2006-017: Draft Annex to ISPM 27– Genus Liriomyza

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
1.	*G*	Editorial		1. The quality of the pictures and drawings should be improved. Better quality files of existing drawings have been prepared and will be provided directly to the IPPC Secretariat. 2. It would also be suitable to have better quality pictures and sometimes additional pictures to illustrate some characters. Comments have been included whenever appropriate. However, it is recognized that specimen must be available for better pictures to be made and this is not always the case. 3. The addition of the figure 14 of PM 7/53 on Liriomyza spp., on male genitalia is suggested, a better quality figure will be provided. It is also suggested that links to figures 9 and 10 of the current Diagnostic Protocol are made.	European Union
2.	*G*	Substantive	I support the document as it is and I have no comments		Guyana, Congo, Singapore, Mexico
3.	*G*	Substantive	Footnotes related to the use of commercial brands should be included in this draft DP.	The following paragraphs mention commercial brands: 145. The footnote should read as follows: "The use of the brands..... in this diagnostic protcol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsment by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results"	Uruguay, Argentina, Chile
4.	*6*	Technical	Agromyzidae is a family of small flies whose larvae feed on the internal tissue of plants, often as leafminers and stem miners. The majority of agromyzid species are either host-specific or restricted to a small group of plants that are related to each other. However, a few highly polyphagous species have become agricultural and horticultural pests of economic importance in many	to be more specific	Kenya

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			parts of the world. These include four species of *Liriomyza* that are listed in plant quarantine legislation in various countries: L. bryoniae, L. huidobrensis, L. sativae and L. trifolii. These are all polyphagous pests of both ornamental and vegetable crops. The species level identification in this protocol is restricted to these four species.		
5.	*9*	Editorial	*Liriomyza huidobrensis* is thought to have originated in South America and has now spread throughout much of the world, including parts of North America, Europe, Africa, Asia and the Pacific (Lonsdale, 2011; CABI, 2013). However, the species as formerly taxonomically defined was recently split into two morphocryptic species – *L. huidobrensis* and *L. langei* – and there is some uncertainty about the precise delineation of their relative distribution. Currently, *L. langei* has been confirmed only from the United States and it~~s~~ seems highly likely that all invasive populations outside the United States are *L. huidobrensis* as now taxonomically defined (Scheffer and Lewis, 2001; Scheffer *et al*., 2001; Takano *et al*., 2008; Lonsdale, 2011). *L. huidobrensis* is highly polyphagous and has been recorded from 14 plant families (Spencer, 1990). The most economically important crops it attacks are sugar beets, spinach, peas, beans, potatoes and ornamental (most commonly gypsophila; rarely carnations and chrysanthemums) (Spencer, 1989), as well as lupins, field peas and broad beans.	Minor edit in 3rd sentence - "is" should be "it"	Canada
6.	*9*	Editorial	*Liriomyza huidobrensis* is thought to have originated in South America and has now spread throughout much of the world, including parts of North America, Europe, Africa, Asia and the Pacific (Lonsdale, 2011; CABI, 2013). However, the species as formerly taxonomically defined was recently split into two morphocryptic species – *L. huidobrensis* and *L. langei* – and there is some uncertainty about the precise delineation of their relative distribution. Currently, *L. langei* has been confirmed only from the United States and it~~s~~ seems highly likely that all invasive populations outside the United States are *L. huidobrensis* as now taxonomically	Typo	Australia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			defined (Scheffer and Lewis, 2001; Scheffer *et al*., 2001; Takano *et al*., 2008; Lonsdale, 2011). *L. huidobrensis* is highly polyphagous and has been recorded from 14 plant families (Spencer, 1990). The most economically important crops it attacks are sugar beets, spinach, peas, beans, potatoes and ornamental (most commonly gypsophila; rarely carnations and chrysanthemums) (Spencer, 1989), as well as lupins, field peas and broad beans.		
7.	*9*	Substantive	Delete all contents of L.langei in the draft.*Liriomyza huidobrensis* is thought to have originated in South America and has now spread throughout much of the world, including parts of North America, Europe, Africa, Asia and the Pacific (Lonsdale, 2011; CABI, 2013). However, the species as formerly taxonomically defined was recently split into two morphocryptic species – *L. huidobrensis* and *L. langei* – and there is some uncertainty about the precise delineation of their relative distribution. Currently, *L. langei* has been confirmed only from the United States and is seems highly likely that all invasive populations outside the United States are *L. huidobrensis* as now taxonomically defined (Scheffer and Lewis, 2001; Scheffer *et al*., 2001; Takano *et al*., 2008; Lonsdale, 2011). *L. huidobrensis* is highly polyphagous and has been recorded from 14 plant families (Spencer, 1990). The most economically important crops it attacks are sugar beets, spinach, peas, beans, potatoes and ornamental (most commonly gypsophila; rarely carnations and chrysanthemums) (Spencer, 1989), as well as lupins, field peas and broad beans.	It is impossible to identify L.langei and L.huidobrensis based on adult morphology (Spencer 1973) and molecular techniques(Kox et al.2005). And it is still controversial on the synonyms of L.langei with L.huidobrensis. Therefore, the disputed species of L.langei at species level should not be included in draft.	China
8.	*9*	Technical	*Liriomyza huidobrensis* is thought to have originated in South America and has now spread throughout much of the world, including parts of North America, Europe, Africa, Asia and the Pacific (Lonsdale, 2011; CABI, 2013). However, the species as formerly taxonomically defined was recently split into two morphocryptic species – *L. huidobrensis* and *L. langei* – and there is some uncertainty about the precise delineation of their relative distribution. Currently, *L. langei* has been	Chabi-Olaye et al., 2008; Europhyte, 2015	Kenya

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			confirmed only from the United States and is seems highly likely that all invasive populations outside the United States are *L. huidobrensis* as now taxonomically defined (Scheffer and Lewis, 2001; Scheffer *et al*., 2001; Takano *et al*., 2008; Lonsdale, 2011). *L. huidobrensis* is highly polyphagous and has been recorded from 14 plant families (Spencer, 1990). The most economically important crops it attacks are sugar beets, spinach, peas, beans, potatoes add herbs and ornamental (most commonly gypsophila; rarely carnations and chrysanthemums) add eryngium, solidago and Dahlia (Spencer, 1989), as well as lupins, field peas and broad beans.		
9.	*11*	Editorial	*Liriomyza trifolii*, also originally from North, Central and South America, has been spread to large parts of Europe, Africa, Asia and the Pacific, most likely as the result of trade in *Chrysanthemum* cuttings (Martinez and Etienne, 2002; EPPO, 2009; Lonsdale, 2011; CABI, 2013). It is highly polyphagous and has been recorded from 25 plant families (Spencer, 1990). The most economically important crops it attacks are beans, celery, chrysanthemums, cucumbers, gerberas, gypsophila, lettuce, onions, potatoes and tomatoes (Spencer, 1989), as well as peanuts, groundnuts, soybeans, lentils, lupins, broad beans and chickpeas.	The reference EPPO 2009 is not included in the reference list The EPPO Secretariat was not able to identify a possible reference that could match the text	European Union
10.	*12*	Editorial	A further (fifth) species, *L. strigata*,is closely related to both *L. bryoniae* and *L. huidobrensis*, and is as such a species that a diagnostician must be able to eliminate when seeking to positively identify the four quarantine species. *L. strigata* is a~~n~~ Eurasian species (Pitkin *et al.* (2013) quoting Spencer (1976), Dempewolf (2001), Ellis (2013) and Pape *et al.* (2013)). The eastern borders of its distribution are not clearly defined, but the range extends beyond the Ural Mountains (Spencer, 1976) and it has been doubtfully recorded in Southeast Asia (Dempewolf, 2004). It is highly polyphagous, having been recorded from 29 plant families worldwide (Spencer, 1990).	Minor edit in first sentence - "an" should be "a"	Canada
11.	*12*	Editorial	A further (fifth) species, *L. strigata*,is closely related to both *L. bryoniae* and *L. huidobrensis*, and is as such a	Regarding 'Pitkin et al. (2013)' : The reference is dated 2014 but using the link the page is dated 2015-	European Union

