2004-017: Draft Annex to ISPM 27:2006 – *Ditylenchus dipsaci* and *Ditylenchus destructor*

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
1.	*G*	Substantive	I support the document as it is and I have no comments		Lao People's Democratic Republic, Georgia, Jamaica, United States of America, Dominica, Mexico, Barbados, New Zealand, Ghana, Korea, Republic of, OIRSA, Malawi, Burundi, Belize, Gabon
2.	*G*	Substantive	1. There are repeated content in the description of hosts of section 1 and section 3.1. And the part of sympotom of section 3.1 may be simplified. 2. The description for extraction methods of the section 3.2.2 may be simplified, and only the name of extraction and the document may be listed .	1.There are repeated content in the two section. 2.Simplify the section 3.2.2.	China
3.	*G*	Substantive	Could it be possible to add some contents such as; • the infection process where the juveniles live in plant parts • the environmental conditions favorable for nematode infection and survival	It's useful for pest information.	Thailand
4.	*G*	Substantive		Canada has reviewed the document and agrees with its content.	Canada
5.	*G*	Technical	Include references for authors Filipjev, 1936 and Kuhn, 1857 Filipjev, I.N. 1936. On the classification of the Tylenchinae. *Proc. Helminth. Soc. Wash*. 3, 80-82. Kuhn, J. 1857. Ueber das vorkmmen von Anguillulen in erkrankten Bluhtenkopfen von dipsacus fullonum *L. z. wiss. Zool*. 9, 129-137.	Need a reference for author of species.	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
6.	5	Editorial	Most nematode species within the large genus *Ditylenchus* Filipjev, 1936 are mycetophagous and have a worldwide distribution. However, the genus contains a few species that are of great importance as ~~parasites~~ pests of higher plants. It is worth mentioning that though there are certain plants (e.g. beets, lucerne, clover) that are affected by both *Ditylenchus dipsaci* and *Ditylenchus destructor*, the two species never occur together in the same plant (Andrássy and Farkas, 1988).	If the term "parasites" is used in its general meaning and not in its biological meaning. This also applies to paragraph 9.	EPPO, European Union, Georgia, Serbia
7.	5	Technical	Most nematode species within the large genus *Ditylenchus* Filipjev, 1936 are mycetophagous and and *Ditylenchus* species are distributed worldwide~~have a worldwide distribution~~. However, the genus contains a few species that are of great importance as parasites of higher plants (Sturhan and Brzeski, 1991). It is worth mentioning that though there are certain plants (e.g. beets, lucerne, clover) that are affected by both *Ditylenchus dipsaci* and *Ditylenchus destructor*, the two species rarely~~never~~ occur together in the same plant (Andrássy and Farkas, 1988).	1) For most species described within this genus the distribution is unknown. However, we do know that Ditylenchus species are found throughout the world 2. Reference added. 3.The two species are sometimes found together on the same host plant in intensive production systems.	EPPO, European Union, Georgia, Serbia
8.	7	Editorial	*D. dipsacisensu lato* (s.l.), or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphologial, biochemical, molecular and karyological analyses of different populations and races of the *D. dipsaci* s.l. have suggested that it is a species complex of at least 30 host races, with limited host ranges. Jeszke *et al*. (2013) divided this complex into two groups, the first containing diploid populations characterized by their “normal” size and named *D. dipsaci sensu stricto* (s.s.). This group comprises most of the populations recorded so far. The second group is polyploidal and currently comprises *Ditylenchus gigas*Vovlas *et al.*~~, Troccoli, Palomares-Rius, De Luca, Liebanas, Landa, Subbotin and Castillo~~, 2011 (the “giant race” of *D. dipsaci* parasitizing *Vicia faba*); *D. weischeri* Chizhov *et al.*~~, Borisov and Subbotin~~, 2010 (parasitizing *Cirs~~c~~ium arvense* (creeping thistle)); and three undescribed *Ditylenchus* spp. called D, E and F, which are associated with plant species of the Fabaceae, Asteraceae and Plantaginaceae respectively (Jeszke *et al.*, 2013). Of all these species only *D. dipsaci* s.s. and its morphologically larger variant *D. gigas* are ~~plant~~ pests of economic importance. ~~This protocol therefore covers *D. dipsaci* s.s. and presents *D. gigas* separately.~~This protocol includes information to distinguish *D. dipsaci* s.s. and *D. gigas*.	1. and 2. Clearer. The other authors are given in the two references (paragraphs [279] and [221]). 3. A misspelled name (Cirsium) 4.Last but one sentence: "plant pests" to be replaced by "pests" (cf. ISPM 5). 5. We question whether s. l. and s.s. should be in italic (latin) 6. Last sentence modified for clarity.	EPPO, European Union, Georgia, Serbia
9.	7	Editorial	*D. dipsacisensu lato* (s.l.), or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphologi~~c~~al, biochemical, molecular and karyological analyses of different populations and races of the *D. dipsaci* s.l. have suggested that it is a species	Spelling mistake	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			complex of at least 30 host races, with limited host ranges. Jeszke *et al*. (2013) divided this complex into two groups, the first containing diploid populations characterized by their “normal” size and named *D. dipsaci sensu stricto* (s.s.). This group comprises most of the populations recorded so far. The second group is polyploidal and currently comprises *Ditylenchus gigas*Vovlas, Troccoli, Palomares-Rius, De Luca, Liebanas, Landa, Subbotin and Castillo, 2011 (the “giant race” of *D. dipsaci* parasitizing *Vicia faba*); *D. weischeri* Chizhov, Borisov and Subbotin, 2010 (parasitizing *Circium arvense* (creeping thistle)); and three undescribed *Ditylenchus* spp. called D, E and F, which are associated with plant species of the Fabaceae, Asteraceae and Plantaginaceae respectively (Jeszke *et al.*, 2013). Of all these species only *D. dipsaci* s.s. and its morphologically larger variant *D. gigas* are plant pests of economic importance. This protocol therefore covers *D. dipsaci* s.s. and presents *D. gigas* separately.		
10.	*7*	Substantive	*simplify the content.* *D. dipsacisensu lato* (s.l.), or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphologial, biochemical, molecular and karyological analyses of different populations and races of the *D. dipsaci* s.l. have suggested that it is a species complex of at least 30 host races, with limited host ranges. Jeszke *et al*. (2013) divided this complex into two groups, the first containing diploid populations characterized by their “normal” size and named *D. dipsaci sensu stricto* (s.s.). This group comprises most of the populations recorded so far. The second group is polyploidal and currently comprises *Ditylenchus gigas*Vovlas, Troccoli, Palomares-Rius, De Luca, Liebanas, Landa, Subbotin and Castillo, 2011 (the “giant race” of *D. dipsaci* parasitizing *Vicia faba*); *D. weischeri* Chizhov, Borisov and Subbotin, 2010 (parasitizing *Circium arvense* (creeping thistle)); and three undescribed *Ditylenchus* spp. called D, E and F, which are associated with plant species of the Fabaceae, Asteraceae and Plantaginaceae respectively (Jeszke *et al.*, 2013). Of all these species only *D. dipsaci* s.s. and its morphologically larger variant *D. gigas* are plant pests of economic importance. This protocol therefore covers *D. dipsaci* s.s. and presents *D. gigas* separately.	simplify the content.	China
11.	*7*	Technical	*D. dipsacisensu lato* (s.l.), or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphologial, biochemical, molecular and karyological analyses of different populations and races of the *D. dipsaci* s.l. have suggested that it is a species complex of at least 30 host races, with limited host ranges. Jeszke *et al*. (2013)	We question the reference in the third sentence to ’a species complex of at least 30 host races’. We suggest there is either a complex of different closely related species or host races, within a species, not both.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			divided this complex into two groups, the first containing diploid populations characterized by their “normal” size and named *D. dipsaci sensu stricto* (s.s.). This group comprises most of the populations recorded so far. The second group is polyploidal and currently comprises *Ditylenchus gigas*Vovlas, Troccoli, Palomares-Rius, De Luca, Liebanas, Landa, Subbotin and Castillo, 2011 (the “giant race” of *D. dipsaci* parasitizing *Vicia faba*); *D. weischeri* Chizhov, Borisov and Subbotin, 2010 (parasitizing *Circium arvense* (creeping thistle)); and three undescribed *Ditylenchus* spp. called D, E and F, which are associated with plant species of the Fabaceae, Asteraceae and Plantaginaceae respectively (Jeszke *et al.*, 2013). Of all these species only *D. dipsaci* s.s. and its morphologically larger variant *D. gigas* are plant pests of economic importance. This protocol therefore covers *D. dipsaci* s.s. and presents *D. gigas* separately.		
12.	*8*	Editorial	*D. dipsaci* lives mostly as an endoparasite in aerial parts of plants (stems, leaves and flowers), but also attacks bulbs, tubers and rhizomes. This nematode is seed-borne in *V. faba* (broad bean), *Medicago sativa* (lucerne/alfafa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon) (Sikora *et al*., 2005; Sousa *et al*., 2003). Of great importance is the fact that the fourth stage juvenile can withstand desiccation for a long time, sometimes 20 years or more (Barker and Lucas, 1984). These nematodes clump together in a cryptobiotic state to form “nematode wool” when the plant tissue begins to dry (Figure 1). The wool can often be observed on the seeds in heavily infested pods and in dry plant debris. The presence of the infective fourth stage juveniles in seed and dry plant material is important in the passive dissemination of the nematode over long distances. The nematode in its desiccated state can survive passage through pigs and cattle on or in infected seed (Palmisano *et al.*, 1971).	On or in infested seeds	EPPO, European Union, Georgia, Serbia
13.	*8*	Substantive	*simplify the content.* *D. dipsaci* lives mostly as an endoparasite in aerial parts of plants (stems, leaves and flowers), but also attacks bulbs, tubers and rhizomes. This nematode is seed-borne in *V. faba* (broad bean), *Medicago sativa* (lucerne/alfafa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon) (Sikora *et al*., 2005; Sousa *et al*., 2003). Of great importance is the fact that the fourth stage juvenile can withstand desiccation for a long time, sometimes 20 years or more (Barker and Lucas, 1984). These nematodes clump together in a cryptobiotic state to form “nematode wool” when the plant tissue begins to dry (Figure 1). The wool can often be observed on the seeds in heavily infested pods and in dry plant debris. The presence of the infective fourth stage juveniles in seed and dry plant material is important in the passive dissemination of the nematode over long distances. The nematode in its desiccated state can survive passage	simplify the content.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			through pigs and cattle on infected seed (Palmisano *et al.*, 1971).		
14.	*8*	Technical	*D. dipsaci* lives mostly as an endoparasite in aerial parts of plants (stems, leaves and flowers), but also attacks bulbs, tubers and rhizomes. This nematode is seed-borne in *V. faba* (broad bean), *Medicago sativa* (lucerne/alfafa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon) (Sikora *et al*., 2005; Sousa *et al*., 2003). Of great importance is the fact that the fourth stage juvenile can withstand desiccation for a long time, sometimes 20 years or more (Barker and Lucas, 1984). These nematodes clump together in a cryptobiotic state to form “nematode wool” when the plant tissue begins to dry (Figure 1). The wool can often be observed on the seeds in heavily infested pods and in dry plant debris, e.g. remaining in the field after harvest. The presence of the infective fourth stage juveniles in seed and dry plant material is important in the passive dissemination of the nematode over long distances. The nematode in its desiccated state can survive passage through pigs and cattle on infected seed (Palmisano *et al.*, 1971).	1. More detailed explanation.	EPPO, European Union, Georgia, Serbia
15.	*9*	Substantive	Simplify the content. Although *D. dipsaci* is seen as a parasite of higher plants, Viglierchio (1971) reported that a Californian population of *D. dipsaci* from *Allium sativum* (garlic) could reproduce on soil fungi (*Verticilium* and *Cladosporium*) under laboratory conditions and Paesler (1957) stated that the nematode is of potential economic importance on *Agaricus bisporus* (mushroom).	Simplify the content.	China
16.	*9*	Technical	Although *D. dipsaci* is seen as a parasite of higher plants, Viglierchio (1971) reported that a Californian population of *D. dipsaci* from *Allium sativum* (garlic) could reproduce on soil fungi (*Verticilium* and *Cladosporium*) under laboratory conditions ~~and Paesler (1957) stated that the nematode is of potential economic importance on *Agaricus bisporus* (mushroom)~~.	We believe that in 1957 material from mushrooms was described as D. myceliophagus	EPPO, European Union, Georgia, Serbia
17.	*13*	Editorial	*Ditylenchus destructor*, or potato rot nematode, attacks almost exclusively the subterranean parts of plants (e.g. tubers, rhizomes and stem-like underground parts). It is a near-cosmopolitan species, common in temperate regions and responsible for severe losses in potato and hop production (EPPO, 2013a). The host range of the nematode is extensive, comprising more than 90 plant species, which include ornamental plants, crop plants and weeds. *Solanum tuberosum* (potato*)* is the principal host, the tubers developing wet or dry rot that will spread to other tubers in storage. Under certain conditions, wet rot organisms may damage the tubers extensively, but will also kill the nematodes. *D. destructor* can survive only when dry rot organisms invade the tuber. Rojancovski and Ciurea (1986) found 55 species of bacteria and fungi associated with *D. destructor* in	A missing word.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			S. tuberosum tubers, with *Fusarium* spp. being the most common.		
18.	*14*	Editorial	Other common hosts are *Ipomoea batatas* (sweet potato), bulbous iris (hybrids and selections derived from *Iris xiphium* and *Irus xiphioides*), *Taraxacum officinale* (dandelion), *Humulus lupulus* (hop), *Tulipa* spp. (tulip), *Leopoldia comosa* (hyacinth), *Gladiolus* spp. (gladiolus), *Dahlia* spp. (dahlia), *Coronilla varia* and *Anthyllis vulneraria* (vetch), *Beta vulgaris* (sugar beet), *Calendula officinalis* (marigold), *Daucus carota* (carrot), *Petroselinum crispum* (parsley) and *Trifolium* spp. (red, white and alsike clover) (Sturhan and Brzeski, 1991). In the absence of higher plants, *D. destructor* reproduces readily on the mycelia of about 70 species of fungi and it is known to destroy the hyphae of cultivated mushroom (Sturhan and Brzeski, 1991). The species is able to survive dessication and low temperatures, but does not form nematode wool as does *D. dipsaci* (Kühn, 1857) Filipjev, 1936. This species, however, overwinters asin eggs, which makes eggs more vital in *D. destructor* than in *D. dipsaci*. *D. destructor* in seed potatoes and flower bulbs is on the list of quarantine pests of many countries and organizations (Sturhan and Brzeski, 1991). *D. destructor* was reported on *Arachis hypogaea* (groundnut/peanut) in South Africa, but these records are now considered to be a separate species, *Ditylenchus africanus* Wendt *et al.*, Swart, Vrain and Webster, 1995, which is morphologically and morphometrically close to *D. destructor*.	1) More correct 2) Clearer. The other authors are given in the reference (cf. paragraph [283]).	EPPO, European Union, Georgia, Serbia
19.	*14*	Technical	Other common hosts are *Ipomoea batatas* (sweet potato), bulbous iris (hybrids and selections derived from *Iris xiphium* and *Irus xiphioides*), *Taraxacum officinale* (dandelion), *Humulus lupulus* (hop), *Tulipa* spp. (tulip), *Leopoldia comosa* (grape hyacinth), *Hyacinthus orientalis* (hyacinth), *Gladiolus* spp. (gladiolus), *Dahlia* spp. (dahlia), *Coronilla varia* and *Anthyllis vulneraria* (vetch), *Beta vulgaris* (sugar beet), *Calendula officinalis* (marigold), *Daucus carota* (carrot), *Petroselinum crispum* (parsley) and *Trifolium* spp. (red, white and alsike clover) (Sturhan and Brzeski, 1991). In the absence of higher plants, *D. destructor* reproduces readily on the mycelia of about 70 species of fungi and it is known to destroy the hyphae of cultivated mushroom (Sturhan and Brzeski, 1991). The species is able to survive dessication and low temperatures, but does not form nematode wool as does *D. dipsaci* (Kühn, 1857) Filipjev, 1936. This species, however, overwinters in eggs, which makes eggs more vital in *D. destructor* than in *D. dipsaci*. *D. destructor* in seed potatoes and flower bulbs is on the list of quarantinea regulated pests of in many countries and organizations (Sturhan and Brzeski, 1991). *D. destructor* was reported on *Arachis hypogaea* (groundnut/peanut) in South Africa, but these records are now considered to be a separate species, *Ditylenchus africanus* Wendt, Swart, Vrain and Webster, 1995, which is morphologically and morphometrically close to *D. destructor*.	1. It is better to call it grape hyacinth to prevent confusion with the ordinary hyacinth, which has also been added. 2. It's the countries who determine the lists of regulated pests.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
20.	*18*	Editorial	**Synonyms:** Synonyms of the type species *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 are listed ~~described~~ in Siddiqi (2000)	More correct.	EPPO, European Union, Georgia, Serbia
21.	*21*	Editorial	Note: *Ditylenchus dipsaci* is now~~has come to be~~ considered a species complex composed of a great number of biological races and populations differing mainly in host preference. Consequently a total of 13 nominal species have been synonymized with *D. dipsaci* and up to 30 biological races have been differentiated, mainly distinguished by host range and generally named after their principal host plant.	More correct.	EPPO, European Union, Georgia, Serbia
22.	*23*	Technical	**Synonyms:** None ~~used in recent years (Sturhan and Brzeski, 1991)~~	There are no synonyms for this species.	EPPO, European Union, Georgia, Serbia
