), mm: 0.52 (0.45–0.61); 0.81 (0.71–1.01); 1.05 (0.89–1.29); 0.58 (0.51–0.66); 1.13 (0.91–1.31) a (body length / greatest body diameter): 42.6 (37–48); 40.0 (33–46); 50.0 (41–58); 41.9 (32.8–50.6); 45.6 (39.4–50.3) b (body length / distance from anterior to ~~oesophago~~pharyngo-intestinal valve): 9.6 (8.3–10.5); 10.3 (9.4–12.8); 13.8 (12.7–16.4); 10.1 (9.1–11.2); 14.7 (11.6–16.8) c (body length / tail length): 27.2 (23–31); 26.0 (23–32); 26.6 (22–32); 25.4 (20.2–29.0); 28.1 (21.9–34.4) Stylet, µm: 12.8 (12.6–13.0); 15.9 (14–18); 12.3 (11–15); 11.2 (10.0–12.5); 14.4 (12–16)	Terminology issue.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Vulva position (V), % of L: 74.7 (73–78); 72.7 (67–78); 73.3 (70–76); 71.5 (70.1–72.9); 72.6 (70.4–74.5)		
81.	*82*	Editorial	*B. xylophilus* is one species of the *xylophilus* group *sensu* Braasch (2001). Although there is current debate among taxonomists on the number of species within this group, at least 15 species (as at May 2014) belong to the *xylophilus* group based on the number of lateral lines (Figure 10), the number and position of caudal papillae and spicule characteristics, and a large vulval flap (Gu *et al*., 2005; Ryss *et al*., 2005; Braasch *et al*., 2009; Braasch and Schönfeld, 2013). At least two *Bursaphelenchus* species (*B. trypophloei* Tomalak & Filipiak, 2011 and *B. masseyi* Tomalak, Worrall & Filipiak, 2013) were recently proposed to be added to the *xylophilus* group, ~~but~~ however this protocol follows the last grouping of Braasch and Schönfeld (2013) who did not consider these species to be valid members of the group due to, ~~who left these two species out because of~~ their spicule morphology. Therefore the members of the *xylophilus* group are:	But replaced by 'however' : more correct. Replacement of the sentence 'who left these two species out because of' by a more precise one.	European Union
82.	*85*	Substantive	Change“15 species” to “13 species and 2 Subspecies”The 15 species of the *xylophilus* group can be distinguished from all other *Bursaphelenchus* species by the shape of the male spicules and by the presence in the female of a vulval flap with a characteristic shape. To separate *B. xylophilus* from the 14 other species listed in the group, the female tail shape (subcylindrical to cylindrical with a normally round terminus, and absence of a mucro) can be used. All other species of the *xylophilus* group have either a conical or a mucronate female tail. However, a few mucronate populations of *B. xylophilus* exist in North America and are difficult to differentiate morphologically from other mucronate species (Figure 5). In addition, *B. xylophilus* females from laboratory cultures normally show a typical round tail terminus, whereas strains obtained from infested or artificially inoculated trees may contain females with mucros of variable length beside	2 subspecies were included on the nematodes listed.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			round-tailed females (Figure 5). More details on this subject can be found in Gu *et al.* (2011).		