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			species that a diagnostician must be able to eliminate when seeking to positively identify the four quarantine species. *L. strigata* is an Eurasian species (Pitkin *et al.* (2013) quoting Spencer (1976), Dempewolf (2001), Ellis (2013) and Pape *et al.* (2013)). The eastern borders of its distribution are not clearly defined, but the range extends beyond the Ural Mountains (Spencer, 1976) and it has been doubtfully recorded in Southeast Asia (Dempewolf, 2004). It is highly polyphagous, having been recorded from 29 plant families worldwide (Spencer, 1990).	05-31.	
12.	*12*	Technical	A further (fifth) species, *L. strigata* *is included in this protocol because it* ~~,~~is closely related to both *L. bryoniae* and *L. huidobrensis*, and is as such a species that a diagnostician must be able to eliminate when seeking to positively identify the four quarantine species. *L. strigata* is an Eurasian species (Pitkin *et al.* (2013) quoting Spencer (1976), Dempewolf (2001), Ellis (2013) and Pape *et al.* (2013)). The eastern borders of its distribution are not clearly defined, but the range extends beyond the Ural Mountains (Spencer, 1976) and it has been doubtfully recorded in Southeast Asia (Dempewolf, 2004). It is highly polyphagous, having been recorded from 29 plant families worldwide (Spencer, 1990).	A modification of this paragraph is suggested to explain why L. strigata is specifically mentioned in the introduction. This paragraph has caused confusion with the experts as some understood that L. strigata was considered as the only species which can be confused with the quarantine species.	European Union
13.	*13*	Technical	Change Liriomyza sativae (Blanchard, 1938) to Liriomyza sativae Blanchard, 1938.**2. Taxonomic Information**	This species has never been newly combinated.	China
14.	*24*	Substantive	**Common name:** tomato leafminer	Possibility of confusion with other pests, proposing to add fly to specify this insect so the name will be tomato leafminer fly	Tunisia
15.	*26*	Technical	**Synonyms:***Liriomyzacucumifoliae* Blanchard, 1938; *Liriomyza decora* Blanchard, 1954; *Liriomyzadianthi* Frick, 1958, *Agromyza huidobrensis* Blanchard, *Liriomyza langei* Frick.	Mentioned as a synonym in the EPPO Database as well as Liriomyza langei Frick. However we understand that all may not need to be listed.	European Union
16.	*27*	Substantive	Delete all contents of L.langei in the draft.The taxonomic relationship between *L. huidobrensis* (Blanchard) and *L. langei* Frick is complex. *L. huidobrensis* was originally described from	It is impossible to identify L.langei and L.huidobrensis based on adult morphology (Spencer 1973) and molecular techniques(Kox et al.2005). And it is still controversial on the synonyms of L.langei with	China

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			specimens taken from *Cineraria* in Argentina by Blanchard (1926). Frick (1951) described *L. langei* from California as a species that he noted was primarily a pest of peas although it had also damaged *Aster*. In 1973, Spencer then synonymized the two species as they were (and de facto remain) morphologically indistinguishable. Following a study of their mitochondrial and nuclear DNA sequences (Scheffer, 2000; Scheffer and Lewis, 2001), supported by later rearing experiments (Takano *et al*., 2008), the two species were formally separated as two cryptic species (Lonsdale, 2011). The name *L. langei* Frick was resurrected and applied to the cryptic species from California, and the name *L. huidobrensis* (Blanchard) was applied to the cryptic species from South and Central America.	L.huidobrensis. Therefore, the disputed species of L.langei at species level should not be included in draft.	
17.	*28*	Editorial	Lonsdale (2011) attempted to delineate diagnostic morphological characters that could differentiate “most” specimens of the two species, but found the characters “subtle and sometimes overlapping” so he recommended the use of molecular data to support identification whenever possible. Scheffer and her collaborators consider that the ranges of the two species do not overlap (although Lonsdale (2011) recorded *L. huidobrensis* from California, once in 1968 and once in 2008, he states that it is unknown if the populations established), and that all of the invasive populations that they had studied were *L. huidobrensis* as so defined (Scheffer and Lewis, 2001; Scheffer *et al.,* 2001). This means that reports from California in the literature predating Scheffer's papers should almost certainly be considered as applying to *L. langei*. *L. langei* is predominantly a Californian species although it has apparently been introduced into Hawaii, Oregon and Washington; populations found in Florida, Utah and Virginia in the mid-1990s did not establish (Lonsdale, 2011). Only *L. huidobrensis* has been confirmed in Mexico (Lonsdale, 2011), but Takano *et al*. (2005) reported that specimens of *L. langei* (described as the Californian clade) were intercepted ~~in Japan in a~~	For clarification	Japan

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			~~package~~ at Japanese inspection site on fresh vegetables originating from Mexico.		
18.	*28*	Substantive	Delete all contents of L.langei in the draft.Lonsdale (2011) attempted to delineate diagnostic morphological characters that could differentiate “most” specimens of the two species, but found the characters “subtle and sometimes overlapping” so he recommended the use of molecular data to support identification whenever possible. Scheffer and her collaborators consider that the ranges of the two species do not overlap (although Lonsdale (2011) recorded *L. huidobrensis* from California, once in 1968 and once in 2008, he states that it is unknown if the populations established), and that all of the invasive populations that they had studied were *L. huidobrensis* as so defined (Scheffer and Lewis, 2001; Scheffer *et al.,* 2001). This means that reports from California in the literature predating Scheffer's papers should almost certainly be considered as applying to *L. langei*. *L. langei* is predominantly a Californian species although it has apparently been introduced into Hawaii, Oregon and Washington; populations found in Florida, Utah and Virginia in the mid-1990s did not establish (Lonsdale, 2011). Only *L. huidobrensis* has been confirmed in Mexico (Lonsdale, 2011), but Takano *et al*. (2005) reported that specimens of *L. langei* (described as the Californian clade) were intercepted in Japan in a package originating from Mexico.	It is impossible to identify L.langei and L.huidobrensis based on adult morphology (Spencer 1973) and molecular techniques(Kox et al.2005). And it is still controversial on the synonyms of L.langei with L.huidobrensis. Therefore, the disputed species of L.langei at species level should not be included in draft.	China
19.	*30*	Technical	Change Liriomyza sativae (Blanchard, 1938) to Liriomyza sativae Blanchard, 1938.**Name:***Liriomyza sativae* (Blanchard, 1938)	This species has never been newly combinated.	China
20.	*34*	Technical	**Synonyms:***Agromyza phaseolunulata* Frost, 1943; *Liriomyza alliovora* Frick, 1955	More synonyms are listed in databases such as Q-bank and EOL. However we understand that all may not need to be listed.	European Union
21.	*38*	Substantive	Female flies use their ovipositor to puncture the leaves of the host plants, causing wounds that serve as sites for feeding (by both female and male flies) or for oviposition. Feeding punctures of *Liriomyza* species are rounded, usually about 0.2 mm in diameter, and appear	It is not clear because there is no reference to possibility of confusion between Liriomyza sp and Chromatomiya species before this paragraph	Tunisia

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			as white speckles on the upper surface of the leaf. Oviposition punctures are usually smaller (0.05 mm) and more uniformly round. Feeding punctures made by the polyphagous agromyzid pest species *Chromatomyia horticola* and *C. syngenesiae* are distinctly larger and more oval than those made by *Liriomyza* flies. The appearance of feeding and oviposition punctures does not differ among *Liriomyza* species, and the pattern of their distribution on the leaf cannot be used to identify species. Feeding punctures cause the destruction of a large number of cells and are clearly visible to the naked eye (EPPO, 2005).		
22.	*38*	Technical	Female flies use their ovipositor to puncture the leaves of the host plants, causing wounds that serve as sites for feeding (by both female and male flies) or for oviposition. Feeding punctures of *Liriomyza* species are rounded, usually about 0.2 mm in diameter, and appear as white speckles on the upper surface of the leaf. Oviposition punctures are usually smaller (0.05 mm) and more uniformly round. Feeding punctures made by the polyphagous agromyzid pest species *Chromatomyia horticola* and *C. syngenesiae* are distinctly larger and more oval than those made by *Liriomyza* flies. The appearance of feeding and oviposition punctures does not differ among *Liriomyza* species, and the pattern of their distribution on the leaf cannot be used to identify species. Feeding punctures cause the destruction of a large number of cells and are clearly visible to the naked eye (EPPO, 2005).	The comparison of feeding punctures between Chromatomyia and Liriomyza would be more obvious with figures that would show the differences of punctures. Consider adding appropriate figures.	European Union
23.	*40*	Editorial	There are three larval stages, all of which feed within the leaves. The larvae predominantly feed on the plant in which the eggs are laid. The larvae of *Liriomyza* spp. leave the leaf when ready to pupariate (Parrella and Bethke, 1984), and their exit hole characteristically takes the form of a semicircular slit; in contrast, the larvae of *C. horticola* and *C. syngenesiae* pupate inside the leaf at the end of the larval mine, with the anterior spiracles usually projecting out from the lower surface of the leaf.*Liriomyza* puparia~~e~~, therefore, may be found	Minor edit to the final sentence - "pupae" should be "puparia"	Canada