23.	*28*	Technical	D. *dipsaci* and D. *destructor* both have the following common symptoms that allow their detection: swelling, distortion, discoloration and stunting of the above-ground plant parts, and necrosis or rotting of the bulbs and tubers (Thorne, 1945).	Add citation. Add to reference list too.) Thorne, G. 1945. Ditylenchus destructor, n. sp., the potato rot nematode, and Ditylenchus dipsaci (Kuhn, 1857) Filipjev, 1936, the teasel nematode (Nematoda: Tylenchidae). Proceedings of the Helminthological Society of Washington 12: 27-34	Australia
24.	*30*	Editorial	~~Common symptoms of *D. dipsaci* infestation are swelling, distortion, discoloration and stunting of above-ground plant parts, and necrosis and rotting of bulbs and tubers.~~ *D. dipsaci* shows parasitic adaptation in its ability to invade solid parenchyma tissue following enzymatic lysis of the pectic or middle lamella layer between adjacent cell walls, leading to separation and rounding of the cells. This causes the typical glistening appearance or mealy texture of infested tissues, reminiscent of the flesh of an over-ripe apple (Southey, 1993).	The deleted sentence gives the same information as paragraph [28].	EPPO, European Union, Georgia, Serbia
25.	*31*	Editorial	According to Vovlas *et al.* (2011), *D. gigas* (giant stem and bulb nematode) infestation of *V. faba* causes swelling and deformation of stem tissue or lesions, which turn reddish-brown then black. In severe infestations the seeds appear dark, distorted and smaller in size that uninfested seeds, and they have speckle-like spots on the surface. Hosts other than *V. faba* are *Lamium purpureum*, *Lamium album*, *Lamium amplexicaule*, *Ranunculus arvensis*, *Convolvulus arvensis* and *Avena sterilis.*	1. Clearer with an added comma in the first sentence.	EPPO, European Union, Georgia, Serbia
26.	*33*	Editorial	*D. destructor* commonly infects the underground parts of plants (tubers and stolons of potato, bulbs of lilies, rhizomes of mint, and roots of hop and lilac), causing discoloration and rotting of plant tissue. The above-ground parts are sometimes also infected, causing dwarfing, thickening and branching of the stem	Last but one sentence: replace the full stop by a comma after "can".	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			and dwarfing, curling and discoloration of the leaves (e.g. in potato) (Sturhan and Brzeski, 1991). More often, however, no symptoms of infection are found in the above-ground parts of plants. *D. africanus*, which infects groundnut in southern Africa, is morphologically very similar to *D. destructor*. It can~~.~~, however, be separated from *D. destructor* by a combination of morphological and molecular characteristics, which are presented in sections 4.1, 4.2 and Tables 2 and 3.For *D. africanus* symptoms on groundnut, see McDonald *et al*. (2005).		
27.	*33*	Technical	*D. destructor* commonly infects the underground parts of plants (tubers and stolons of potato, ~~bulbs of lilies,~~ rhizomes of mint, and roots of hop and lilac), causing discoloration and rotting of plant tissue. The above-ground parts are sometimes also infected, causing dwarfing, thickening and branching of the stem and dwarfing, curling and discoloration of the leaves (e.g. in potato) (Sturhan and Brzeski, 1991). More often, however, no symptoms of infection are found in the above-ground parts of plants. ~~*D. africanus*, which infects groundnut in southern Africa, is morphologically very similar to *D. destructor*. It can. however, be separated from *D. destructor* by a combination of morphological and molecular characteristics, which are presented in sections 4.1, 4.2 and Tables 2 and 3.For *D. africanus* symptoms on groundnut, see McDonald *et al*. (2005).~~	1. Lily bulbs are not to our knowledge hosts of D. destructor. Lilium is also not mentioned as host for D. destructor in this draft. 2. It is recommended to delete the entire information about D. africanus in this paragraph. The morphological features are already covered in Tables 2 and 3, so no need for it here.	EPPO, European Union, Georgia, Serbia
28.	*35*	Editorial	According to Sturhan and Brzeski (1991), the principal hosts of *D. dipsaci* are Gramineae: *Avena sativa*, *Secale cereale* (rye), *Zea mays* (maize), *Triticum aestivum* (wheat); Liliaceae: *A. cepa*, *A. sativum*, *Tulipa* spp.; Leguminosae: *Medicago sativa*, *Vicia* spp., *Pisum sativum*, *Trifolium* spp.; Solanaceae: *Solanum tuberosum*, *Nicotiana* spp.; Cruciferae: *Brassica campestris*; and Amarilidaceae: *Narcissus* spp. Other hosts include *D. carota*, *Fragaria* spp. (strawberry), *B. vulgaris*, *Malus domestica* (apple) and *Prunus p~~é~~ersica* (peach) in nurseries, *Hyacinthus orientalis*, *Allium ampeloprasum* (leek), *Phlox drummondii*, *Phlox paniculata*, *Dianthus* spp. (carnation), *Apium graveolens* (celery), *Hydrangea* spp., *Lens culinaris* (lentil), *Brassica napus* (rape), *Petroselinum crispum* and *Helianthus annuus* (sunflower). Various generations of *D. dipsaci* may be present in a host plant during a season, following each other. If affected parts of the plant die due to injuries by the pest, nematodes leave the host before it dies completely. When lacking host plants, the nematodes can enter~~introduce themselves~~ into non-host plants and feed there for a certain time, though they are unable to reproduce in non-host plants (Andrássy and Farkas, 1988).The most common symptoms of *D. dipsaci* infestation are stunted, chlorotic plants; thickened, stunted, gall-containing and distorted stems, petioles and flowers; and necrotic lesions in and rotting of bulbs and rhizomes. *D. dipsaci* may also infest seeds, from, for example, *Phaseolus vulgaris*, *V. faba* and *Allium* spp. Small seeds generally show no visible symptoms of infestation but larger seeds may have a	1. "pérsica" to be replaced by "persica". 2. Simpler language.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			shrunken skin with discoloured spots.		
29.	*35*	Technical	According to Sturhan and Brzeski (1991), the principal hosts of *D. dipsaci* are Gramineae: *Avena sativa*, *Secale cereale* (rye), *Zea mays* (maize), *Triticum aestivum* (wheat); Liliaceae: *A. cepa*, *A. sativum*, *Tulipa* spp.; Leguminosae: *Medicago sativa*, *Vicia* spp., *Pisum sativum*, *Trifolium* spp.; Solanaceae: *Solanum tuberosum*, *Nicotiana* spp.; Cruciferae: *Brassica campestris*; and Amarilidaceae: *Narcissus* spp. Other hosts include *D. carota*, *Fragaria* spp. (strawberry), *B. vulgaris*, *Malus domestica* (apple) and *Prunus pérsica* (peach) in nurseries, *Hyacinthus orientalis*, *Allium ampeloprasum* (leek), *Phlox drummondii*, *Phlox paniculata*, *Dianthus* spp. (carnation), *Apium graveolens* (celery), *Hydrangea* spp., *Lens culinaris* (lentil), *Brassica napus* (rape), *Petroselinum crispum* and *Helianthus annuus* (sunflower). Various generations of *D. dipsaci* may be present in a host plant during a season, following each other. If affected parts of the plant die due to injuries by the pest, nematodes leave the host before it dies completely. When lacking host plants, the nematodes can introduce themselves into non-host plants and feed there for a certain time, though they are unable to reproduce in non-host plants (Andrássy and Farkas, 1988).The most common symptoms of *D. dipsaci* infestation are stunted, chlorotic plants; thickened, stunted, gall-containing and distorted stems, petioles and flowers; and necrotic lesions in and rotting of bulbs and rhizomes, often appearing as brown rings when bulbs are sliced. *D. dipsaci* may also infest seeds, from, for example, *Phaseolus vulgaris*, *V. faba*, ~~and~~ *Allium* spp. and Medicago sativa. Small seeds generally show no visible symptoms of infestation but larger seeds may have a shrunken skin with discoloured spots.	1. In the second sentence, we would like to know what information there is to show that Malus (and Prunus) are hosts for D. dipsaci ? We do not have any evidence and if it is an occasional finding it should not be mentioned. If it is important, then also describe symptoms in the section below and refer to literature. 2. Specific symptom in bulbs: infested bulb have brown rings when sliced 3. Another host species added.	EPPO, European Union, Georgia, Serbia
30.	*36*	Editorial	**3.1.1.1 Symptoms specific to Gramineae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
31.	*39*	Editorial	**3.1.1.2Symptoms specific to Liliaceae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
32.	*40*	Editorial	***Allium cepa*, *Allium sativum* and *Allium cepa* var. *aggregatum* (shallot):** It is characteristic in most *Allium* spp. that leaves and bulbs become deformed on infestation with *D. dipsaci* (Figures 2 and 3). The base of young plants becomes swollen and leaves become distorted. Older infected bulbs show swelling (bloat) of scales with open cracks often occurring at the root disc of the bulbs (Potter and Olthof, 1993). *A. cepa* attacked by *D. dipsaci* have a frosted appearance caused by the dissolution of cells that results from nematode feeding (Ferris and Ferris, 1998). Infested bulbs tend to rot readily in storage (Bridge and Hunt, 1986). The	Cf. paragraph [51].	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			inner scales of the bulb are usually more severely attacked than the outer scales. As the season advances the bulbs become soft and when cut open show browning of the scales in concentric circles. Conversely, *D. dipsaci* does not induce deformation of leaves or swelling in*A. sativum*, but does cause leaf yellowing and death (Netscher and Sikora, 1990). Mollov *et al.* (2012) reported *D. dipsaci* for the first time from *A. sativum* in Minnesota, USA. The symptoms of the above-ground plant were stunting and chlorosis, while the symptoms of the bulbs were necrosis, underdevelopment and distortion. *Allium* spp. may have foliar spickels (*i.e.* blister-like swellings on the leaves).		
33.	*40*	Technical	***Allium cepa*, *Allium sativum* and *Allium cepa* var. *aggregatum* (shallot):** It is characteristic in most *Allium* spp. that leaves and bulbs become deformed on infestation with *D. dipsaci* (Figures 2, 2A and 3). The base of young plants becomes swollen and leaves become distorted. Older infected bulbs show swelling (bloat) of scales with open cracks often occurring at the root disc of the bulbs (Potter and Olthof, 1993). *A. cepa* attacked by *D. dipsaci* have a frosted appearance caused by the dissolution of cells that results from nematode feeding (Ferris and Ferris, 1998). Infested bulbs tend to rot readily in storage (Bridge and Hunt, 1986). The inner scales of the bulb are usually more severely attacked than the outer scales. As the season advances the bulbs become soft and when cut open show browning of the scales in concentric circles. Conversely, *D. dipsaci* does not induce deformation of leaves or swelling in*A. sativum*, but does cause leaf yellowing and death (Netscher and Sikora, 1990). Mollov *et al.* (2012) reported *D. dipsaci* for the first time from *A. sativum* in Minnesota, USA. The symptoms of the above-ground plant were stunting and chlorosis, while the symptoms of the bulbs were necrosis, underdevelopment and distortion. *Allium* spp. may have foliar spickels. No symptoms of infestation are observed on infested *Allium* seeds.	A reference to a new picture and additional information on the expression of symptoms added.	EPPO, European Union, Georgia, Serbia
34.	*41*	Editorial	***Tulipa* spp.** (Southey, 1993): Symptoms of *D. dipsaci* attack on tulip, both on growing plants and bulbs, are quite different from those on *Narcissus* spp~~*spp*~~.. In the field, infestation is best detected at flowering. The first sign is a pale or purplish lesion on one side of the stem immediately below the flower, which bends in the direction of the lesion. The lesion increases in size, the epidermis splits – revealing typical loose tissue beneath – and the damage spreads downwards and often upwards on to the petals. In more severe attacks, similar lesions extend down stems from leaf axils and growth may become distorted. Infestations start at the base of new bulbs, which arise as lateral offset buds from the base of the previous stems. The infection can be seen and felt on removal of the outer brown scales, as grey or brown soft patches on the outer fleshy scales. Infected bulbs do not show brown rings as they do in narcissus and hyacinth.	"spp." shouldn't be in italics.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
35.	*42*	Editorial	**3.1.1.3Symptoms specific to Leguminosae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
36.	*43*	Editorial	***Medicago sativa*:***D. dipsaci* is the most important nematode pest of *M. sativa.* Infestation occurs readily in heavier soils and during times of high rainfall or in sprinkler-irrigated areas. “White flagging” associated with loss of leaf chlorophyll is often a feature of infested crops under conditions of moisture stress (Griffin, 1985). Infested fields often show irregular areas of sparse growth. Typical symptoms of nematode attack include basal swelling, dwarfing and twisting of stalks and leaves, shortening of internodes, and the formation of many axillary buds, producing an abnormal number of tillers to give the plant a bushy appearance (McDonald and Nicol, 2005). Infested plants sometimes do not grow tall enough for hay (Ferris and Ferris, 1998), and they often fail to produce flower spikes (McDonald and Nicol, 2005). *D. dipsaci* predisposes lucerne to *Phytophtora megasperma*. Damage by *D. dipsaci* is increased by the occurrence of other, sap~~h~~rophagous nematodes (*Rhabditis*, *Cephalobus* and *Panagrolaimus* species) on the diseased, broken plants, which also hasten the death of the plants (Andrássy and Farkas 1988).	Spelling mistake	EPPO, European Union, Georgia, Serbia
37.	*43*	Technical	***Medicago sativa*:***D. dipsaci* is the most important nematode pest of *M. sativa.* Infestation occurs readily in heavier soils and during times of high rainfall or in sprinkler-irrigated areas. “White flagging” associated with loss of leaf chlorophyll is often a feature of infested crops under conditions of moisture stress (Griffin, 1985). Infested fields often show irregular areas of sparse growth. Typical symptoms of nematode attack include basal swelling, dwarfing and twisting of stalks and leaves, shortening of internodes, and the formation of many axillary buds, producing an abnormal number of tillers to give the plant a bushy appearance (McDonald and Nicol, 2005). Infested plants sometimes do not grow tall enough for hay (Ferris and Ferris, 1998), and they often fail to produce flower spikes (McDonald and Nicol, 2005). *D. dipsaci* predisposes lucerne to *Phytophtora megasperma*. Damage by *D. dipsaci* is increased by the occurrence of other, saphrophagous nematodes (*Rhabditis*, *Cephalobus* and *Panagrolaimus* species) on the diseased, broken plants, which also hasten the death of the plants (Andrássy and Farkas 1988). No symptoms of infestation are observed in infested Medicago seeds.	New information on the expression of symptoms added.	EPPO, European Union, Georgia, Serbia
38.	*45*	Editorial	**3.1.1.4Symptoms specific to Solanaceae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
39.	*48*	Editorial	**3.1.1.5Symptoms specific to Cruciferae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
40.	*50*	Editorial	**3.1.1.6Symptoms specific to Amarilidaceae**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
41.	*52*	Editorial	**3.1.1.7Symptoms specific to other hosts**	Should not be underlined.	EPPO, European Union, Georgia, Serbia
42.	*54*	Editorial	**Family Asparagacae, subfamily Sciloideae (hyacinths) and other bulbs** (Southey, 1993):Bulb symptoms are the same as in *Narcissus* spp., but distinct swellings are not usually seen on the plant leaves. The foliage may show pale yellow streaks, distortion and often slight swelling. Other liliaceous bulbs generally show the same symptoms as hyacinths. Symptoms of infestation in Amarylliaceae are similar to those in *Narcissus* spp.; for example, *Galanthus* spp. and *Nerine* spp. show swellings on their leaves and concentric, brown rings in bulbs.	Last but one sentence: "hyacinth," to be replaced by "hyacinths." (i.e. add an "s" and replace the comma by a full stop).	EPPO, European Union, Georgia, Serbia
43.	*55*	Technical	***Beta vulgaris* and *Daucus carota***(Cooke, 1993):*D. dipsaci* feeding results in the death of the growing point in seedlings (leading to the formation of multiple crowns); cotyledons and leaves may become twisted, swollen and distorted; and galls may develop on leaves or petioles of slightly older plants. Later in the season, feeding on the crown may cause a rot known as crown canker, crown rot or collar rot. This is first visible as raised, greyish pustules, usually among the leaf scars. Rotting then develops outwards and downwards, expanding across the shoulder of the plant, allowing the crown to become detached when pulled. In *D. carota*, additional symptoms may include straddled leaves and discoloration of the head of the main root. Symptoms mainly occur 2-4 cm below and above the ground. Severe infestation causes leaf death and~~severe~~ crown rot, especially in autumn (Figure 6).	Expanded description of symptoms.	EPPO, European Union, Georgia, Serbia
44.	*56*	Editorial	***Phlox paniculata* and other ornamental plants** (Southey, 1993):On phlox, infested shoots show typical thickening and brittleness of stems and shortening of internodes that have a tendency to split. Characteristic and unique to this host is the crinkling and reduction of laminae of the upper leaves, the uppermost of which may be reduced to attenuated filaments. Examples of plants recorded as hosts, with malformed growth, swelling and so forth, are species and cultivars of *Anemone, Calceolaria, Cheiranthus, Gypsophila, Helenium, Heuchera, Lychnis, Lysimachia* and *Penstemon* (Roberts, 1981). Edwards (1937) reported stunting, leaf malformation, rotting and failure to flower in *Primula* spp.. Woody plants are not often attacked, but *Hydrangea* may be infested with *D. dipsaci*, causing distortion of non-woody shoots, swelling of petioles and main veins, and pronounced crinkling of leaf laminae. The crinkled leaves are usually the first sign of infection. Another woody plant, *Yucca smaliana*, shows leaf distortion and	Add a full stop after "Primula spp.".	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			blister-like swellings.		