83.	*88*	Substantive	It is suggested that all the molecular methods in paragraph “4.2 Molecular identification ” should be verified. **4.2 Molecular identification**	Avoid list the unreliable and no extensively used methods on this international standard.	China
84.	*88*	Technical	**4.2 Molecular identification**	horizontal comments The use of positive and negative controls for molecular methods (conventional and real-time PCR) should be mentioned earlier in the text (or at least it should be referred to the section 4.2.6. earlier)	European Union
85.	*91*	Technical	The most recent approach for molecular identification relies on sequencing and barcoding analysis (section 4.2.8). This approach requires access to sequencing facilities and to reliable sequences (such as those found in Q bank) as well as highly skilled staff to analyse the sequences in such a way as to avoid false results.	Access to reliable sequence is equally important and a database based on sequences from reference material is available and was the result of an EU research project Q-bol. A proposal is made to add a reference to this database	European Union
86.	*92*	Editorial	When molecular techniques are used to detect PWN in wood products for quarantine purposes, it is critical to distinguish between living and dead nematodes. Several phytosanitary treatments kill PWN in wood, and current DNA-based detection methods are unable to differentiate whether a positive result is due to living nematodes or DNA remnants of dead nematodes. The use of molecular methods based on RNA that *can* distinguish between living and dead nematodes present in wood is~~are~~ preferable for questions of quarantine regulation (Leal *et al*., 2013) (section 4.2.4). This problem needs to be taken into account when choosing the nematode extraction method (e.g. the Baermann funnel technique relies on living nematodes; see also section 3.5) and the molecular technique for determination. Whenever possible, a positive molecular result should be validated by morphological identification.	Is (singular) necessary	Australia
87.	*92*	Substantive	Change “PWN” to “*B.xylophilus*”.When molecular techniques are used to detect PWN in wood products for quarantine purposes, it is critical to distinguish between living and dead nematodes. Several phytosanitary treatments kill PWN in wood, and current DNA-based detection methods are unable to differentiate whether a	There are two types of Bursaphelenchus xylophilus abbreviation, we suggest to use “B.xylophilus” only.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			positive result is due to living nematodes or DNA remnants of dead nematodes. The use of molecular methods based on RNA that *can* distinguish between living and dead nematodes present in wood are preferable for questions of quarantine regulation (Leal *et al*., 2013) (section 4.2.4). This problem needs to be taken into account when choosing the nematode extraction method (e.g. the Baermann funnel technique relies on living nematodes; see also section 3.5) and the molecular technique for determination. Whenever possible, a positive molecular result should be validated by morphological identification.		
88.	*92*	Substantive	When molecular techniques are used to detect PWN in wood products for quarantine purposes, it is critical to distinguish between living and dead nematodes. Several phytosanitary treatments kill PWN in wood, and current DNA-based detection methods are unable to differentiate whether a positive result is due to living nematodes or DNA remnants of dead nematodes. The use of molecular methods based on RNA that *can* distinguish between living and dead nematodes present in wood are preferable for questions of quarantine regulation (Leal *et al*., 2013) (section 4.2.4). This problem needs to be taken into account when choosing the nematode extraction method (e.g. the Baermann funnel technique relies on living nematodes; see also section 3.5) and the molecular technique for determination. Whenever possible, a positive molecular result should be validated by morphological identification.	It is critical for the NPPO of the importing country to determine whether the nematodes have been found dead in the imported wood due to a successful phytosanitary treatment in the country of origin, or the nematodes died during its extraction for analysis in the importing country. The use of molecular methods based on RNA can distinguish between living and dead nematodes present in wood. In some cases, importing developing countries may have to reject to consignment due to inability to determine whether the samples taken from living or dead nematodes.	Bahrain
89.	*92*	Technical	When molecular techniques are used to detect PWN in wood products for quarantine purposes, it is critical to distinguish between living and dead nematodes. Several phytosanitary treatments kill PWN in wood, and current DNA-based detection methods are unable to differentiate whether a positive result is due to living nematodes or DNA remnants of dead nematodes. The use of molecular methods based on RNA that *can* distinguish between living and dead nematodes present in wood are preferable for questions of quarantine regulation (Leal *et al*., 2013)	Section 3.5 only refers to the detection on nematodes in their vector. Section 3.4 refers to the detection of the nematode in wood sample and the living status of the nematode is also an issue. The addition of a reference to section 3.4 is consequently proposed.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			(section 4.2.4). This problem needs to be taken into account when choosing the nematode extraction method (e.g. the Baermann funnel technique relies on living nematodes; see also sections 3.4 and 3.5) and the molecular technique for determination. Whenever possible, a positive molecular result should be validated by morphological identification.		