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			in crop debris, in the soil or sometimes on the leaf surface.		
24.	*40*	Technical	There are three larval stages, all of which feed within the leaves. The larvae predominantly feed on the plant in which the eggs are laid. The larvae of *Liriomyza* spp. leave the leaf when ready to pupariate (Parrella and Bethke, 1984), add L.sativae and L. trifolii may pupate on plant leaves and their exit hole characteristically takes the form of a semicircular slit; in contrast, the larvae of *C. horticola* and *C. syngenesiae* pupate inside the leaf at the end of the larval mine, with the anterior spiracles usually projecting out from the lower surface of the leaf.*Liriomyza* pupae, therefore, may be found in crop debris, in the soil or sometimes on the leaf surface.	Literature is avalaible to support	Kenya
25.	*44*	Editorial	• puparia~~e~~: in crop debris, in the soil or sometimes on the external leaf surface	Replace "pupae" with "puparia"	Canada
26.	*45*	Technical	• adult: free-flying, on leaf surfaces while producing feeding and oviposition punctures. more diagrams needed	include diagrams for an egg inserted below the leaf surface, larvae inside mines on leaves, pupae and adult liriomyza	Kenya
27.	*47*	Substantive	Delete Line 3,"Adult females are often identifiable with certainty only to genus level".*Liriomyza* flies can be collected as immature life stages in association with mined leaf samples or as adults. Because the morphological characters used to diagnose species are based on male genitalia, adult males are needed in order to confirm species identification. Adult females are often identifiable with certainty only to genus level. Collecting multiple specimens from a plant or a location will increase the likelihood of obtaining male flies, which is important unless molecular methods are to be used for diagnosis of immature life stages.	Morphology characters of both male and female adults may be applied to diagnosis.	China
28.	*49*	Substantive	1.Add the rearing method for *Liriomyza* spp. in the draft. 2.Change"they can be collected by using sticky traps" to "they can be collected by using yellow sticky traps".Adult flies are normally found on the foliage, and can be collected by hand or swept from the foliage with	1.A great number of references had been cited in the draft standard. It is difficult to find the references for user and not advantage to the use of the standard. Some literatures just for information may not be listed in the draft. 2.The speices of Liriomyza were strongly	China

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			a hand net into glass vials, or collected with a vacuum sampler. Alternatively, they can be collected by using sticky traps, particularly in glasshouses. However, the most practical and reliable method for collecting leafminer flies such as *Liriomyza* species is to collect mined leaves containing live larvae.These can be placed in a large jar for rearing to adult flies in the laboratory. Techniques for rearing agromyzidsare described in Griffiths (1962) and Fisher et al. (2005).	attracted by color of yellow.	
29.	*49*	Technical	Adult flies are normally found on the foliage, and can be collected by hand or swept from the foliage with a hand net into glass vials, or collected with a vacuum sampler. Alternatively, they can be collected by using sticky traps, particularly in glasshouses. However, the most practical and reliable method for collecting leafminer flies such as *Liriomyza* species is to collect mined leaves containing live larvae.These can be placed in a large jar for rearing to adult flies in the laboratory. Techniques for rearing agromyzidsare described in Griffiths (1962) and Fisher et al. (2005). However, live material should not be moved out of quarantine areas.	e.g. L. sativae is in a quarantine zone in the Torres Strait and moving them live for rearing to an unifested area would be prohibited and risk spreading the pest further	Australia
30.	*50*	Substantive	Add dry needle specimens as another stored method for adult.Adults and larvae can be placed in 70% ethanol and stored indefinitely, although their colour fades gradually with time. Vials of specimens in ethanol should be sealed to avoid leakage and packed with cushioning material in a strong box.	Because the gray pubscense of leafminer adults on mesonotum is easily dissolved at 70% ethanol, some key characters are disappeared. Therefore, the dry needle specimens for adult are suggested to be added.	China
31.	*50*	Technical	Adults and larvae can be placed in 70% ethanol and stored indefinitely, although their colour fades gradually with time. Vials of specimens in ethanol should be sealed to avoid leakage and packed with cushioning material in a strong box. Dry storage is also possible.	The fact that dry storage is possible should also be mentioned.	European Union
32.	*51*	Editorial	Specimens required for molecular diagnostic work should be killed and either preserved in 96–100% ethanol and stored frozen (at about –-20 or -80 ºC) or preserved on FTA cards (M. Blacket, personal communication, September 2014). New text not submitted	Specialist comments that specimens do not have to be frozen when in 96-100% ethanol for diagnostic work to be carried out. So the and of and/or could be deleteted.	New Zealand
33.	*53*	Editorial	If the intention is to collect and preserve plant samples, leaves with suspect feeding punctures or mines should	Specialist comments that this is not relevant for this section. The same material is mentioned above under	New Zealand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			be picked and placed between sheets of newspaper to permit slow drying. ~~For laboratory rearing of adult flies, mined leaves containing larvae, or pupae, can be placed in a large jar and kept in a constant temperature room for regular checking.~~	collection of adults - therefore could be removed here.	
34.	*53*	Technical	If the intention is to collect and preserve plant samples, leaves with suspect feeding punctures or mines should be picked and placed between sheets of newspaper to permit slow drying. For laboratory rearing of adult flies, mined leaves containing larvae, or pupae, can be placed in a large jar and kept in a constant temperature room for regular checking.add after emergence, adults should be preserved after, not more than 12 hours	Literature available for support	Kenya
35.	*56*	Substantive	Identification of leafminer species by morphological examination is restricted to adult male specimens because there are no adequate keys for the species-level identification of adult females or for eggs, larvae or pupae. Identification of adult material is possible by examination of morphological characters, in particular the genitalia of the male fly. The morphological characters of the male genitalia are examined under a high-power microscope (at about 100× magnification). Using this protocol with good quality preparations should allow adults of the four quarantine species of *Liriomyza* to be identified with certainty by morphological examination alone (with the exception of *L. huidobrensis* and *L. langei* for the reasons discussed in section 1).	As it is mentioned, identification of leafminer species by morphological examination is restricted to adult male specimens because there are no adequate keys for the species-level identification of adult females or for eggs, larvae or pupae. In case of infested imported plants with different life stages or adult other than adult male, what countries (which do not use the molecular methods) can use for identification?	Bahrain
36.	*57*	Substantive	Molecular methods for identification can be applied to all life stages, including the immature stages for which morphological identification to species level is not possible. Additionally, in cases where adult specimens are atypical or damaged, molecular assays may provide further relevant information about identity. However, the specificity of molecular assays may be limited as they will have been developed for a purpose and evaluated against a restricted number of species, using samples from different geographic regions. Therefore, the results	As it is mentioned, the specificity of molecular assays may be limited as they will have been developed for a purpose and evaluated against a restricted number of species, using samples from different geographic regions. It is required to give more clarification about using samples from different geographical regions.	Bahrain

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			from molecular assays need to be carefully interpreted.		
37.	*59*	Technical	Examination of the male genitalia (in particular, the distiphallus, see Figure 9) is necessary in order to obtain a positive identification for any of the four target species of *Liriomyza*. A brief account of a satisfactory method of preparing specimens (based on Malipatil and Ridland, 2008) is outlined below. More details on or variations to the method are provided by Spencer (1981, 1992), Spencer and Steyskal (1986) and EPPO (2005). Evidence of distiphallic structure should be compared with characters of external morphology (Table 1) in order to confirm the species identification.	Figure 9 provides the illustration of distiphallus and could quoted here, Mr Collins (one of the author of the protocol) considered that rearrangement of the pictures is however needed.	European Union
38.	*64*	Technical	The abdomen should be removed from the body to enable clearing of tissues and observation. This can be accomplished by using fine dissecting needles (which can be made by gluing the blunt end of pointed micro pins into the end of a wooden matchstick, first making a shallow hole with a normal pin), to carefully separate the abdomen from the rest of the fly. The abdomen can be boiled in 10% potassium hydroxide (KOH) for 2–4 min or, alternatively, left in cold 10% KOH overnight to clear the tissues. Transferring the treated abdomen to cold (about 4 °C) glacial acetic acid for 2–3 min will neutralize the KOH. Excess glacial acetic acid can be removed by blotting the abdomen. The abdomen is then ready for transfer to a drop of Hoyer's medium (50 ml water, 30 g gum arabic, 200 g chloral hydrate, 20 ml glycerine) on a cavity slide.	1. Alternative procedure of temporary preparation is proposed to avoid or reduce the used of harmful or toxic solutions 2. IPPC protocol should avoid as much as possible recommending chemistry that is known to be toxic (e.g Hoyer's medium).. In any case there is more than one way for clearing or mounting procedures and whatever is proposed in the IPPC text should be indicated as one of many possibility. We suggest the addition of the following sentence 3. Alternative methods and chemicals can also produce suitable slide mounts. A procedure recommended in French laboratories involves less toxic chemical and is presented below. The abdomen can be boiled in 10% potassium hydroxide (KOH) for 2–4 min or, alternatively, left in cold 10% KOH overnight to clear the tissues. transferring the treated abdomen in bath of distilled water will neutralize the KOH. The abdomen is then ready for transfer to a drop of glycerol on a cavity slide.	European Union
39.	*64*	Technical	10% Sodium hydroxide (NaOH) is recommended to add as one of selective solutions. The abdomen should be removed from the body to enable clearing of tissues and observation. This can be accomplished by using fine dissecting needles (which can be made by gluing the blunt end of pointed micro pins into the end of a wooden matchstick, first making a shallow hole with a normal pin), to carefully separate the abdomen from the	NaOH has the same function as KOH and can be used to clear the tissues.	China