45.	*59*	Editorial	***Solanum tuberosum* and *Dahlia* spp.:**No symptoms are visible during the growth period. The nematodes enter potato tubers usually via the stolons. Most of the nematodes are located at the edge of the browning and undamaged parts If a small sample from this part of the tuber is taken and placed in water, the mass of small nematodes is conspicuous even with a simple magnifying glass. The earliest symptoms of *D. destructor* infection are small, white, chalky or light-coloured spots that can be seen just below the skin of the tuber (Brodie, 1998). These spots later become larger and gradually darker (through grey, dark brown and black), and acquire a spongy texture (Figure 7). This is mostly a result of secondary invasion by bacteria, fungi and saprophytic nematodes (Brodie, 1998). On severely affected tubers there are typically slightly sunken areas with cracked, wrinkled, papery skin. The skin is not attacked but becomes thin and cracks as underlying infected tissues dry and shrink (Brodie, 1998). In contrast, the skin of *Solanum tuberosum* infested with *D. dipsaci* is usually not cracked. The nematodes continue to reproduce inside the tubers after harvest and may build up to large numbers.	Wrong word, corrected.	EPPO, European Union, Georgia, Serbia
46.	*59*	Technical	***Solanum tuberosum* and *Dahlia* spp.:**No symptoms are visible during the growth period. The nematodes enter potato tubers usually via the stolons. Most of the nematodes are located at the edge of the browning and undamaged parts If a small sample from this part of the tuber is taken and placed in water, the mass of small nematodes is conspicuous even with a simple magnifying glass. The earliest symptoms of *D. destructor* infection are small, white, chalky or light-coloured spots that can be seen just below the skin of the tuber (Brodie, 1998). These spots later become larger and gradually darker (through grey, dark brown and black), and acquire a spongy texture (Figure 7). This is mostly a result of secondary invasion by bacteria, fungi and saprophytic nematodes (Brodie, 1998). On severely affected tubers there are typically slightly sunken areas with cracked, wrinkled, papery skin. The skin is not attacked but becomes thin and cracks as lying infected tissues dry and shrink (Brodie, 1998). Finally, mummification of whole tubers may occur. Such fully damaged tubers float in water (Figure 7A). In contrast, the skin of *Solanum tuberosum* infested with *D. dipsaci* is usually not cracked. The nematodes continue to reproduce inside the tubers after harvest and may build up to large numbers. Symptoms may be more visible after storage. Secondary infections of fungi, bacteria and free-living nematodes occur in general on infested tubers.	Expanded information on symptoms.	EPPO, European Union, Georgia, Serbia
47.	*63*	Technical	*D. destructor* infestation of ornamental *Liatris spicata* corms (“Gayflower”, “Blazing Star” or “Button Snakeroot”) in cold storage in South Africa showed a blackish rot with living nematodes at different stages in the tissue adjacent to the decaying areas (pers. comm., F.A. van der Vegte, 1983).	No reference provided for pers comm. F.A. van der Vegte, 1983.	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
48.	*65*	Substantive	~~To extract the nematodes, the affected scales of bulbs (inner scales mainly) and the garlic cloves are cut into small pieces and put in a container (e.g. Petri dish) with tap water at room temperature. After 1 h or more the nematodes can be observed with a stereomicroscope (at least 40× magnification).~~	The part content of the[65] is repeated with [67], [69],[71].	China
49.	*65*	Technical	To extract the nematodes, the affected scales of bulbs (inner scales mainly) and the garlic cloves are cut into small pieces and put in a container (e.g. Petri dish) with tap water at room temperature. To obtain a clear suspension the pieces may be placed on a sieve of 200-250 µm aperture covered with filter paper, as a support (Oostenbrink dish technique). After 1 h or more the nematodes can be observed with a stereomicroscope (at least 40× magnification).	Additional information added.	EPPO, European Union, Georgia, Serbia
50.	*66*	Substantive	~~**3.2.2 Extraction from soil and plant material**~~	Not need subheading	China
51.	*67*	Substantive	The Baermann funnel method and variation of the technique the funnel and The Seinhorst mistifier technique ae reference technique~~The Baermann funnel method is a reference technique~~ for extraction of nematodes from soil and plant material (bulbs, roots, potato peelings). ~~A funnel with a piece of rubber tubing is attached to the stem and closed by a spring or screw clip. The funnel is placed in a support and almost filled with tap water. Soil or plant tissue cut into small pieces is placed in a muslin or tissue paper, which is folded to enclose the material and is gently submerged in the water in the funnel. Active nematodes pass through the cloth and sink to the bottom of the funnel stem. After some hours, or overnight, a small quantity of water containing the nematodes is run off and observed under microscope~~ (Flegg and Hooper, 1970).	Reason the same with general comment。	China
52.	*67*	Technical	The Baermann funnel method is a reference technique for extraction of nematodes from soil and plant material (bulbs, roots, potato peelings and seeds). A funnel with a piece of rubber tubing is attached to the stem and closed by a spring or screw clip. The funnel is placed in a support and almost filled with tap water. Soil or plant tissue cut into small pieces is placed in a muslin or tissue paper, which is folded to enclose the material and is gently submerged in the water in the funnel. Active nematodes pass through the cloth and sink to the bottom of the funnel stem. After some hours, or overnight, a small quantity of water containing the nematodes is run off and observed under microscope (Flegg and Hooper, 1970).	An example added.	EPPO, European Union, Georgia, Serbia
53.	*68*	Technical	~~In a variation of the technique the funnel is replaced by a dish. Lumps of soil are broken up and stones and plant debris removed. Soil (50 ml) is spread evenly on a circle of single-ply paper towel supported on a coarse-meshed plastic screen standing in a plastic container. Water is added to the container until the soil is thoroughly wet but not immersed. The container is covered with a large Petri dish top to reduce evaporation of water. This set-up is left for at least 24 h after which~~	The content of this paragraph is repeated with the [67] .	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			~~the soil is discarded and the nematode suspension is poured from the container into a dish for examination with the aid of a dissection microscope. The soil can be replaced by finely chopped plant tissue (Kleynhans, 1997).~~		
54.	*69*	Technical	~~The Seinhorst mistifier technique for bulbs and roots differs from the Baermann funnel method in that plant sap and toxic decomposition products are washed away. It should be used in preference to the Baermann funnel method for plants as *Narcissus* spp. In this method a Baermann funnel or Oostenbrink dish is placed in a mist or fog of wáter to avoid the depletion of oxygen. The mist is produced by nozzles spraying water over the plant material or by nozzles spraying water upwards so that droplets fall softly back onto the plant material. Live nematodes leave the plant tissue and are washed into the funnel or dish where they sediment. The nematodes are collected every 24 to 48 h in a glass beaker by opening the screw clip on the funnel stem or by collecting the specimens on a 20–25 µm sieve. Extraction can be continued for up to four weeks. This technique is described by Hooper (1986).~~	The content of this paragraph is repeated with the [67] .	China
55.	*70*	Editorial	Another method to extract *Ditylenchus* spp. from plant material was adapted from a description by Oliveira *et al.* (2013). ~~Plant material is cut in 1 cm pieces and they are placed in 500 ml jars filled with tap water. Two holes are punched into the lids of these jars, one providing access to the tube of an aquarium pump and one acting as an outlet for air. The material is kept for 72 h under continuous aeration from the pump. The resulting suspension is poured through a 1 000 µm sieve to remove plant debris and then through a 38 µm sieve to extract the nematodes from the suspension. This method of aerating the suspension prevents the rotting of the plant material so there is a minimal increase of bacterial and fungal feeders and many of the nematodes stay alive. The agitation through the aeration of the suspension containing the plant material results in more nematodes being dislodged from the root tissue and therefore in a much more accurate estimate of the infestation of the plant material.~~	The content of this paragraph is repeated with the [67] .	China
56.	*71*	Editorial	The nematodes can be extracted from plant material by the Coolen and D'Herde (1972) method. ~~The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 mL tap water in a domestic blender at the lowest mixing speed for 2 min. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 mL centrifuge tubes. About 1 mL kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 3 000 r.p.m. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1 750 r.p.m. for 4 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied.~~	The content of this paragraph is repeated with the [67] .	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
57.	*71*	Technical	The nematodes can be extracted from plant material by the Coolen and D'Herde (1972) method. The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 mL tap water in a domestic blender at the lowest mixing speed for 2 min. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 mL centrifuge tubes. About 1 mL kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 3 000 r.p.m. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1 750 r.p.m. for 4 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied.	A big disadvantage of this method is that Ditylenchus gets damaged in the blender (cut in pieces) because it is a large nematode. This method is more suitable for extraction of small nematodes, for example Meloidogyne.	EPPO, European Union, Georgia, Serbia
58.	*71*	Technical	The nematodes can be extracted from plant material by the Coolen and D'Herde (1972) method. The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 mL tap water in a domestic blender at the lowest mixing speed for ~~2~~ 1 min. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 mL centrifuge tubes. About 1 mL kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 3 000 r.p.m. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1 750 r.p.m. ~~for 4~~ 1 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied.	""Lowest mixing speed for 2 min." - We propose to revise the time of 2 min. In bulbs, for example, more than 30 seconds can cause prejudice to the identification, once it can grind nematodes that are relatively big. The experience and other protocols indicate centrifugation for 1 min.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina
59.	*73*	Editorial	For extraction of nematodes from soil, the following method (after Kleynhans, 1997) can be used. ~~Soil (250 ml) is washed through a course-meshed sieve (2 mm) into a 5-litre bucket. Tap water is added to make a volume of 5 litres. The suspension is stirred, then allowed to settle for 30 s before being poured through a 45 µm sieve. This procedure is repeated with the soil in the bucket for two more times, but shortening the setting times to 20 and then 10 s. The residue is transferred from the 45 µm sieve to 50 ml centrifuge tubes. If the solution in the tubes is very sandy, 5 ml kaolin can be added to the tubes (and thoroughly mixed) to assist in the settling of the nematodes. The tubes are centrifuged for 7 min at 1 750 r.p.m. The supernatant is decanted from each tube and discarded. A sugar solution (450 g/litre water) is added to the tubes and this sugar and soil mixture is thoroughly shaken before centrifuging again for 3 min at 1 750 r.p.m. The supernatant is poured through a 45 µm sieve and the residue, with nematodes in it, is collected in a beaker for examination. This is a basic technique and depending on the skill of the technician and type of soil, up to 40% of nematodes may be lost.~~ Hooper *et al*. (2005) describes different extraction methods adapted to take advantage of size, density and motility of nematodes.	simplifies the content.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
60.	*73*	Technical	For extraction of nematodes from soil, the following method (after Kleynhans, 1997) can be used. Soil (250 ml) is washed through a course-meshed sieve (2 mm) into a 5 litre bucket. Tap water is added to make a volume of 5 litres. The suspension is stirred, then allowed to settle for 30 s before being poured through a 45 µm sieve. This procedure is repeated with the soil in the bucket for two more times, but shortening the setting times to 20 and then 10 s. The residue is transferred from the 45 µm sieve to 50 ml centrifuge tubes. If the solution in the tubes is very sandy, 5 ml kaolin can be added to the tubes (and thoroughly mixed) to assist in the settling of the nematodes. The tubes are centrifuged for 7 min at 1 750 r.p.m. The supernatant is decanted from each tube and discarded. A sugar solution (450 g/litre water) is added to the tubes and this sugar and soil mixture is thoroughly shaken before centrifuging again for 3 min at 1 750 r.p.m. The supernatant is poured through a 45 µm sieve and the residue, with nematodes in it, is collected in a beaker for examination. This is a basic technique and depending on the skill of the technician and type of soil, up to 40% of nematodes may be lost. Other methods which may be used for the extraction of nematodes from soil include Flegg modified Cobb technique and Oostenbrink elutriator (EPPO, 2013c). Hooper *et al*. (2005) describes different extraction methods adapted to take advantage of size, density and motility of nematodes.	More techniques added.	EPPO, European Union, Georgia, Serbia
61.	*75*	Substantive	This paragraph should be listed after Para.76 Identification of *Ditylenchus* spp. by morphological means is restricted to adult specimens and preferably both male and female nematodes of a species are examined under a high-power microscope. Good-quality slide preparations should allow adult *D. destructor* and *D. dipsaci* to be identified with certainty by morphological examination alone. The morphological identification of *Ditylenchus* juveniles in a sample should only confirms their development. As mycophagous *Ditylenchus* spp. frequently contaminate decaying plant material, care must be taken in the identification of specimens in both plant and soil samples.	It's more logical.	China
62.	*75*	Technical	Identification of *Ditylenchus* spp. by morphological means is restricted to adult specimens and preferably both male and female nematodes of a species are examined under a high-power microscope. Good-quality slide preparations should allow adult *D. destructor* and *D. dipsaci* to be identified with certainty by morphological examination alone. The morphological identification of *Ditylenchus* juveniles in a sample should only confirms their development. As mycophagous *Ditylenchus* spp. frequently contaminate decaying plant material, care must be taken in the identification of specimens in both plant and soil samples.	The third sentence is not clear, please rephrase.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
63.	*77*	Substantive	This paragraph should be listed after Para.74 The identification of *D. dipsaci* and *D. destructor* should always be based on morphological methods. Molecular methods developed for identifying these species can be used for low infestation levels or when only juveniles are present. Molecular techniques can be applied to damaged and atypical adults, and all life stages, including the juvenile stages, for which morphological identification to species is not possible.	In fact, this paragraph is the content of the Para.74	China
64.	*77*	Technical	The identification of *D. dipsaci* and *D. destructor* should preferably~~always~~ be based on morphological methods. Molecular methods developed for identifying these species can be used for low infestation levels or when only juveniles are present. Molecular techniques can be applied to damaged and atypical adults, and all life stages, including the juvenile stages, for which morphological identification to species is not possible.	Why “should”. It can be done, but it can also be done by molecular methods provided that these methods are properly validated. This formulation gives you no choice then to identify morphologically, it seems.	EPPO, European Union, Georgia, Serbia
65.	*79*	Substantive	~~**Temporary preparations**~~	This part includes the temporary and permanent methods. And it isn’t consistent with the title.	China
66.	*84*	Technical	For light microscopy, live nematodes are extracted from soil or plant material, killed by gentle heat (65-70^{o}C), fixed in FAA (35% distilled water, 10% of 40% formalin, 5% glacial acetic acid, 50% of 95% alcohol) (Andrássy, 1984), transferred into glycerol (Hooper *et al*., 2005) and mounted in anhydrous glycerine between coverslip slides as described by Seinhorst (1959) and Goodey (1963).	1. What is meant by gentle heat? 2. Clarification. A dehydration step after fixing has become normal. The reference by Hooper et al. covers several alternatives for nematode preservation including the more modern ones.	EPPO, European Union, Georgia, Serbia
67.	*85*	Technical	For light microscopy identification work, magnification of 500× to 1 000× (oil immersion lens) in combination with Differential Interference Contrast is recommended.	Differential Interference Contrast is recommended.	EPPO, European Union, Georgia, Serbia
68.	*87*	Technical	Keys for diagnosis for *Ditylenchus* species can be found in Viscardi and Brzeski (1993) and Brzeski (1998). A key to distinguish *Ditylenchus* spp. from other tylenchid and aphelenchid genera are presented in Table 1 below.	A reference added.	EPPO, European Union, Georgia, Serbia
69.	*89*	Technical	**1** Outlet of dorsal oesophageal gland near base of stylet; median bulb roundish, ovoid or absent — Tylenchida - 2 Outlet of dorsal oesophageal gland in median bulb; median bulb a prominent feature, usually oblong — Aphelenchida	1 and 5 We recommend using pharynx instead of oesophagus according to the EPPO Pictorial Glossary of Morphological Terms in Nematology 4 Median bulb without valve There are Ditylenchus species without a valve. Suggest adding an asterisk below the table with an explanation. 5 upper box better use weak than frail 5 lower box better use stylet than	EPPO, European Union, Georgia, Serbia