90.	*93*	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. ~~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.	Text deleted as per general comment.	Uruguay, Argentina, Chile
91.	*98*	Substantive	• ITS1-forward (F): 5′-CGT AAC AAG GTA GCT GTA G-3′ (Ferris *et al*., 1993) Is it the only ITS primer pairs specific for *B. xylophilus*?	Literature: Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus. Journal of Nanjing Forestry University.31(4):128-132	China
92.	*99*	Substantive	• ITS2-reverse (R): 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain, 1993) Is it the only ITS primer pairs specific for B. xylophilus?	Literature: Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus. Journal of Nanjing Forestry University.31(4):128-132	China
93.	*102*	Editorial	*B. hunanensis* and *B. lini* are proposed to be regrouped and therefore no longer belong to the genus *Bursaphelenchus.* Burgermeister *et al*. (2009) give a comprehensive summary of the patterns and ITS-RFLP~~PL~~ DNA fragment sizes for 44 *Bursaphelenchus* species. An example of species differentiation by ITS-RFLP restriction fragment patterns for *B. xylophilus*, *B. mucronatus mucronatus* and *B. mucronatus kolymensis* isolates is provided in Table 2.	Corrigendum	Japan
94.	*111*	Substantive	• X-F: 5′-ACG ATG ATG CGA TTG GTG AC-3′ Is it the only PCR primer pairs specific for B.	Literature: Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus. Journal of Nanjing Forestry University.31(4):128-132	China

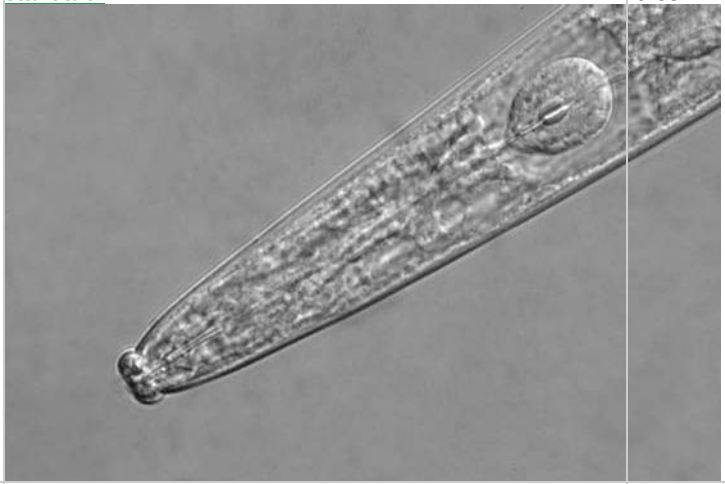
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			xylophilus?		
95.	*112*	Substantive	• X-R: 5′-TAT TGG TCG CGG AAC AAA CC-3′ Is it the only PCR primer pairs specific for B. xylophilus?	Literature: Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus. Journal of Nanjing Forestry University.31(4):128-132	China
96.	*129*	Substantive	BsatF: 5′-TGA CGG AGT GAA TTG ACA AGA CA-3′ Is it the only primer pairs and Taqman prober specific for B. xylophilus?	Literature: Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus. Journal of Nanjing Forestry University.31(4):128-132	China
97.	*130*	Substantive	BSatRV: 5′-AAG CTG AAA CTT GCC ATG CTA AA-3′Is it the only primer pairs and Taqman prober specific for B. xylophilus?	Literature:Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus.Journal of Nanjing Forestry University.31(4):128-132	China
98.	*131*	Substantive	Fluorogenic TaqMan probe BSatS: 5′-FAM-ACA CCA TTC GAA AGC TAA TCG CCT GAG A-TAMRA-3′ Is it the only primer pairs and Taqman prober specific for B.xylophilus?	Literature:Wang Yan, et al. 2007. Comparative analysis on three kinds of molecular detection technique of Bursaphelenchus xylophilus.Journal of Nanjing Forestry University.31(4):128-132	China