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			rest of the fly. The abdomen can be boiled in 10% potassium hydroxide (KOH) for 2–4 min or, alternatively, left in cold 10% KOH overnight to clear the tissues. Transferring the treated abdomen to cold (about 4 °C) glacial acetic acid for 2–3 min will neutralize the KOH. Excess glacial acetic acid can be removed by blotting the abdomen. The abdomen is then ready for transfer to a drop of Hoyer's medium (50 ml water, 30 g gum arabic, 200 g chloral hydrate, 20 ml glycerine) on a cavity slide.		
40.	*65*	Substantive	Under a binocular stereoscopic microscope and using the fine dissecting needles, the genital complex is carefully dissected out from the surrounding membranes, cuticle and associated musculature. Using the fine dissecting needles, the genital complex is positioned for lateral viewing under a compound microscope at up to 400× magnification. The genital complex is repositioned for ventral viewing of the distiphallus at 400× magnification. New text not supplied	Specialist comments: Dissections are better done in ethanol or if on a cavity slide in glycerol. Hoyre's is too viscus and would be difficult to transfer to Hoyer's again when making semi-permanent slides and may be damaged.	New Zealand
41.	*65*	Technical	Under a binocular stereoscopic microscope and using the fine dissecting needles, the genital complex is carefully dissected out from the surrounding membranes, cuticle and associated musculature. Using the fine dissecting needles, the genital complex is positioned for lateral viewing under a compound microscope at up to 400× magnification~~. The genital complex is repositioned for ventral viewing of the distiphallus at 400× magnification~~, without the addition of a cover slip. The distiphallus needs to be viewed in different orientations (e.g; lateral, dorsal/ventral) which requires repositioning under a lower magnification.~~.~~	For a good identification, it is important to orientate the distiphallus. At 400x magnification, it is impossible to do so. The added sentence reminds that this positioning of the distiphallus is necessary and it explains how to do it.	European Union
42.	*66*	Technical	To make semi-permanent slides (e.g. for routine identification), the genital complex should be transferred to a drop of ~~Hoyer's medium~~glycerol on a clean flat slide. The genitalia are immersed gently in the mountant, and a round coverslip is lowered carefully	To avoid the use of toxic product, we suggest to replace the Hoyer's medium by glycerol	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			over it to evenly spread the mountant.		
43.	*67*	Technical	If permanent slide mounts are required, the abdomen should be cleared in KOH and neutralized in cold glacial acetic acid as described above. Then, the abdomen can be transferred to 70% ethanol and, using the fine dissecting needles under a binocular stereoscopic microscope, the genital complex carefully dissected from the surrounding membranes, cuticle and associated musculature. The dissected genitalia should be transferred first to absolute ethanol for 2–4 min, and then to clove oil (in which, if necessary, the genitalia can be stored for any length of time). The genitalia should be transferred to a drop of Euparal on a clean flat slide and orientated in the mountant. A round coverslip should be lowered carefully onto the drop, commencing at its edge, evenly spreading the mountant. Finally, the slide should be placed in an incubator (about 45 ºC) to dry for two weeks. All slide mounts must be labelled with adequate data, detailing host, locality, date of collection and name of collector and code/label to link back to remaining specimen.	Add the phrase "and code/label to link back to the remaining specimen".	Canada
44.	*67*	Technical	If permanent slide mounts are required, the abdomen should be cleared in KOH and neutralized in cold glacial acetic acid as described above. Then, the abdomen can be transferred to 70% ethanol and, using the fine dissecting needles under a binocular stereoscopic microscope, the genital complex carefully dissected from the surrounding membranes, cuticle and associated musculature. The dissected genitalia should be transferred first to absolute ethanol for 2–4 min, and then to clove oil (in which, if necessary, the genitalia can be stored for any length of time). ~~The genitalia should be transferred to a drop of Euparal on a clean flat slide and orientated in the mountant. A round coverslip should be lowered carefully onto the drop, commencing at its edge, evenly spreading the mountant. Finally, the slide should be placed in an incubator (about 45 ºC) to dry for two weeks.~~ The genitalia	1 Euparal is a toxic product. A non toxic procedure is proposed instead. 2 Species is crucial as well as identifier. Collector is not always crucial in a framework of quarantine diagnostics	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			is transferred to 70% ethanol (approximately 10 minutes), then to 95% ethanol (approximately 10 minutes) and finally in clove oil (at least 5 minutes). The genitalia can then be permanently mounted on a slide in a drop of Canada balsam under a cover slip. All slide mounts must be labelled with adequate data, detailing species, host, locality/country of origin, date of collection and name of ~~collector~~identifier.		
45.	*68*	Technical	The remainder of the fly specimen should be mounted onto a card point with an appropriate label cross-referenced to its genitalia mounted on the slide or stored in ethanol.	An alternative option to store the specimen in ethanol should be included;	European Union
46.	*72*	Technical	The following combination of characters define the family Agromyzidae (Hennig, 1958; Spencer, 1987) (Figure 7):	To help using the key illustrations would be useful and correspondence between the key terminology and the figures ensured. The paragraph where pictures would be most useful are indicated	European Union
47.	*74*	Technical	• vibrissae present	A new figure should be added	European Union
48.	*75*	Technical	• 1–7 frontal setae present	A new figure should be added	European Union
49.	*76*	Technical	• wing with costal break present at the apex of Sc	A new figure should be added	European Union
50.	*77*	Technical	• wing cell cup small; wing veins A_1+CuA_2 not reaching wing margin	A new figure should be added	European Union
51.	*78*	Technical	• male with pregenital sclerites with a fused tergal complex of tergites 6–8, with only two spiracles between tergite 5 and the genital segment (Fig. 6a).	This characteristic is illustrated with figure 6a. A reference should be made to it.	European Union
52.	*79*	Technical	• female with the anterior part of abdominal segment 7 forming an oviscape (Fig. 6a).	This characteristic is illustrated with figure 6a. A reference should be made to it.	European Union
53.	*83*	Substantive	Adult flies of the genus *Liriomyza* have the following morphological characters (EPPO, 2005):	1. Consider adding appropriate illustration for the different points of the key to allow an easy use. 2. Two figures are available in the current which may improve the understanding EPPO diagnostic protocol Fig 3 and 4	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
54.	*83*	Substantive	Adult flies of the genus *Liriomyza* have the following morphological characters (EPPO, 2005; Brown et al., 2010):	To add the paper cited regarding the subcostal vein as mentioned below (after paragraph 86).	Japan
55.	*86*	Technical	• scutellum yellow in most species, rarely dark • the subcostal vein reaches the costal vein	Add the following description about the subcostal vein, which is characteristic of Phytomyzinae including Liriomyza. It is appropriate to add this description since it is a useful key and an important point in narrowing down for identification.	Japan
56.	*88*	Technical	• discal cell (dm) small	Show where dm is in figure	European Union
57.	*89*	Technical	• second (outer) crossvein (dm-cu) present in most species	Show where dm-cu is in figure	European Union
58.	*90*	Technical	• stridulating organ present in males (a “scraper”, a chitinized ridge on the hind femora; and a “file”, a line of low chitinized scales on the connecting membrane between the abdominal tergites and sternites).	Could this be shown in a figure?	European Union
59.	*92*	Technical	There are several genera that may be confused with Liriomyza. The closely related genera Phytomyza, Chromatomyia and Phytoliriomyza can generally be separated from Liriomyza by their proclinate (forward pointing) fronto-orbital setulae (always reclinate or occasionally upright or missing in Liriomyza), and by the scutellum, which is generally grey or black but occasionally slightly yellowish centrally (entirely yellow in most Liriomyza). In Phytomyza and Chromatomyia, the costa extends only to R_{4+5}, whereas in Phytoliriomyza and Liriomyza it extends to vein M1+2 (Spencer, 1977). Phytoliriomyza species are gall-forming (on a stem or leaf) internal feeders, whereas Chromatomyia, Phytomyza and Liriomyza species are typically leafminers.	1. To clarify the possible confusion with other genera, it would be appreciated that illustrations are provided 2. Can R4+5 be shown in a figure? 3 vein M is it M1+2?	European Union
60.	*94*	Substantive	Some paragraph should be added to dwell on those morphological characters at species level (including ground color of both outer or inner vertical setae, color of mesonotum and anepisternum ,vein Cu1A) before Table 1. And the corresponding graphs also should be provided. ***4.1.4.1 Morphological characters of adult***	It is necessary to improve the diagnostic practicability for the four quarantine Liriomyza species.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			Liriomyza *spp.*		
61.	*97*	Technical	Identification of the adults can also be carried out with keys. Malipatil and Ridland (2008) provide a key to 17 species of economic importance, including a few species endemic to Australia. In addition, an identification system for pest species from around the world based on photomicrographs is available at Dempewolf (2004). With particular reference to keys for *Liriomyza* species, there are some extensive regional back-catalogues and keys available through the works of Spencer. These cover the regional background fauna, which obviously differs from region to region, and by doing so differentially affects the positive process of eliminating non-target taxa. A full list of these works is listed in Spencer (1973). Considering the host plant on which the fly is detected can help identify agromyzid species that may occur in the same biological context as the finding.	the host plant is important in the diagnostic. The addition of a sentence at the end is proposed	European Union
62.	*98*	Technical	**Table 1. Adult morphological characters of selected *Liriomyza* species**[1]	It is suggested that figure 15 in EPPO 2005 used as an illustration.	European Union
63.	*99*	Editorial		1. Ensure upon finalization that the table is readable (landscape rather than portrait) (delete the middle column with the names) 2. Delete the middle column.	European Union