<table>
<tr><th>Comm. no.</th><th>Para. no.</th><th>Comment type</th><th>Comment</th><th>Explanation</th><th>Country</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td rowspan="2">2</td><td>Anterior part of oesophagus (procorpus) and median bulb not united into single unit; stylet never exceptionally long</td><td>3</td></tr>
<tr><td>Procorpus gradually widened and fused with median bulb; stylet very long, its base often located in anterior part of median bulb</td><td>Other genera</td></tr>
<tr><td rowspan="2">3</td><td>Adult female vermiform</td><td>4</td></tr>
<tr><td>Adult female saccate or pyriform sessile parasite on roots</td><td>Other genera</td></tr>
<tr><td rowspan="2">4</td><td>Valvular median bulb</td><td>5</td></tr>
<tr><td>Median bulb without valve</td><td>Other genera</td></tr>
<tr><td rowspan="2">5</td><td>Oesophageal glands contained within basal bulb, not overlapping or slightly overlapping intestine; cephalic framework rarely conspicuous; stylet frail to moderately strong</td><td>6</td></tr>
<tr><td>Oesophageal glands lobe-like, overlapping intestine; cephalic framework strong; spear massive</td><td>Other genera</td></tr>
<tr><td>6</td><td>Single prodelphic ovary; vulva posterior</td><td>7</td></tr>
<tr><td></td><td>Ovaries two, amphidelphic; vulva slightly post-equatorial</td><td>Other genera</td></tr>
<tr><td>7</td><td>Female not swollen; crustaformeria in female in form of quadricollumella with four rows of four cells each; bursa in males enveloping one-third or more of tail</td><td>Ditylenchus</td></tr>
<tr><td></td><td>Female swollen; crustaformeria with more than 20 cells</td><td>Other genera</td></tr>
</table>