99.	*132*	Substantive	PCR mix and cycling parameters for isolated nematodes PCR is carried out in a total volume of 25 µl containing 1 µl genomic DNA. Each reaction contains 2.5 µl of 10× reaction buffer (qPCR Core kit, Eurogentec), 5 mM $MgCl_2$, 200 µM each dNTP, 0.5 U Taq polymerase (qPCR Core kit) and 200 nM each primer and probe. Real-time PCR tests are performed in a DNA Engine Opticon 2 thermal cycler (MJ Research). Cycling parameters are 95 °C for 10 min, followed by 30 cycles of (95 °C for 15 s and 59 °C for 30 s). Data are analysed using the Opticon 2 Monitor software version 3.1 according to the manufacturer's instructions. Extracts are tested undiluted and diluted 1:10 in nuclease-free water. PCR mix and cycling conditions for direct detection wood extract. PCR mix and conditions for direct detection on wood sample as described by François et al. (2007)	The amplification mix and conditions described here only correspond to the case of isolated nematodes. In paragraph 126, it is mentioned that this test can be also used for direct detection on wood sample. For this type of sample, another amplification mix and conditions should be added, as described in Francois et al. (2007).	European Union

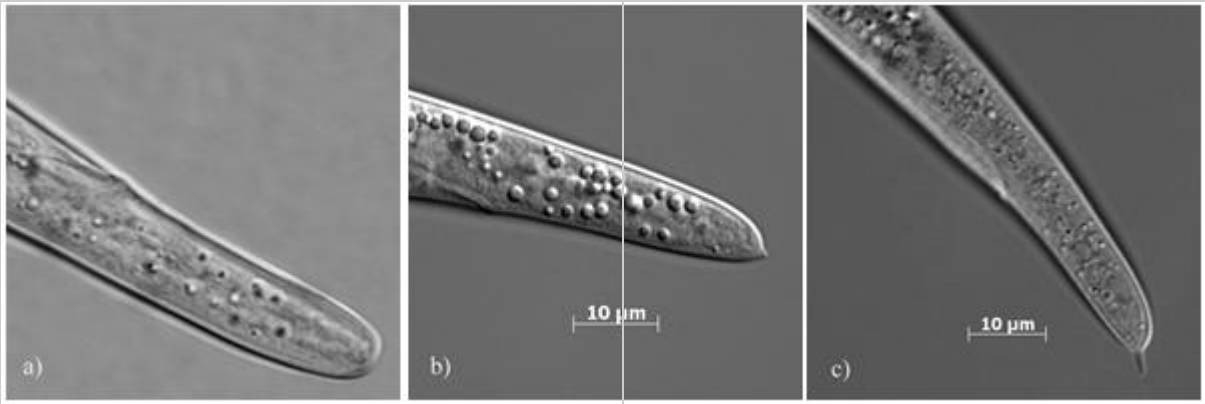
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
100.	*135*	Editorial	For DNA extraction, the method of Burgermeister *et al.* (2005) is used with the following changes: (1) incubation of sample homogenate is at ~~–~~+~~56~~ ºC overnight instead of for 3 h; (2) carrier RNA is used only when DNA is extracted from single nematodes; (3) elution buffer (10 mM Tris-HCl, pH 8.0) is applied to the membrane of the mini-column and incubated for 5 min before centrifugation to elute the sample DNA; and (4) DNA extracts are heated at 55 ºC for 5 min to remove any residual ethanol that could later affect the measurement of DNA quantity and quality and PCR amplification. (5) Samples are eluted in 30 µl (for single nematodes) and 50 µl (for samples containing more than one nematode).	The original paper of Burgermeister et al. (2005) mentions +56°C, not - .	European Union
101.	*140*	Technical	(lower case letters indicate the locked nucleic acids)	The indication of locked nucleic acids (LNA) is confusing (it is difficult to know where they start and end). Consider to use another indication (such as {}] and also verify in the original publication if the LNA are corrected indicated.	European Union
102.	*141*	Technical	~~The following internal control primers (see section 4.2.1) can be included to ensure the test performs as expected:~~	This paragraph is probably not needed, as paragraph 145 provides elements to verify the quality of the genomic DNA. Paragraphs 141-142-143 are redundant with paragraph 145.	European Union