	Male distiphallus	Vertical setae	Anepisternum	Vein Cu 1A	Third antennal segment		Frons and orbits	Femur	Mesonotum	Male abdominal tergites
L. bryoniae	Two distal bulbs; bulb rims circular	Both vertical setae on yellow ground	Predominantly yellow, small black mark at front lower margin	*a* twice length of *b*	Small, yellow	*L. bryoniae*	Frons bright yellow, orbits slightly paler	Bright yellow with some brownish striations	Black, largely shining but with distinct matt undertone	Second and third visible tergites divided by a yellow medial furrow

Comm. no.	Para. no.	Comment type	Comment			Explanation				Country			
			L. huidobrensis[2]	Two distal bulbs, meeting only at their rims; bulb rims drawn out antero-ventrally	Both vertical setae on black ground	Yellow with variable black patch generally across the lower three-quarters	a 2–2.5 times the length of *b*	Slightly enlarged, usually darkened	*L. huidobrensis* *	Fronts yellow, generally more orange than pale lemon-yellow; upper orbits slightly darkened at least to upper orbital setae	Yellow, variably darkened with black striations	Black, matt	Only the second visible tergite divided by a yellow medial furrow
			L. sativae	One distal bulb with a slight constriction between upper and lower halves in dorso-ventral view; bulb appears more strongly sclerotized with a shorter basal stem	Outer vertical setae on black ground that may just reach inner vertical setae, which are otherwise on yellow	Predominantly yellow, with dark area varying in size from a small bar along the lower margin to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin	a 3–4 times length of *b*	Small, yellow	*L. sativae*	Fronts and orbits bright yellow	Bright yellow	Black, shining	Only the second visible tergite divided by a yellow medial furrow
			L. strigata	Two distal bulbs, meeting from their	Black coloration behind the eyes extending to at least the outer	Yellow, but with black patch variable on lower and front	a 2–3 times the length	Small, yellow	*L. strigata*	Fronts and orbits yellow	Yellow with some brownish striations	Black, shining but slightly matt	–

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

	bases; bulb rims drawn out antero-ventrally	inner vertical setae on yellow ground	this can extend along the lower half								
L. trifolii	One distal bulb with marked constriction between lower and upper halves in dorso-ventral view; bulb appears less distinctly sclerotized with a longer basal stem	Both vertical setae on yellow ground	Yellow, small blackish grey mark at front lower margin	a 3–4 times length of *b*	Small, yellow	*L. trifolii*	Frons and orbits yellow	Yellow, occasional slight brownish striations	Matt black with grey undertone	Second to fifth visible tergites divided by a yellow medial furrow

64. *99* Editorial

Rearrange the table by using the five species' names as heading in row 1 and names of morphological characters in column 1, the corresponding descriptions of the characters for the species in the appropriate table cells. Current arrangement of the table is difficult to read with each individual row two deep and column too narrow so that many single words are split into two lines. The suggested modification would greatly improve the readability.

Australia

Male distiphallus	Vertical setae	Anepisternum	Vein Cu 1A	Third antennal segment	Frons and orbits	Femur	Mesonotum	Male abdominal tergites	Wing length	
L. bryoniae	Two distal bulbs; bulb rims circular	Both vertical setae on yellow ground	Predominantly yellow, small black mark at front lower margin	a twice length of *b*	Small, yellow	*L. bryoniae*	Frons bright yellow, orbits slightly paler	Bright yellow with some brownish striations	Black, largely shining but with distinct matt undertone	Second and third visible tergites divided by yellow medial furrow

Comm. no.	Para. no.	Comment type	Comment			Explanation				Country			
			L. huidobrensis[2]	Two distal bulbs, meeting only at their rims; bulb rims drawn out antero-ventrally	Both vertical setae on black ground	Yellow with variable black patch generally across the lower three-quarters	a 2–2.5 times the length of *b*	Slightly enlarged, usually darkened	*L. huidobrensis* *	Frons yellow, generally more orange than pale lemon-yellow; upper orbits slightly darkened at least to upper orbital setae	Yellow, variably darkened with black striations	Black, matt	Only the second visible tergite divided by yellow medial furrow
			L. sativae	One distal bulb with a slight constriction between upper and lower halves in dorso-ventral view; bulb appears more strongly sclerotized with a shorter basal stem	Outer vertical setae on black ground that may just reach inner vertical setae, which are otherwise on yellow	Predominantly yellow, with dark area varying in size from a small bar along the lower margin to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin	a 3–4 times length of *b*	Small, yellow	*L. sativae*	Frons and orbits bright yellow	Bright yellow	Black, shining	Only the second visible tergite divided by yellow medial furrow
			L. strigata	Two distal bulbs, meeting from their rims to their bases; bulb rims drawn out antero-ventrally	Black coloration behind the eyes extending to at least the outer vertical setae, but inner vertical setae on yellow ground	Yellow, but with black patch variable on lower and front margins, and this can extend along the lower half	a 2–3 times the length of *b*	Small, yellow	*L. strigata*	Frons and orbits yellow	Yellow with some brownish striations	Black, shining but slightly matt	–
			L. trifolii	One distal bulb with marked constriction between lower and upper halves in dorso-ventral view; bulb appears less distinctly	Both vertical setae on yellow ground	Yellow, small blackish grey mark at front lower margin	a 3–4 times length of *b*	Small, yellow	*L. trifolii*	Frons and orbits yellow	Yellow, occasional slight brownish striations	Matt black with grey undertone	Second to fifth visible tergites divided by yellow medial furrow

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			sclerotized with a longer basal stem		
65.	*99*	Editorial		The seventh column overlaps the first column, it should be removed	Japan

Male distiphallus	Vertical setae	Anepisternum	Vein Cu 1A	Third antennal segment	Frons and orbits	Femur	Mesonotum	Male abdominal tergites	Wing length	
L. bryoniae	Two distal bulbs; bulb rims circular	Both vertical setae on yellow ground	Predominantly yellow, small black mark at front lower margin	*a* twice length of *b*	Small, yellow	~~*L. bryoniae*~~	Frons bright yellow, orbits slightly paler	Bright yellow with some brownish striations	Black, largely shining but with distinct matt undertone	Second ar third visibl tergites divided by yellow medial furrow
L. huidobrensis[2]	Two distal bulbs, meeting only at their rims; bulb rims drawn out antero-ventrally	Both vertical setae on black ground	Yellow with variable black patch generally across the lower three-quarters	a 2–2.5 times the length of *b*	Slightly enlarged, usually darkened	~~*L. huidobrensis **~~	Frons yellow, generally more orange than pale lemon-yellow; upper orbits slightly darkened at least to upper orbital setae	Yellow, variably darkened with black striations	Black, matt	Only the second visible tergite divided by yellow medial furrow
L. sativae	One distal bulb with a slight constriction between upper and lower halves in dorso-ventral view; bulb appears more strongly sclerotized with a shorter basal stem	Outer vertical setae on black ground that may just reach inner vertical setae, which are otherwise on yellow	Predominantly yellow, with dark area varying in size from a small bar along the lower margin to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin	a 3–4 times length of *b*	Small, yellow	~~*L. sativae*~~	Frons and orbits bright yellow	Bright yellow	Black, shining	Only the second visible tergite divided by yellow medial furrow