* A few non plant-parasitic species of Ditylenchus do not have valvular median bulb</td><td>spear</td><td></td></tr>
<tr><td>70.</td><td>91</td><td>Technical</td><td>Description of Ditylenchus dipsaci(after Sturhan and Brzeski, 1991, ~~and~~ Wendt et al., 1995 and Brzeski, 1998).Details and views are provided in Figure 8.</td><td>New reference added.</td><td>EPPO, European Union,</td></tr>
</table>

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
					Georgia, Serbia
71.	*92*	Editorial	*Morphological diagnostic characters*: The number of lateral incisures (four) (Figure 8F), the comparatively long stylet, the length of the postvulval sac and the pointed tail (Figure 8D) are the distinguishing characters for this species (Andrássy, 2007). *D. dipsaci* can be distinguished from *D. gigas* by the shorter body (1.0–1.7 vs 1.5–1.7 mm) (Vovlas *et al*., 2011). When observed in the lateral view, the spicule is more arched in *D. dipsaci* than in *D. destructor* (Figure 8C). See Karssen and Willemsen (2010) for more information on the spiculum and its use in the identification of *D. dipsaci* and *D. destructor*.	A comma is missing after "Volvas et al.".	EPPO, European Union, Georgia, Serbia
72.	*92*	Technical	*Morphological diagnostic characters*: The number of lateral incisures (four) (Figure 8F), the comparatively long stylet, the length of the postvulval sac and the pointed tail (Figure 8D) are the distinguishing characters for this species (Andrássy, 2007). *D. dipsaci* can be distinguished from *D. gigas* by the shorter body (1.0–1.7 vs 1.5–1.7 mm) (Vovlas *et al*. 2011). ~~When observed in the lateral view, the spicule is more arched in *D. dipsaci* than in *D. destructor* (Figure 8C).~~ See Karssen and Willemsen (2010) for more information on the spiculum and its use in the identification of *D. dipsaci* and *D. destructor*. Exchange the order of the Para. 92 and 93.	In general, describe the General Morphology, and then describe the “Morphological diagnostic characters”. The spicule is more arched in D. dipsaci than in D. destructor , as identification characteristics of the lack of scientific basis.	China
73.	*92*	Technical	*Morphological diagnostic characters*: The number of lateral incisures (four) (Figure 8F), the comparatively long stylet, the length of the postvulval sac and the pointed tail (Figure 8D) are the distinguishing characters for this species (Andrássy, 2007). *D. dipsaci* can be distinguished from *D. gigas* by the shorter body of females (1.0–1.7 vs 1.56 ~~–1.7~~2.2 mm) (Vovlas *et al*. 2011). When observed in the lateral view, the spicule is more arched in *D. dipsaci* than in *D. destructor* (Figure 8C). See Karssen and Willemsen (2010) for more information on the spiculum and its use in the identification of *D. dipsaci* and *D. destructor*.	1. The body length relates to females 2. There is an inconsistency between the measurements in the text and those given in Table 2. The measurements indicated by Volvas and al. (2011) are " 1.0-1.7 vs 1.6-2.2" (cf. table 2), which gives a narrower zone of overlapping and so better allows to distinguish the two species by the difference of their body length. On the other hand, it is indicated in the table 2 for D. dispaci "1,0-1,3"; it would be necessary to choose. Reference to additional characteristics may be relevant: for example (in the draft EPPO protocol): ‘Ditylenchus gigas is morphologically close to D. dipsaci from which it differs by its longer body size (1.5-2.2 mm vs. 1.0-1.7) and longer vulva-anus distance (202-266 vs. 132-188 µm).’ 3. Note	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				that in the seeds of Vicia faba essentially larvae of the fourth stage are present .	
74.	*94*	Editorial	Place Para.94 after Para.91 Measurements (criteria described in EPPO, 2013b): (*Ex* Oat, *Avena sativa* L., after Blake, 1962, in Hooper, 1972). (*n* = 48♀♀): L = 1.3 mm ± 0.009; a = 62 ± 5.6; b = 15 ± 1.4; c = 14 ± 2.1; V = 80 ± 1.5. (*n* = 23♂♂): L = 1.3 mm ± 0.017; a = 63 ± 11.3; b = 15 ± 1.7; c = 14 ± 2.1; T = 72.	According to the expression habit.	China
75.	*95*	Editorial	**Description of *Ditylenchus destructor*(after Sturhan and Brzeski, 1991). Details and views are provided in Figure 9.** *Morphological diagnostic characters*: *D. destructor* is similar to *D. dipsaci*, but differs from that species by the lateral field showing six incisures (Figure 9F), the longer postvulval sac and the finely rounded tail terminus (Figure 9D). Morphologically *D. destructor* differs from *D. africanus* mainly in the stylet length, which may overlap slightly, and the spicule length, which implies that males must be present in the population. As PCR technology is sufficiently sensitive to resolve differences between closely related genera, Wendt *et al*. (1995) used restriction fragment length polymorphisms (RFLPs) generated by seven restriction enzymes on the internal transcribed spacer of ribosomal (r)DNA to separate *D. destructor* from *D. africanus*. When observed in the lateral view, the spicule is less arched in *D. dipsaci* than in *D. destructor* (Figure 9C).	In the second paragraph, 3rd sentence, the availability of a PCR test is useful but could be shortened and a reference made to paragraph 113. Sufficient details are already given in paragraph 113.	EPPO, European Union, Georgia, Serbia
76.	*95*	Technical	**Exchange the order of the Para. 95 and 96.** **Description of *Ditylenchus destructor*(after Sturhan and Brzeski, 1991). Details and views are provided in Figure 9.** *Morphological diagnostic characters*: *D. destructor* is similar to *D. dipsaci*, but differs from that species by the lateral field showing six incisures (Figure 9F), the longer postvulval sac and the finely rounded tail terminus (Figure 9D). Morphologically *D. destructor* differs from *D. africanus* mainly in the stylet length, which may overlap slightly, and the spicule length, which implies that males must be present in the population. As PCR technology is sufficiently sensitive to resolve differences between closely related genera, Wendt *et al*. (1995) used restriction fragment length polymorphisms (RFLPs) generated by seven restriction enzymes on the internal transcribed spacer of ribosomal (r)DNA to separate *D. destructor* from *D. africanus*.	In general, describe the General Morphology, then introduce the “Morphological diagnostic characters”.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			When observed in the lateral view, the spicule is less arched in *D. dipsaci* than in *D. destructor* (Figure 9C).		
77.	*95*	Technical	**Description of *Ditylenchus destructor*(after Sturhan and Brzeski, 1991 and Brzeski,1998). Details and views are provided in Figure 9.** *Morphological diagnostic characters*: *D. destructor* is similar to *D. dipsaci*, but differs from that species by the lateral field showing six incisures (Figure 9F), the longer postvulval sac and the finely rounded tail terminus (Figure 9D). Morphologically *D. destructor* differs from *D. africanus* mainly in the stylet length, which may overlap slightly, and the spicule length, which implies that males must be present in the population. As PCR technology is sufficiently sensitive to resolve differences between closely related genera, Wendt *et al*. (1995) used restriction fragment length polymorphisms (RFLPs) generated by seven restriction enzymes on the internal transcribed spacer of ribosomal (r)DNA to separate *D. destructor* from *D. africanus*. When observed in the lateral view, the spicule is less arched in *D. dipsaci* than in *D. destructor* (Figure 9C).	New reference added.	EPPO, European Union, Georgia, Serbia
78.	*96*	Editorial	*General morphology*: Adults of *D. destructor* are minute, worm-like animals, 0.8–1.4 mm long, 23–47 µm wide and slightly ventrally arcuate. Considerable morphometric variation occurs in adults according to their host and age. Males and females are similar in general appearance. Lateral field with six incisures (Figure 9F), reduced to two on the neck and tail regions ~~(Figure 9F)~~. Cuticular and head annulation fine, head often narrower than adjacent body, about four head annules discerned by scanning electron microscopy (Wendt *et al*., 1995). Stylet 10–12 µm long, occasionally specimens with stylets of 14 µm have been described. Stylet cone 45–50% of the stylet length, knobs distinct, rounded and sloping backwards. Median bulb muscular, with thickenings of lumen walls (or valve) about 3 µm long. Posterior bulb overlaps intestine for a short distance on the dorsal body side, although specimens with an offset glandular bulb are occasionally seen (Figure 9A). Excretory pore opposite oesophageal glands. Postvulval sac extending about three-quarters of the vulva–anus distance (Figure 9E). Eggs twice as long as wide (Adrássy, 2007). Lips of vulva thick, elevated (Figure 9B). Anterior ovary outstretched, sometimes reaching the oesophageal region. Postvulval part of uterine sac 40–98% of vulva–anus distance, not functioning as a spermatheca (Figure 9E). Male bursa surrounds 50–90% of the tail length. Spicules are 24–27 µm long. Testis outstretched approaching the base of esophagus. Tail of both sexes conical, three to five anal body widths long, usually ventrally curved, terminus rounded.	Please see Figure 9F.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
79.	*96*	Editorial	*General morphology*: Adults of *D. destructor* are minute, worm-like animals, 0.8–1.4 mm long, 23–47 µm wide and slightly ventrally arcuate. Considerable morphometric variation occurs in adults according to their host and age. Males and females are similar in general appearance. Lateral field with six incisures, reduced to two on the neck and tail regions (Figure 9F). Cuticular and head annulation fine, head often narrower than adjacent body, about four head annules discerned by scanning electron microscopy (Wendt *et al*., 1995). Stylet 10–12 µm long, occasionally specimens with stylets of 14 µm have been described. Stylet cone 45–50% of the stylet length, knobs distinct, rounded and sloping backwards. Median bulb muscular, with thickenings of lumen walls (or valve) about 3 µm long. Posterior bulb overlaps intestine for a short distance on the dorsal body side, although specimens with an offset glandular bulb are occasionally seen (Figure 9A). Excretory pore opposite oesophageal glands. Postvulval sac extending about three-quarters of the vulva–anus distance (Figure 9E). Eggs twice as long as wide (Andrássy, 2007). Lips of vulva thick, elevated (Figure 9B). Anterior ovary outstretched, sometimes reaching the oesophageal region. Postvulval part of uterine sac 40–98% of vulva–anus distance, not functioning as a spermatheca (Figure 9E). Male bursa surrounds 50–90% of the tail length. Spicules are 24–27 µm long. Testis outstretched approaching the base of esophagus. Tail of both sexes conical, three to five anal body widths long, usually ventrally curved, terminus rounded.	Spelling	Australia
80.	*96*	Technical	*General morphology*: Adults of *D. destructor* are minute, worm-like animals, 0.8–1.4 mm long, 23–47 µm wide and slightly ventrally arcuate. Considerable morphometric variation occurs in adults according to their host and age. Males and females are similar in general appearance. Lateral field with six incisures, reduced to two on the neck and tail regions (Figure 9F). Cuticular and head annulation fine, head often narrower than adjacent body, about four head annules discerned by scanning electron microscopy (Wendt *et al*., 1995). Stylet 10–12 µm long, occasionally specimens with stylets of 14 µm have been described. Stylet cone 45–50% of the stylet length, knobs distinct, rounded and sloping backwards. Median bulb muscular, with thickenings of lumen walls (or valve) about 3 µm long. Posterior bulb overlaps intestine for a short distance on the dorsal body side, although specimens with an offset glandular bulb are occasionally seen (Figure 9A). Excretory pore opposite oesophageal glands. Postvulval sac extending about three-quarters of the vulva–anus distance (Figure 9E). Eggs twice as long as wide (Adrássy, 2007). Lips of vulva thick, elevated (Figure 9B). Anterior ovary outstretched, sometimes reaching the oesophageal region. Postvulval part of uterine sac 40–98% of vulva–anus distance, not functioning as a spermatheca (Figure 9E). Male bursa surrounds 50–90% of the tail length. Spicules are 24–27 µm long. The spiculum shape of *D. dipsaci* differs from *D. destructor* having a ventral tumulus i	Additional information added.	EPPO, European Union, Georgia, Serbia