103.	*142*	Technical	• ~~ITS1-F: 5′-CGT AAC AAG GTA GCT GTA G-3′~~	This paragraph is probably not needed, as paragraph 145 provides elements to verify the quality of the genomic DNA. Paragraphs 141-142-143 are redundant with paragraph 145.	European Union
104.	*143*	Technical	• ~~ITS2-R: 5′-TTT CAC TCG CCG TTA CTA AGG-3′~~	This paragraph is probably not needed, as paragraph 145 provides elements to verify the quality of the genomic DNA. Paragraphs 141-142-143 are redundant with paragraph 145.	European Union
105.	*147*	Technical	The following tests detect only living nematodes. Options are given for conventional and real-time RT PCR.	Insert “reverse transcriptase (RT)” or “RT” before PCR.	European Union
106.	*149*	Editorial	A conventional reverse transcription ~~(~~RT~~)~~-PCR method for the detection of living PWN based on a *hsp70* gene sequence was described by Leal *et al*. (2013). In this test, the forward and reverse primers are placed on either side of the *hsp70* intron so that genomic DNA can be easily differentiated from cDNA by amplicon size. Its specificity was evaluated against six non-target *Bursaphelenchus*	Delete brackets (RT)-PCR.	European Union

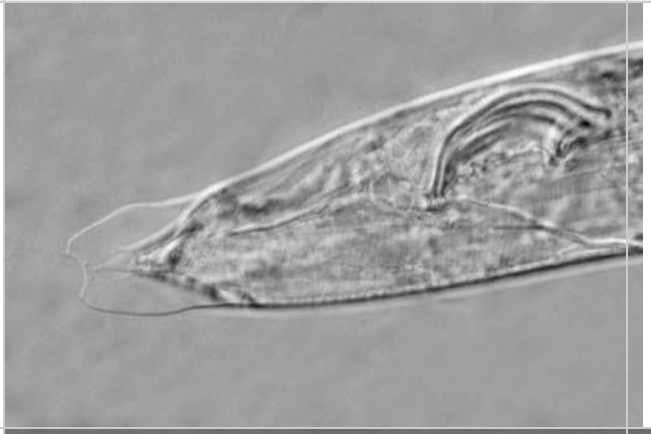
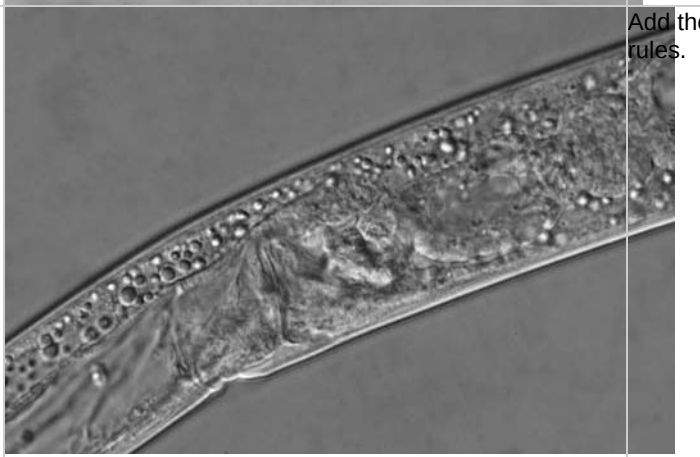
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			species and six isolates of *B. xylophilus*. The limit of detection of this test is 0.4 nematodes per reaction, measured in three of three replicates.		
107.	*154*	Technical	The following control primers target the actin gene and can be included to ensure the test performs as expected when testing isolated gDNA. They produce an amplicon of 228 bp:	It is also important to mention here the control for the RT-reaction (i.e. a PCR reaction with no reverse transcriptase), especially when the objective is to detect living organism. It is suggested to add as a control the RNA solution provided with the kit.	European Union
108.	*165*	Technical	The following internal control primers may be included to ensure the test performs as expected:	It is also important to mention here the control for the RT-reaction (i.e. a PCR reaction with no reverse transcriptase), especially when the objective is to detect living organism. It is suggested to add as a control the RNA solution provided with the kit.	European Union
109.	*199*	Technical	**4.2.8 Sequencing**	New paragraph added the current paragraph does not provide enough guidance or indications about the limits of barcoding, how to use it etc. There is guidance provided in the Q-bank database on sequencing including a molecular decision scheme. Sequences generated in Q-bank have been generated from reference material from reference collections. A new paragraph is suggested.	European Union