Comm. no.	Para. no.	Comment type	Comment											Explanation	Country
			L. strigata	Two distal bulbs, meeting from their rims to their bases; bulb rims drawn out antero-ventrally	Black coloration behind the eyes extending to at least the outer vertical setae, but inner vertical setae on yellow ground	Yellow, but with black patch variable on lower and front margins, and this can extend along the lower half	a 2–3 times the length of *b*	Small, yellow	~~*L. strigata*~~	Frons and orbits yellow	Yellow with some brownish striations	Black, shining but slightly matt	–		
			L. trifolii	One distal bulb with marked constriction between lower and upper halves in dorso-ventral view; bulb appears less distinctly sclerotized with a longer basal stem	Both vertical setae on yellow ground	Yellow, small blackish grey mark at front lower margin	a 3–4 times length of *b*	Small, yellow	~~*L. trifolii*~~	Frons and orbits yellow	Yellow, occasional slight brownish striations	Matt black with grey undertone	Second to fifth visible tergites divided by yellow medial furrow		
66.	99	Editorial		**Male distiphallus**	**Vertical setae**	**Anepisternum**	**Vein Cu 1A**	**Third antennal segment**		**Frons and orbits**	**Femur**	**Mesonotum**	**Male abdominal tergites**	Presentation of the table is not clear, orientation of the table has to be changed . if it is possible, an illustration in the table will be suitable	Tunisia
			L. bryoniae	Two distal bulbs; bulb rims circular	Both vertical setae on yellow ground	Predominantly yellow, small black mark at front lower margin	*a* twice length of *b*	Small, yellow	*L. bryoniae*	Frons bright yellow, orbits slightly paler	Bright yellow with some brownish striations	Black, largely shining but with distinct matt undertone	Second and third visible tergites divided by a yellow medial furrow		
			L. huidobrensis[2]	Two distal bulbs, meeting only at their	Both vertical setae on black ground	Yellow with variable black patch generally across the lower	a 2–2.5 times the	Slightly enlarged, usually darkened	*L. huidobrensis* *	Frons yellow, generally more	Yellow, variably darkened with black	Black, matt	Only the second visible tergite		

Comm. no.	Para. no.	Comment type	Comment			Explanation					Country			
				rims; bulb rims drawn out antero-ventrally		three-quarters	length of *b*			orange than pale lemon-yellow; upper orbits slightly darkened at least to upper orbital setae	striations		divided by a yellow medial furrow
			L. sativae	One distal bulb with a slight constriction between upper and lower halves in dorso-ventral view; bulb appears more strongly sclerotized with a shorter basal stem	Outer vertical setae on black ground that may just reach inner vertical setae, which are otherwise on yellow	Predominantly yellow, with dark area varying in size from a small bar along the lower margin to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin	a 3–4 times length of *b*	Small, yellow	*L. sativae*	Frons and orbits bright yellow	Bright yellow	Black, shining	Only the second visible tergite divided by a yellow medial furrow
			L. strigata	Two distal bulbs, meeting from their rims to their bases; bulb rims drawn out antero-ventrally	Black coloration behind the eyes extending to at least the outer vertical setae, but inner vertical setae on yellow ground	Yellow, but with black patch variable on lower and front margins, and this can extend along the lower half	a 2–3 times the length of *b*	Small, yellow	*L. strigata*	Frons and orbits yellow	Yellow with some brownish striations	Black, shining but slightly matt	–

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			L. trifolii | One distal bulb with marked constriction between lower and upper halves in dorso-ventral view; bulb appears less distinctly sclerotized with a longer basal stem | Both vertical setae on yellow ground | Yellow, small blackish grey mark at front lower margin | a 3–4 times length of *b* | Small, yellow | *L. trifolii* | Frons and orbits yellow | Yellow, occasional slight brownish striations | Matt black with grey undertone | Second to fifth visible tergites divided by a yellow medial furrow	
67.	*99*	Technical		1. Species should be grouped phylogenetically rather than in alphabetical order? 2. Vertical setae : Would it help to include an illustration guide? 3. Anepisternum : Also called mesopleural area? (Spencer 1973 – fig 7). Include an illustration guide? 4. Frons : Not explained in Figure 7 5. 'a twice length of b' : Refer to a and b in Figure 7	European Union

Male distiphallus	Vertical setae	Anepisternum	Vein Cu 1A	Third antennal segment	Frons and orbits	Femur	Mesonotum	Male abdominal tergites	Wing length	
L. bryoniae	Two distal bulbs; bulb rims circular	Both vertical setae on yellow ground	Predominantly yellow, small black mark at front lower margin	*a* twice length of *b*	Small, yellow	*L. bryoniae*	Frons bright yellow, orbits slightly paler	Bright yellow with some brownish striations	Black, largely shining but with distinct matt undertone	Second ar third visibl tergites divided by yellow medial furrow
L. huidobrensis[2]	Two distal bulbs, meeting only at their rims; bulb rims drawn out antero-ventrally	Both vertical setae on black ground	Yellow with variable black patch generally across the lower three-quarters	a 2–2.5 times the length of *b*	Slightly enlarged, usually darkened	*L. huidobrensis* *	Frons yellow, generally more orange than pale lemon-yellow; upper orbits slightly darkened at least to upper orbital setae	Yellow, variably darkened with black striations	Black, matt	Only the second visible tergite divided by yellow medial furrow

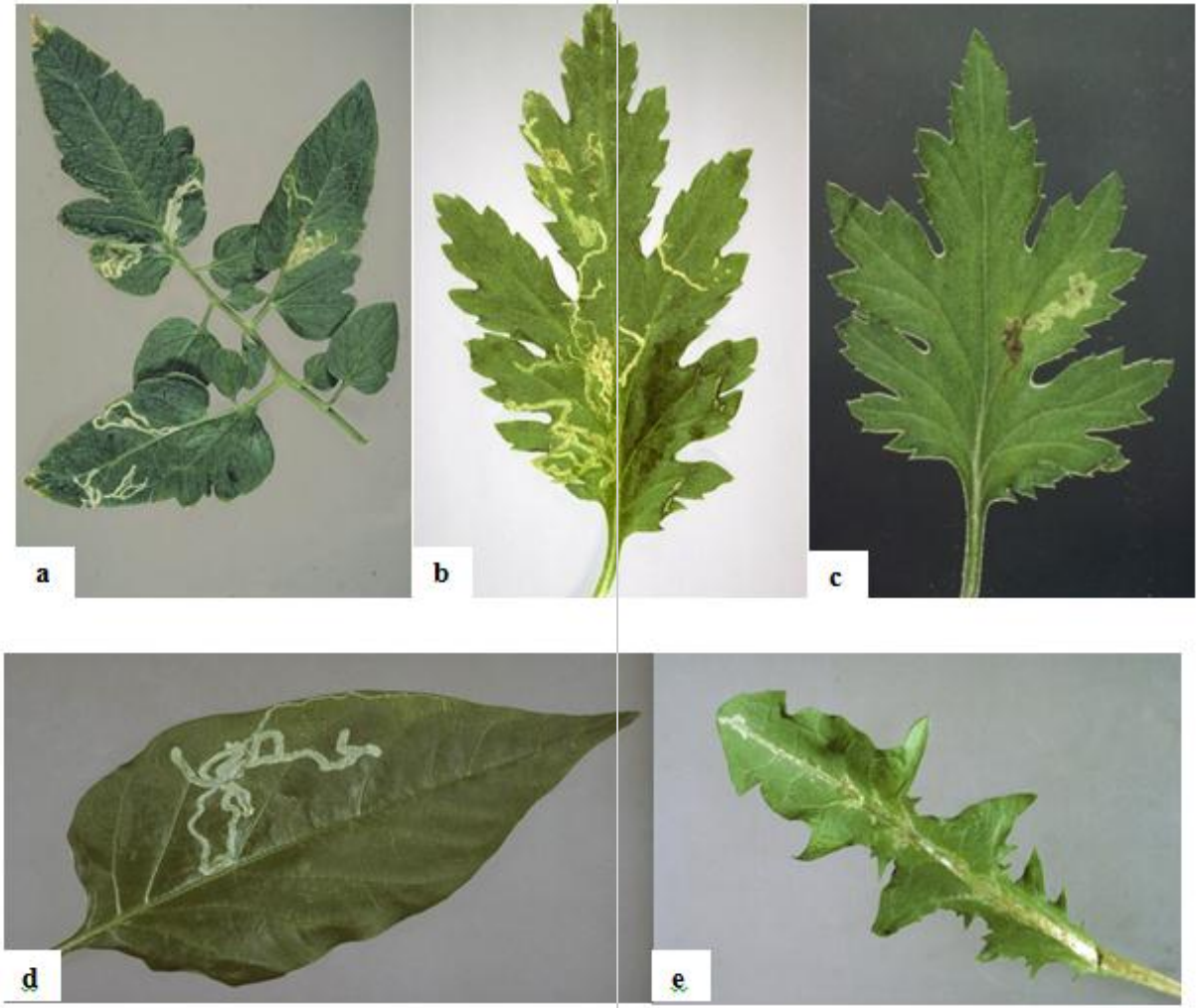
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			L. sativae / One distal bulb with a slight constriction between upper and lower halves in dorso-ventral view; bulb appears more strongly sclerotized with a shorter basal stem / Outer vertical setae on black ground that may just reach inner vertical setae, which are otherwise on yellow / Predominantly yellow, with dark area varying in size from a small bar along the lower margin to a patch along the entire lower margin, well up the front margin and narrowly up the hind margin / a 3–4 times length of *b* / Small, yellow / *L. sativae* / Frons and orbits bright yellow / Bright yellow / Black, shining / Only the second visible tergite divided by yellow medial furrow		
			L. strigata / Two distal bulbs, meeting from their rims to their bases; bulb rims drawn out antero-ventrally / Black coloration behind the eyes extending to at least the outer vertical setae, but inner vertical setae on yellow ground / Yellow, but with black patch variable on lower and front margins, and this can extend along the lower half / a 2–3 times the length of *b* / Small, yellow / *L. strigata* / Frons and orbits yellow / Yellow with some brownish striations / Black, shining but slightly matt / –		
			L. trifolii / One distal bulb with marked constriction between lower and upper halves in dorso-ventral view; bulb appears less distinctly sclerotized with a longer basal stem / Both vertical setae on yellow ground / Yellow, small blackish grey mark at front lower margin / a 3–4 times length of *b* / Small, yellow / *L. trifolii* / Frons and orbits yellow / Yellow, occasional slight brownish striations / Matt black with grey undertone / Second to fifth visible tergites divided by yellow medial furrow		
68.	*108*	Editorial	The distiphallus is a very small, fragile structure enclosed by membranes. It is the terminal part of the aedeagus (the intromittent organ, part of the male genitalia) (Figure 9) and its complex three-dimensional structure is of considerable diagnostic value. Indeed, the distiphallus provides a single character by which all four target species can be identified reliably. The basic structure of	Delete extra full stop.	Canada