<table>
<tr><th>Comm. no.</th><th>Para. no.</th><th>Comment type</th><th>Comment</th><th>Explanation</th><th>Country</th></tr>
<tr><td></td><td></td><td></td><td>n the calomus area (Figure 10) (Karssen and Willemsen, 2010). Testis outstretched approaching the base of esophagus. Tail of both sexes conical, three to five anal body widths long, usually ventrally curved, terminus rounded.</td><td></td><td></td></tr>
<tr><td>81.</td><td>99</td><td>Editorial</td><td>Table 2. Comparative diagnostic characteristics of Ditylenchus destructor, Ditylenchus africanus, Ditylenchus myceliophagus, Ditylenchus gigas and Ditylenchus dipsaci according to Hooper (1972, 1973), Hesling (1974), Sturhan and Brzeski (1991), Wendt et al. (1995) and Vovlas et al. (2011).</td><td>It would be desirable to indicate which publication corresponds to which column.</td><td>EPPO, European Union, Georgia, Serbia</td></tr>
<tr><td>82.</td><td>100</td><td>Editorial</td><td>
<table>
<tr><th>Characters</th><th>D. destructor</th><th>D. africanus</th><th>D. myceliophagus</th><th>D. gigas</th><th>D. dipsaci</th></tr>
<tr><td>Body length female (mm)</td><td>0.8–1.9</td><td>0.7–1.1</td><td>0.6–1.4</td><td>1.6–2.2</td><td>1.0–1.3</td></tr>
<tr><td>Number of lateral lines</td><td>6</td><td>6–15</td><td>6</td><td>4</td><td>4</td></tr>
<tr><td>Form of tail terminus</td><td>Rounded</td><td>Rounded</td><td>Rounded</td><td>Pointed to finely rounded</td><td>Pointed</td></tr>
<tr><td>c (body length/tail length) of female ?</td><td>–9–30 ?</td><td>8.8–16.9 ?</td><td>10.5–20.5–17 ?</td><td>15.8–27.6 ?</td><td>–14–18</td></tr>
<tr><td>Posterior bulb</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Slightly overlapping</td><td>Not overlapping</td></tr>
<tr><td>Stylet length (µm) of female</td><td>10–14</td><td>8–10</td><td>7–8</td><td>10.5–13.0</td><td>10–12</td></tr>
<tr><td>PUS/vulva–anus length (%)</td><td>53–90</td><td>37–85</td><td>30–69</td><td>*About 50 %</td><td>40–70</td></tr>
<tr><td>Spiculum length (µm)</td><td>24–27</td><td>17–21</td><td>15–20</td><td>23.5–28</td><td>23–28</td></tr>
<tr><td>Bursa length (as % of tail length)</td><td>50–70</td><td>48–66</td><td>20–55</td><td>72–76</td><td>40–70</td></tr>
<tr><td>Host preference (helpful information in case of confusing</td><td>Higher plants and mycelia of</td><td>Groundnut and fungi</td><td>Mycelia of fungi</td><td>Higher plants</td><td>Higher plants and fungi</td></tr>
</table>
</td><td>C values of D. destructor, D. Myceliophagus and D. dipsaci in Line 5 may be wrong, please check carefully. PUS/vulva–anus length (%) of D. gigas should be about 50, not 50%, please delete %.</td><td>China</td></tr>
</table>

<table>
<tr><th>Comm. no.</th><th>Para. no.</th><th>Comment type</th><th>Comment</th><th>Explanation</th><th>Country</th></tr>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>morphological criteria)</td><td>fungi</td><td></td><td></td><td></td><td></td></tr>
</table>
</td><td></td><td></td></tr>
<tr><td>83.</td><td>100</td><td>Editorial</td><td>
<table>
<tr><th>Characters</th><th>D. destructor</th><th>D. africanus</th><th>D. myceliophagus</th><th>D. gigas</th><th>D. dipsaci</th></tr>
<tr><td>Body length female (mm)</td><td>0.8–1.9</td><td>0.7–1.1</td><td>0.6–1.4</td><td>1.6–2.2</td><td>1.0–1.3</td></tr>
<tr><td>Number of lateral lines</td><td>6</td><td>6–15</td><td>6</td><td>4</td><td>4</td></tr>
<tr><td>Form of tail terminus</td><td>Rounded</td><td>Rounded</td><td>Rounded</td><td>Pointed to finely rounded</td><td>Pointed</td></tr>
<tr><td>c (body length/tail length) of female</td><td>–9–30</td><td>8.8–16.9</td><td>10.5–20.5–~~17~~</td><td>15.8–27.6</td><td>–14–18</td></tr>
<tr><td>Posterior bulb</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Slightly overlapping</td><td>Not overlapping</td></tr>
<tr><td>Stylet length (µm) of female</td><td>10–14</td><td>8–10</td><td>7–8</td><td>10.5–13.0</td><td>10–12</td></tr>
<tr><td>PUS/vulva–anus length (%)</td><td>53–90</td><td>37–85</td><td>30–69</td><td>*About 50 %</td><td>40–70</td></tr>
<tr><td>Spiculum length (µm)</td><td>24–27</td><td>17–21</td><td>15–20</td><td>23.5–28</td><td>23–28</td></tr>
<tr><td>Bursa length (as % of tail length)</td><td>50–70</td><td>48–66</td><td>20–55</td><td>72–76</td><td>40–70</td></tr>
<tr><td>Host preference (helpful information in case of confusing morphological criteria)</td><td>Higher plants and mycelia of fungi</td><td>Groundnut and fungi</td><td>Mycelia of fungi</td><td>Higher plants</td><td>Higher plants and fungi</td></tr>
</table>
</td><td>Editrial correction</td><td>Japan</td></tr>
<tr><td>84.</td><td>100</td><td>Technical</td><td>
<table>
<tr><th>Characters</th><th>D. destructo</th><th>D. africanus</th><th>D. myceliophag</th><th>D. gigas</th><th>D. dipsaci</th></tr>
</table>
</td><td>1. What do the – (dashes) mean? 2. For D dipsaci, c = 11-20 should be used rather than</td><td>EPPO, European Union, Georgia, Serbia</td></tr>
</table>