110.	*200*	Technical	Several genomic regions have been directly sequenced from isolated nematodes (single for Wu *et al*. (2013) or bulk from cultures on fungus for Ye *et al.* (2007)) for the purpose of species identification of *B. xylophilus* and differentiation of different *Bursaphelenchus* spp. These regions include internal transcribed spacers (ITS-1, ITS-2, 5.8S) of ribosomal DNA (Abelleira *et al*., 2011; Wu *et al*., 2013) or the D2-D3 region of the 28S rRNA gene (Ye *et al.*, 2007). The targeted region is amplified by PCR, and the amplicons are sequenced either directly or after they are cloned. Sequence data can then be analysed using the Basic Local Alignment Search Tool (BLASTN) available at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and compared with *Bursaphelenchus* sequences available in the NCBI database (e.g. accession numbersHQ646254 and KC460340 for the above-mentioned ITS region and AY508105 to AY508109 for the 28S rRNA region). For the ITS gene, if the sample's pairwise sequence divergence	Regarding the sentence "For the 28S gene, if the sample's pairwise sequence divergence compared with known B. xylophilus sequences is less than 0.5% but more than 0.5% with all other species, it is identified as B. xylophilus. " : Can such a strong statement be made for databases for which there is no requirements of confirmation of the identity of the specimen from which sequences were generated ?	European Union

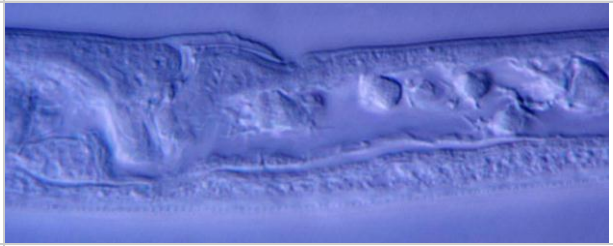
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			compared with known *B. xylophilus* sequences is less than 2% but more than 2% with all other species, it is identified as *B. xylophilus*. For the 28S gene, if the sample's pairwise sequence divergence compared with known *B. xylophilus* sequences is less than 0.5% but more than 0.5% with all other species, it is identified as *B. xylophilus*. Any other results should be further investigated. Guidance on sequencing as well as a molecular decision scheme are available in the Q-bank database (http://www.q-bank.eu/Nematodes/). The Q-bank database generated from reference material from reference collections.		
111.	*214*	Substantive	1.Add Jianjun Ge as another Chinese *Bursaphelenchus xylophilus*. expert （Jianjun Ge, jianjun11@163.com). 2.Delete "Taiwan".In addition to the experts mentioned above, regional experts on this nematode are listed in Table 3. **Table 3.** List of regional and national experts on *Bursaphelenchus xylophilus* (not exhaustive) **Region or country** **Africa** **Australia**	Taiwan belongs to China.	China

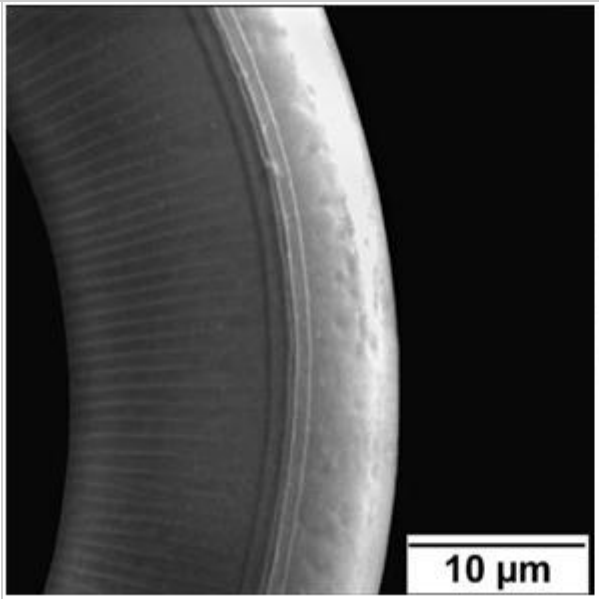
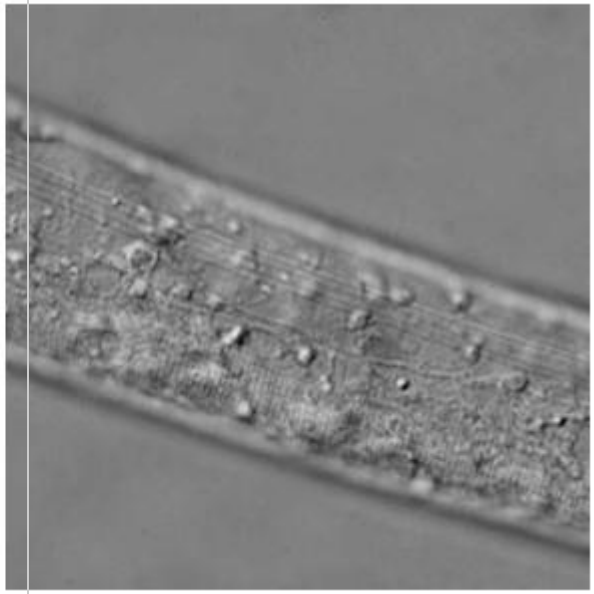
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			China	Department of Forest Protection, Nanjing Forestry University, No. 159 Longpan Road, Nanjing, 210037 China (Boguang Zhao; e-mail: 13505186675@126.com).	