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			the distiphallus differs in the two natural species groups: in group 1, there are two distal bulbs side by side (Figure 10), while in group 2, there is only one distal bulb, which has a medial constriction dividing it into distinct lower and upper sections (Figure 11). A key that facilitates identification of the four target species using the distiphallus is provided below. For convenience, the key also includes *L. strigata*which is closely related to *L. bryoniae* and L. *huidobrensis* and which is also polyphagous and therefore to be found on similar host plants..		
69.	*108*	Technical	The distiphallus is a very small, fragile structure enclosed by membranes. It is the terminal part of the aedeagus (the intromittent organ, part of the male genitalia) (Figure 9) and its complex three-dimensional structure is of considerable diagnostic value. Indeed, the distiphallus provides a single character by which all four target species can be identified reliably. The basic structure of the distiphallus differs in the two natural species groups: in group 1, there are two distal bulbs side by side (Figure 10), while in group 2, there is only one distal bulb, which has a medial constriction dividing it into distinct lower and upper sections (Figure 11). A key that facilitates identification of the four target species using the distiphallus is provided below. For convenience, the key also includes *L. strigata* which is closely related to *L. bryoniae* and L. *huidobrensis* and which is also polyphagous and therefore to be found on similar host plants..	The resolution of pictures 10 and 11 is not good enough. A better resolution and a better shot would be welcomed.	European Union
70.	*110*	Substantive	A key for four quarantine species of Liriomyza including the morphological characters of male and female adult is suggested to be listed in the draft.**Diagnostic key for identification of *Liriomyza* spp. using the male distiphallus**	The key will improve the diagnostic practicability of this standard.	China
71.	*110*	Technical	**Diagnostic key for identification of *Liriomyza* spp. using the male distiphallus**	The definition of pictures 10 and 11 is not good enough. A better resolution and a better shot would be welcomed. The distiphallus are difficult to identify on these pictures, even for an experienced operator.	European Union