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				14-18 (Sturhan and Brzeski 1991 and Brzeski, 1998).	
85.	*100*	Technical		This information seems to be not useful for species identification.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina

	r		***us***		
Body length female (mm)	0.8–1.9	0.7–1.1	0.6–1.4	1.6–2.2	1.0–1.3
Number of lateral lines	6	6–15	6	4	4
Form of tail terminus	Rounded	Rounded	Rounded	Pointed to finely rounded	Pointed
c (body length/tail length) of female	–9–30	8.8–16.9	10.5–20.5–17	15.8–27.6	–14–18
Posterior bulb	Short, dorsally overlapping	Short, dorsally overlapping	Short, dorsally overlapping	Slightly overlapping	Not overlapping
Stylet length (µm) of female	10–14	8–10	7–8	10.5–13.0	10–12
PUS/vulva–anus length (%)	53–90	37–85	30–69	*About 50 %	40–70
Spiculum length (µm)	24–27	17–21	15–20	23.5–28	23–28
Bursa length (as % of tail length)	50–70	48–66	20–55	72–76	40–70
Host preference (helpful information in case of confusing morphological criteria)	Higher plants and mycelia of fungi	Groundnut and fungi	Mycelia of fungi	Higher plants	Higher plants and fungi

Characters	***D. destructor***	***D. africanus***	***D. myceliophagus***	***D. gigas***	***D. dipsaci***
Body length female (mm)	0.8–1.9	0.7–1.1	0.6–1.4	1.6–2.2	1.0–1.3

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			<table><tr><td>Number of lateral lines</td><td>6</td><td>6–15</td><td>6</td><td>4</td><td>4</td></tr><tr><td>Form of tail terminus</td><td>Rounded</td><td>Rounded</td><td>Rounded</td><td>Pointed to finely rounded</td><td>Pointed</td></tr><tr><td>c (body length/tail length) of female</td><td>–9–30</td><td>8.8–16.9</td><td>10.5–20.5–17</td><td>15.8–27.6</td><td>–14–18</td></tr><tr><td>Posterior bulb</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Short, dorsally overlapping</td><td>Slightly overlapping</td><td>Not overlapping</td></tr><tr><td>Stylet length (µm) of female</td><td>10–14</td><td>8–10</td><td>7–8</td><td>10.5–13.0</td><td>10–12</td></tr><tr><td>PUS/vulva–anus length (%)</td><td>53–90</td><td>37–85</td><td>30–69</td><td>*About 50 %</td><td>40–70</td></tr><tr><td>Spiculum length (µm)</td><td>24–27</td><td>17–21</td><td>15–20</td><td>23.5–28</td><td>23–28</td></tr><tr><td>Bursa length (as % of tail length)</td><td>50–70</td><td>48–66</td><td>20–55</td><td>72–76</td><td>40–70</td></tr><tr><td>~~Host preference (helpful information in case of confusing morphological criteria)~~</td><td>~~Higher plants and mycelia of fungi~~</td><td>~~Groundnut and fungi~~</td><td>~~Mycelia of fungi~~</td><td>~~Higher plants~~</td><td>~~Higher plants and fungi~~</td></tr></table>		
86.	*103*	Technical	**4.2 Molecular identification of *D. dipsaci and D. destructor***	It would be useful to add methodology for specimen conservation to date of extraction.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina
87.	*110*	Editorial	Please check carefully the statements in this paragraph. The molecular analysis of rDNA sequences including the internal transcribed spacer (ITS)1-5.8S-ITS2 region, the D2–D3 fragment of the s8S gene, the small 18S subunit, the partial mitochondrial gene for cytochrome c oxidase I (mitochondrial (mt)DNA) and *hsp90* gene sequences (nuclear (n)DNA) clearly distinguishes *D. gigas* from *D. dipsaci* s.s. (Vovlas *et al*., 2011).	It should be ITS1-5.8S-ITS2 region or ITS1-5.8S rRNA-ITS2 region? 28S gene or 28S rRNA gene? small 18S subunit or 18S rRNA gene? Which is the right one?	China

<table>
<tr><th>Comm. no.</th><th>Para. no.</th><th>Comment type</th><th>Comment</th><th>Explanation</th><th>Country</th></tr>
<tr><td>88.</td><td>115</td><td>Substantive</td><td>Please check carefully if D. dipsaci should be D. Destructor or not in this paragraph.

Powers et al. (2001) first sequenced the ITS1 region for D. dipsaci. More than 50 sequence accessions of rRNA fragments obtained from D. destructor collected from different localities and host plants are presently available in the GenBank.</td><td>The logic of this paragraph seems unreasonable. The first part is on D.dipsaci, the next part becomes talking about D. Destructor.</td><td>China</td></tr>
<tr><td>89.</td><td>117</td><td>Substantive</td><td>Several juveniles or adults are transferred into a microtube and used for extraction of DNA. DNA extraction is described by Webster et al. (1990~~); other DNA extraction methods are provided in each test described below~~.</td><td>Because no DNA extraction methods are provided in the following text.</td><td>China</td></tr>
<tr><td>90.</td><td>123</td><td>Editorial</td><td>The amplicons are 900 base pairs (bp) for both D. dipsaci and D. myceliophagus, and 1 200 bp for D. destructor.</td><td>An added comma for more clarity.</td><td>EPPO, European Union, Georgia, Serbia</td></tr>
<tr><td>91.</td><td>125</td><td>Technical</td><td>Please check carefully ‘…40 cycles of 45s ant 96℃, 30s at 50℃ and 4 min at 72℃…’, 4 min is correct ?

The PCR cycling parameters consist of a first cycle of 1.5 min at 96 °C, 30 s at 50 °C and 4 min at 72 C; 40 cycles of 45 s at 96 °C, 30 s at 50 °C and 4 min at 72 °C; and a final cycle of 45 s at 96 °C, 30 s at 50 °C and 10 min at 72 °C.[1] After DNA amplification, 2–5 µl of the product is run on a 1% agarose gel. The remainder is stored at –20 °C and used for RFLP. Several restriction enzymes are useful for identifying D. destructor and D. dipsaci from other Ditylenchus species; for example, HaeIII, HpaII, HinfI and RsaI (Wendt et al., 1993). The lengths of the restriction fragments generated by these diagnostic enzymes are given in Table 3.</td><td>Usually, 1-2min at 72℃ is applicable.</td><td>China</td></tr>
<tr><td>92.</td><td>127</td><td>Substantive</td><td><table>
<tr><th>Enzyme</th><th>D. destructor</th><th>D. myceliophagus</th><th>D. dipsaci</th><th>D. gigas (giant race of D. dipsaci)</th><th>D. africanus</th></tr>
<tr><td>Unrestricted PCR product</td><td>1 200</td><td>900</td><td>900</td><td>900</td><td>1 000</td></tr>
<tr><td>HaeIII</td><td>450, 170</td><td>450, 200</td><td>900</td><td>800, 200</td><td>650, 540</td></tr>
<tr><td>HpaII</td><td>1 000</td><td>900</td><td>320, 200, 180</td><td>600, 200</td><td>950</td></tr>
</table></td><td>According to the published paper, D.destructor includes at least two genotypes, i.e. genotype A and B. In this standard, the RFLP pattern of genotype B is provided, but the pattern of genotype A is not. Please add the RFLP pattern of genotype A of Ditylenchus destructor. Reference1: Ji, L., Wang, J.C., Yang, X.L., Huang, G.M. & Lin, M.S. 2006. PCR-RFLP patterns for differentiation of three Ditylenchus species. Journal of Nanjing Agricultural University, 29: 39-43 (in Chinese). reference2: Sergei A. SUBBOTIN, et al. 2011. Length variation and repetitive sequences of Internal Transcribed Spacer of ribosomal RNA gene, diagnostics and relationships of populations of potato rot</td><td>China</td></tr>
</table>

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			<table><tr><td>*Hin*fI</td><td>780, 180</td><td>630, 310</td><td>440, 350, 150</td><td>350, 150</td><td>450, 340, 150, 130, 100</td></tr><tr><td>*Rsa*I</td><td>600, 250, 170</td><td>900</td><td>450, 250, 140</td><td>490, 450</td><td>690, 450</td></tr></table> In table 3, add information on the RFLP pattern of another genotype of *D.destructor*.	nematode, Ditylenchus destructor Thorne, 1945 (Tylenchida: Anguinidae). Nematology, 13(7): 773-785	
93.	*140*	Editorial	The amplicon is approximately 242 bp for *D. dipsaci* (normal race) and 198 bp for *D. dipsaci* (giant race). For both primer sets, no amplification is observed with non-target species, and non-target race (Esquibet *et al*., 2003).	A comma to be deleted.	EPPO, European Union, Georgia, Serbia
94.	*149*	Substantive	The 25 µl PCR mixture is composed of: 1X from 10X PCR buffer including 15 mM $MgCl_2$, 0.2 mM each dNTP, 60 nM each primer and 1 U Taq DNA polymerase. The PCR is performed in a 96-well Peltier type thermocycler (PTC100, MJ Research) with the following cycling parameters: initial 4 min at 94 °C; 35 cycles of 15 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.	The commercial brands should be mentioned in a correspondent footnote or deleted.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina
95.	*157*	Substantive	The 25 µl PCR mixture is composed of: 1× Taq buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 10 pmol each primer (PF1-PR1 primer set) and 1.5 U Taq DNA polymerase (Fermentas). The PCR assay was developed on a 96-well Peltier type thermocycler (PTC200, MJ Resarch), with the following cycling parameters: 3 min at 94 °C; 30 cycles of 2 min at 94 °C, 30 s at 62 °C and 2 min at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.	The commercial brands, “Fermentas” and “PTC200, MJ Resarch”, should be mentioned in a correspondent footnote or deleted.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina
96.	*161*	Editorial	Two specific primer sets are used, one for the identification of *D. dipsaci* alone and one for the identification *of D. gigas* and *D. dipsaci*. The use of both primer sets allows to separate *D. gigas* from *D. dipsaci*., and they are:	One "of" and a blank (after the first occurrence of "gigas") are missing.	EPPO, European Union, Georgia, Serbia
97.	*170*	Substantive	The 20 µl PCR mixture is composed of: 1.5 mM amplification buffer with final $MgCl_2$ concentration of 5 mM, 200 µM each dNTP, 0.5 µM each primer (in case of simplex PCR withDdpS1-rDNA2 or DdpS2-rDNA2; in case of duplex PCR, the final concentration of DdpS1 primer is 0.5µM wheras it is 1µM for DdpS2 and rDNA2) and 1 U Taq DNA polymerase (MP Biomedicals). The PCR was developed on a 96-well Peltier type thermocycler (GeneAmp 9600 PCR System, Perkin Elmer),with the following cycling parameters: 1 min at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 60 C and 45 s at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.	The commercial brands should be mentioned in a correspondent footnote or deleted.	COSAVE, Uruguay, Chile, Brazil, Peru, Argentina
98.	*183*	Substantive	The 25 µl PCR mixture is composed of: 1× PCR buffer (Fermentas), 1.5 mM $MgCl_2$, 200 µM each dNTP, 10 pmol each primer (either DIT_2 or DIT_5 primer set), 1.5 U Taq DNA polymerase (Fermentas) and 50 ng DNA as template. The PCR is	The commercial brands should be mentioned in a correspondent footnote or deleted.	COSAVE, Uruguay, Chile,