			European Union	NemaLab-ICAM, Departamento Biologia, Universidade de Évora, 7002-554 Évora, Portugal (Manuel Mota; e-mail: mmota@uevora.pt).	
			Japan	Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan (Mitsuteru Akiba; e-mail: akiban@ffpri.affrc.go.jp).	
			Republic of Korea (South Korea)	Division of Forest Insect Pests and Disease, Korea Forest Research Institute (207 Cheongnyangni 2-dong, Dongdaemun-gu, Seoul 130-712, Korea (ROK) (Hyerim Han; e-mail: hrhan@forest.go.kr).	
			Russia	To be added at a later stage	
			South America	To be added at a later stage	
			Taiwan	To be added at a later stage	
			United States	To be added at a later stage	
112.	*260*	Technical	**Kishi, Y.** 1995. *The pine wood nematode and the Japanese pine sawyer.* Forest Pests in Japan No. 1. Tokyo, Thomas Company Limited. 302 pp. Kondo. E. & Ishibashi. N. 1978. Ultrastructural differences between the propagative and dispersal forms in the pine wood nematode. *Bursaphelenchus lignicolus* with reference to the survival. Appl. Ent. Zool. 13: 1-11.	nex reference added	European Union
113.	*272*	Editorial	**Nickle, W.R., Golden, A.M., Mamiya, Y. & Wergin, W.P.** 1981. On the taxonomy and morphology of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle ~~WR~~ (1970). *Journal of Nematology,* 13~~2~~: 385–392.	Corrigendum	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
114.	*296*	Technical	**Figure 1.** Life cycle of *Bursaphelenchus xylophilus* from egg to adult nematodes.	Replace L by J in the figure.	European Union
115.	*297*	Technical	~~L~~J$_x$, ~~larvae~~juvenile of x stage.	Terminology issue.	European Union
116.	*300*	Technical	**Figure 2.** *Bursaphelenchus xylophilus*: (A) female; (B) male; (C) male tail; (D) ventral view of male tail, tip with ~~caudal alae~~bursa; (E) ventral view of spicules; (F) female, anterior portion; (G) female vulva; and (H), (I) and (J) female tail.	Terminology issue	European Union
117.	*305*	Editorial	Add the scales of the photo in this standard. 	Add the scales in the photo according to the publication rules.	China
118.	*306*	Technical	**Figure 4.** *Bursaphelenchus xylophilus* anterior region with stylet and ~~median bulb~~metacorpus.	Terminology issue.	European Union
119.	*308*	Editorial	Add the scales of the photo in this standard.	Add the scales in the photo according to the publication rules.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
					
120.	*311*	Editorial	Add the scales of the photo in this standard. 	Add the scales in the photo according to the publication rules.	China
121.	*314*	Editorial	Add the scales of the photo in this standard.	Add the scales in the photo according to the publication rules.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
					
122.	*317*	Editorial	 Add the scales of the photo in this standard.	Add the scales in the photo according to the publication rules.	China
123.	*320*	Editorial	**Add the scales of the photo in this standard.A**	Add the scales in the photo according to the publication rules.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
					
124.	*321*	Editorial	Add the scales of the photo in this standard.**B** 	Add the scales in the photo according to the publication rules.	China
125.	*324*	Editorial	Add the scales of the photo in this standard.	Add the scales in the photo according to the publication rules.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			 		
126.	*330*	Editorial	Add the scales of the photo in this standard.	Add the scales in the photo according to the publication rules.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country