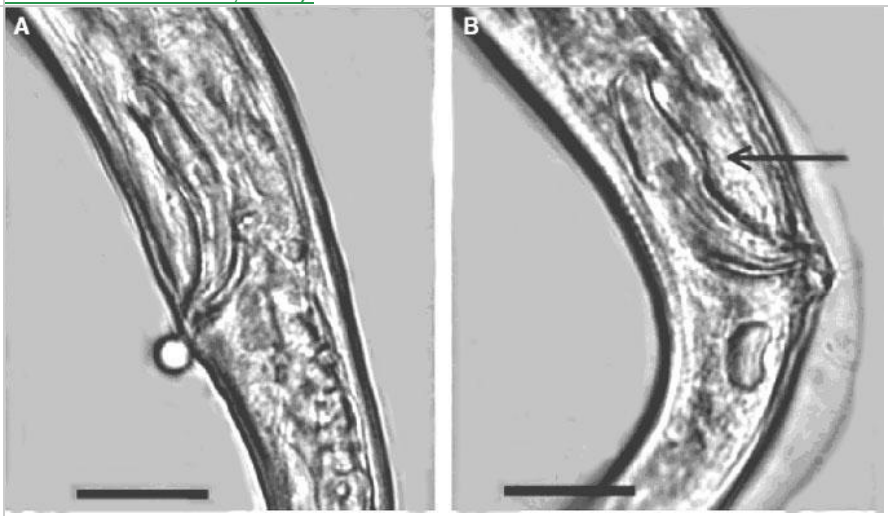
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			performed in a 96-well Peltier type thermocycler (PTC200, MJ Research), with the following cycling parameters: 3 min at 94 °C; 30 cycles of 1 min at 94 °C, 30 s at 60 °C and 1 min at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.		Brazil, Peru, Argentina
99.	*184*	Substantive	**4.2.8 Controls for molecular tests** **Add internal control.**	For conventional PCR,internal controls (House Keeper Gene (HKG)) such as the general eukaryotic 28S rRNA gene or the COI gene should be incorporated into the PCR protocols to eliminate the possibility of PCR false negatives due to nucleic acid extraction failure or degradation or the presence of extraction inhibitors.	China
100.	*191*	Technical	This control is used to monitor contamination during nucleic acid extraction. This requires nucleic acid extraction and subsequent amplification of extraction buffer only. It is recommended that multiple controls are included when large numbers of positives are expected. Add:1. conventional PCR identification of D.destructor. 2.DNA barcoding method for D.destructor and D.dipsaci identification. 3.usage of molecular test results.	1.Because related method for D.destructor has been established. Reference: Sergei A. SUBBOTIN, et al. 2011. Length variation and repetitive sequences of Internal Transcribed Spacer of ribosomal RNA gene, diagnostics and relationships of populations of potato rot nematode, Ditylenchus destructor Thorne, 1945 (Tylenchida: Anguinidae). Nematology, 13(7): 773-785 2.Because the test results of DNA barcoding are more reliable and extensively accepted, especially in China. Reference1: Subbotin S. A., et al. 2005. Molecular diagnostics, taxonomy, and phylogeny of the stem nematode Ditylenchus dipsacispecies complex based on the sequences of the internal transcribed spacer rDNA. Phytopathology, 95: 1308-1315. Reference2: Vovlas N. et al . 2011. Ditylenchus gigasn. sp. parasitizing broad bean: a new stem nematode singled out from theDitylenchus dipsaci species complex using a polyphasic approach with molecular phylogeny. Plant Pathology, 60, 762–775. References3: WANG,J.,et al. 2007. Alignments of rDNA-ITS sequences and phylogeny of different geo-populations of Ditylenchus destructorin China.] Journal of	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				Agricultural University of Hebei30, 79-84. And other related references 3.It is necessary to give an explanation for how to use the molecular test results. Molecular methods need to be used in combination with morphology, or can be used alone as the basis for pest identification.	
101.	*200*	Editorial	Biosystematics Division, ARC-PPRI, Private Bag X134, Queenswood, 0121 Republic of South Africa	Paragraphs [200] and [201] should be merged.	EPPO, European Union, Georgia, Serbia
102.	*201*	Editorial	(Ms Antoinette Swart; e-mail: SwartA@arc.agric.za).	Paragraphs [200] and [201] should be merged.	EPPO, European Union, Georgia, Serbia
103.	*206*	Editorial	This protocol was drafted by Antoinette Swart (Nematology Unit,Biosystematics Division, ARC-PPRI, Private Bag X134, Queenswood, 0121 Republic of South Africa) ~~and~~, Eliseo Jorge Chaves (INTA-Estación Experimental de Balcarce, Laboratorio de Nematología, Casilla de Correo 276, 7620 Balcarce, Argentina) and Renata C.V. Tenente (EMBRAPA, Recursos Genéticos e Biotecnología, Caixa Postal 2372 (70849-970) Brasilia, DF, Brazil).	First "and" to be replaced by a comma and ", Brazil" to be added.	EPPO, European Union, Georgia, Serbia
104.	*220*	Technical	**Brown, D.J.F., Dalmasso, A. & Trudgill, D.L.** 1993. Nematode pests of soft fruits and vines. *In* K. Evans, D.L. Trudgill & J.M. Webster, eds. *Plant parasitic nematodes in temperate agriculture*, pp. 427–462. Wallingford, UK, CAB International. 656 pp. Brzeski, M.W. (1998) Nematodes of Tylenchina in Poland and temperate Europe. Muzeum i Instytut Zoologii Polska Akademia Nauk, Warsaw (PL), 397 pp.	New reference added.	EPPO, European Union, Georgia, Serbia
105.	*225*	Editorial	**Courtney, W. D.** 1962. Stem nematode of red clover in the Pacific Northwest. *Bulletin of the Washington State Agricultural Experiment Station, ~~no. 640~~* 640: 1–17.	Consistency with the other references.	EPPO, European Union, Georgia, Serbia
106.	*226*	Editorial	**De Ley, P. & Blaxter, M.** 2003. A new system for Nematoda: Combining morphological characters with molecular trees, and translating clades into ranks and taxa. *Nematological Monographs and Perspectives,* 2: 1–21.	To be put after paragraph [227] (alphabetical order of the authors).	EPPO, European Union, Georgia, Serbia
107.	*230*	Technical	**EPPO** (European and Mediterranean Plant Protection Organization). 2013b. *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology.* EPPO Technical Document No. 1056 (Rev. 4). Available at http://www.eppo.int/QUARANTINE/diag_activities/EPPO_TD_1056_Glossary.pdf.	New reference added.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			EPPO (European and Mediterranean Plant Protection Organization). 2013c. EPPO Standard PM 7/119(1) Nematode extraction. Bulletin OEPP/EPPO Bulletin, 43, 471-485.		
108.	*246*	Editorial	**Ji, L., Wang, J.C., Yang, X.L., Huang, G.M. & Lin, M.S.** 2006. [PCR-RFLP patterns for differentiation of three Ditylenchus species. Journal of Nanjing Agricultural University,~~three species: *Ditylenchus.*] *Nanjing Agricultural University Journal*~~, 29: 39-43 (in Chinese).	Some small errors exist in the title of the reference paper and its journal name.	China
109.	*252*	Editorial	**Marek, M., Zouhar, M., Rysanek, P. & Havranek, P.** 2005. Analysis of ITS sequences of nuclear rDNA and development of a PCR-based assay for the rapid identification of the stem nematode *Ditylenchus dipsaci* (Nematoda: Anguinidae) in plant tissues. *Helminthologia,* 42: 49–56.	A full stop is missing after "2005".	EPPO, European Union, Georgia, Serbia
110.	*275*	Editorial	**Subbotin, S.A., Madani, M., Krall, E., Sturhan, D. & Moens, M.** 2005~~.~~. Molecular diagnostics, taxonomy and phylogeny of the stem nematode *Ditylenchus dipsaci* species complex based on the sequences of the ITS-rDNA. *Phytopathology,* 95: 1308–1315.	The full stop after "2005" shouldn't be in bold.	EPPO, European Union, Georgia, Serbia
111.	*282*	Editorial	**Wendt, K.R., Vrain, T.C. & Webster, J.M.** 1993. Separation of three species of *Ditylenchus* and some host races of *D. dipsaci* by restriction fragment length polymorphism. *Journal of Nematology,* 25: 555–563.	Paragraph [282] should be after paragraph [283] (alphabetical order of the authors).	EPPO, European Union, Georgia, Serbia
112.	*284*	Editorial	**Zouhar, M., Marek, M., Licinio, J. & Ryšánek, P.** 2002. Using point mutations in rDNA for differentiation of bioraces of *Ditylenchus dipsaci* from the Czech Republic. *Plant Protection Science,* 38 (Special 2): 358–360.	Paragraph [284] should be after paragraph [285] (alphabetical order of the authors).	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
113.	*292*	Technical	 Photo G. Caubel, Nemapix (1999). (New Figure 2A added)	A new photograph and its description added.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Figure 2A Young *Allium cepa* plants infected by *Ditylenchus dipsaci* Photo E. Hennig. The State Plant Health and Seed Inspection Service, Torun, Poland		
114.	*294*	Editorial	**Figure 3.** Narcissus bulb infected by *Ditylenchus dipsaci.*	It looks like garlic (cf. paragraph [40] where this figure is quoted).	EPPO, European Union, Georgia, Serbia
115.	*307*	Technical	 Photo S. Ayoub, Nemapix (2000). (New Figure 7A added) Figure 7A. Potatoes of various level of infestation by *Ditylenchus destructor* Photo H. Andersen, Denmark	New photograph □and its description added.	EPPO, European Union, Georgia, Serbia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
116.	*311*	Technical	**Figure 9.***Ditylenchus destructor* Thorne, 1945 (after Sturhan and Brzeski, 1991). (A), female, oesophageal region; (B), head of female; (C), male, spicule region; (D), tail tips of two females; (E), posterior region of female; (F), lateral field at midbody. Each unit marking on bars, 10 µm. (New Figure 10 added) Figure 10: *Ditylenchus* spiculum photographs. (A) *D. dipsaci;* (B) *D. destructor,* arrow=tumulus. Each bar=12µm (after Karssen & Willemsen, 2010).	New phtotograph added.	EPPO, European Union, Georgia, Serbia
117.	*312*	Technical	 **Fig 10: Ditylenchus spiculum photographs. (A) D. dipsaci, (B) D. destructor, arrow = tumulus. Each bar = 12µm (after Karssen & Willemsen, 2010)** **Footnote 1:** The PCR cycling conditions are those described in the original article (Wendt *et al.*, 1993). Improvement of thermocyclers and reagents for PCR may lead to revision of these cycling parameters.	Add a Fig. 10 before this last paragraph	EPPO, European Union, Georgia, Serbia