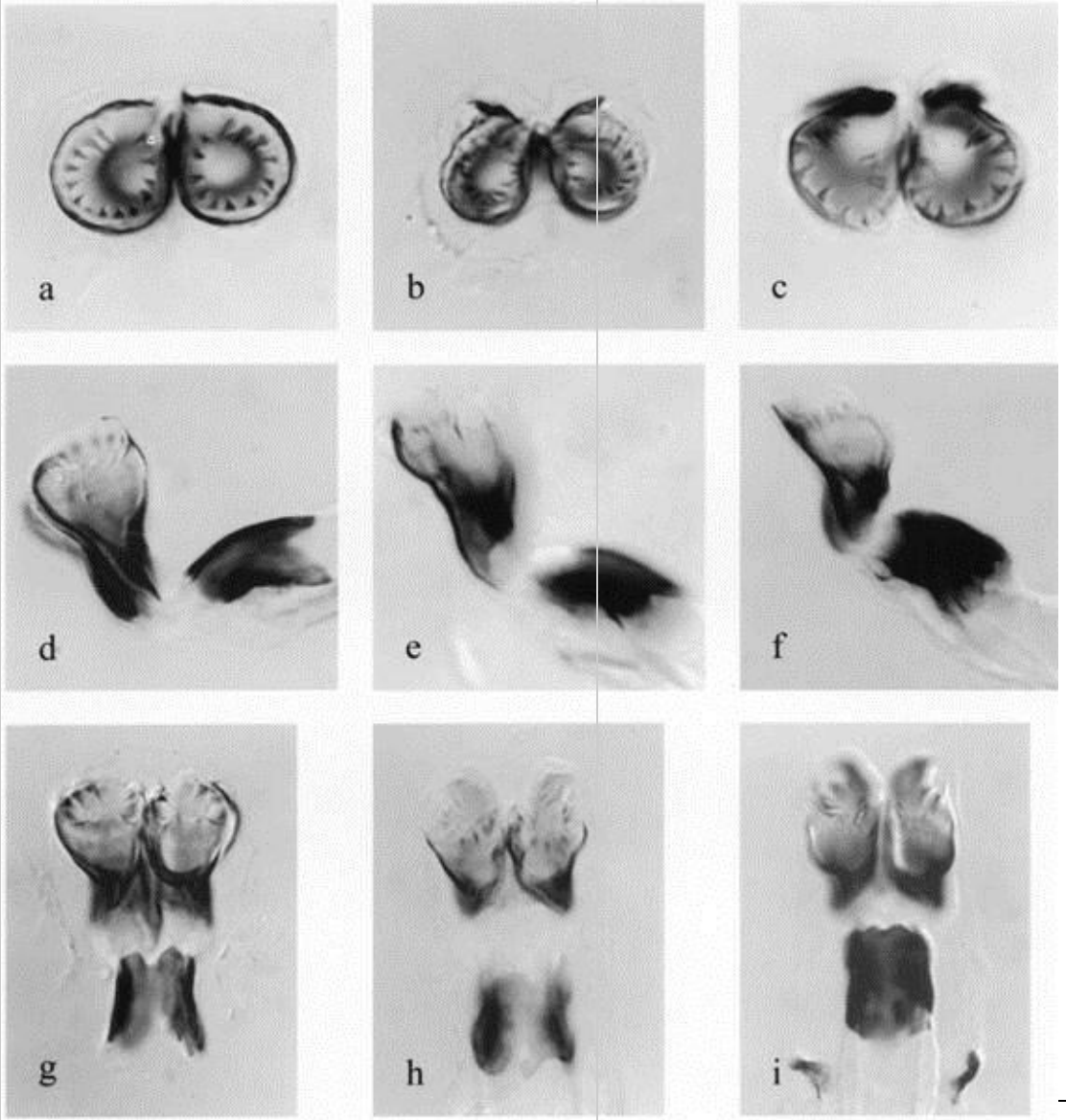
Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
72.	*110*	Technical	Delete the key.**Diagnostic key for identification of *Liriomyza* spp. using the male distiphallus**	The characters of distphallus had been described in Para. 108.The key is redundant.	China
73.	*122*	Editorial	Of the four life stages (egg, larva, pupa and adult) only the adult male flies can be positively identified to species level using morphological features (the shape of the male genitalia). The morphological characteristics of larvae and pupae can be used to distinguish between the members of the two natural species groups described above (section 4.1.4.2). This information can contribute towards ~~a~~ species identification but is insufficient by itself to allow species identification. To complement morphological identification, molecular assays can be used to distinguish between the species included in the protocol (section 4.2)	Remove unnecessary "a" from penultimate sentence.	Canada
74.	*126*	Technical	There are three larval instars, which feed as they tunnel through the leaf tissue. The newly emerged larvae (Figure 2(a)) are about 0.5 mm long but reach 3.0 mm when fully grown. They are typical of agromyzids in their gross form (see section 4.1.2). Pupae (Figure 2(b)) are oval cylinders in shape, about 2.0 mm in length, very slightly flattened ventrally, with projecting anterior and posterior spiracles. In practice, for larvae and pupae, the two natural groups can be distinguished from each other morphologically (but not the species within the groups) as follows.	1. To allow an easy use of this protocol, an additional illustration could be included from the EPPO current protocol (Figure 12 PM 7/53). 2. Regarding Figure 2(a) : In the legend of Fig 2 this is mentioned as the third larval instar. 3. Regarding "the two natural groups can be distinguished from each other morphologically " : An illustration showing the characteristics of the two species group would help.	European Union
75.	*130*	Technical	Larvae of *L. sativae* and *L. trifolii* are translucent when newly emerged and yellow-orange later. Each posterior spiracle is tricorn-shaped with three pores, each on a distinct projection, the outer two elongate. Puparia are yellowish-orange, sometimes a darker golden brown. The form of the larval spiracles is retained in the puparium but the detail is less obvious.	Regarding "yellow-orange later." : Is this over the entire body? can this be clarified?	European Union
76.	*130*	Technical	Add“4.1.4.4 electrophoresis for identification of four species of Liriomyza spp.” after Para.130.Larvae of *L. sativae* and *L. trifolii* are translucent when newly emerged and yellow-orange later. Each posterior spiracle is tricorn-shaped with three pores, each on a distinct projection, the outer two elongate. Puparia are yellowish-orange, sometimes a darker golden brown. The form of the larval spiracles is retained in the puparium but the detail is less obvious.	According to OEPP/EPPO 1992 “Quarantine procedures No.42, Identification of Liriomyza spp.”, the four species of L. bryoniae, L. huidobrensis, L. trifolii and L. sativae , can be identified quickly and exactly by electrophoresis based on other substantiation （eg：morphology, host plants, et al.）. Reference：Ulenberg, S.A. 1992. Quarantine procedure—Identification of Liriomyza spp.Bulletin OEPP/EPPO	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
				Bulletin（No. 42）， 22：235-238.	
77.	*132*	Substantive	More evidences of confirmation tests for molecular identification should be provided in the draft.Various polymerase chain reaction (PCR)-based molecular methods have been used to identify *Liriomyza* species, including PCR-restriction fragment length polymorphism (RFLP), end-point PCR using species-specific primers, real-time PCR, and DNA sequence comparison. Of these methods, the ones that can be used to distinguish between the four target species (i.e. *L. bryoniae*, *L. huidobrensis*, *L. sativae* and *L. trifolii*) or between *L. huidobrensis* and *L. langei* are described below. Each assay is described as published, as these conditions define the original level of performance. No assay reported for these species has been formally validated for analytical sensitivity and reproducibility.	The molecular protocol has just been cited from the published. It is not confirmed by reference laboratory or NPPOs/RPPOs.	China
78.	*133*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. ~~Use of names of reagents chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	Text deleted as per general comment	Uruguay, Argentina, Chile
79.	*173*	Substantive	Delete all contents of L.langei in the draft.**4.2.5 Distinguishing cryptic species *L. langei* and *L. huidobrensis***	It is impossible to identify L.langei and L.huidobrensis based on adult morphology (Spencer 1973) and molecular techniques (Kox et al.2005). And it is still controversial on the synonyms of L.langei with L.huidobrensis. Therefore, the disputed species of L.langei at species level should not be included in draft.	China
80.	*187*	Technical	Efforts to generate a more taxonomically comprehensive resource of DNA sequence records for the 5' region of the *Liriomyza COI* gene used in animal DNA barcode studies are ongoing (e.g. Bhuiya *et al.*, 2011, Maharjan *et al.* 2014). There are currently DNA barcode records for 31 species of *Liriomyza* (including the four target species) available on the Barcode of Life database (BOLD) (http://www.boldsystems.org). Alternatives barcodes and p	1. QBANK, an European database for barcodes of plant pests and invasive species, provides procedures for DNA amplification of the relevant barcodes, but also reference sequences that were produced from reference material. This database is curated and regularly updated. This provides an additional tool to BOLD. 2. Barcoding note : Recently, a range of problems have emerged using the COI gene for diagnostics. For	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
			rocedures are provided on Q-bank (www/q-bank.eu), a curated database, including sequences obtained from reference material. A recent study (Maharjan *et al.* 2014) included details for the separation of *L. huidobrensis; L. trifolii, L. sativae, L. bryoniae* and *L. chinensis*. Despite these advances in DNA sequencing resources, the methodology is not described in detail here for *Liriomyza* species identification because interpretation rules for the resources have not yet been published in the scientific literature.	example, in some groups barcoding primers seem to pick up fragments which might not be the homologous mtdna and this could results in misidentifications. At this moment “genbank” COI data show that, at least, some of the target species are already mixed in the phylogenetic trees. Whether this is due to misidentifications or because of, e.g., nuclear encoded fragments is not clear to me. Could the authors consider adding a comment?	
81.	*205*	Substantive	**Boykin, L.M., Armstrong, K., Kubatko, L. & De Barro, P.** 2012. DNA barcoding invasive insects: Database roadblocks. *Invertebrate Systematics,* 26: 506–514. Brown, B. V., Borkent, A., Cumming, J. M., Wood, D. M., Woodley, N. E. & Zumbado, M. 2010. Manual of Central American Diptera, Vol. 2.	To add the paper cited for the subcostal vein mentioned above (after paragraph 86) .	Japan
82.	*260*	Editorial	**Figure 2.** Examples of stages of *Liriomyza* spp.: (a) third larval instar of *L. bryoniae*; (b) pupa of *Liriomyza* sp.; and (c) adult of *L. bryoniae*.	illustration of the other species can be added	Tunisia
83.	*260*	Technical	Add the Scale of the three images.**Figure 2.** Examples of stages of *Liriomyza* spp.: (a) third larval instar of *L. bryoniae*; (b) pupa of *Liriomyza* sp.; and (c) adult of *L. bryoniae*.	The scale will provide accurate size of the different stages of Liriomyza spp..	China
84.	*263*	Editorial	**Figure 3.** Typical characteristics of mines of (a) *Liriomyza bryoniae*, (b) *Liriomyza huidobrensis* and (c) *Liriomyza strigata.*	Figures are not clear, differences between the different types of mines are not clear and annotations are unreadable	Tunisia
85.	*268*	Substantive	Change Photo e into that on an identified host.	As the reference object of standard, it should be an certain one.	China

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
					
86.	*272*	Editorial	**Figure 6.** Abdomen in (a) male and (b) female *Liriomyza*. Source: courtesy Fera Science Ltd.	Addition of the source to be consistent with the other figures.	European Union

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
87.	*272*	Technical	**Figure 6.** Abdomen in (a) male and (b) female *Liriomyza*.	Name tergites, referred to in lines 78-79.	European Union
88.	*274*	Substantive	**Figure 7.** Adult morphology of Agromyzidae (*Agromyza* sp.).	This figure is too complicated (too many arrow and legends) for any easy use. Fig. 3 in PM 7/53 is simpler and only focusses on the essential diagnostic characters. It is proposed to replace the Figure 7 by Figure 3 from PM 7/53. The EPPO secretariat will provide this picture to the IPPC Secetariat.	European Union
89.	*274*	Technical	**Figure 7.** Adult morphology of Agromyzidae(*Agromyza* sp.). It would be preferable to have a diagonal view as well as a side view as the morphological figure.	The colors of bases of outer/inner vertical setae are diagnostic characteristics, however, it is difficult to identify the location of the setae with only the side view.	Japan
90.	*280*	Technical	**Figure 9.** Male genitalia of *Liriomyza huidobrensis* (lateral view).	The information of the type of view is missing.	European Union
91.	*282*	Substantive	Delete the photo j and k.	Photo j and g, k and h show the distiphallus of the same species, a type specimen is enough here.	China

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

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country
92.	*283*	Substantive	**Figure 10.** Distiphallus of *Liriomyza* spp. (×400 magnification): (a) *L. bryoniae*, anterior view; (b) *L. huidobrensis*, anterior view; (c) *L. strigata*, anterior view; (d) *L. bryoniae*, lateral view; (e) *L. huidobrensis*, lateral view; (f) *L. strigata*, lateral view; (g) *L. bryoniae*, dorso-ventral view; (h) *L. huidobrensis*, dorso-ventral view; (i) *L. strigata*, dorso-ventral view; (j) *L. bryoniae*, dorso-ventral view (in a different plane to (g)); and (k) *L. huidobrensis*, dorso-ventral view (in a different plane to (h)).	The pictures are old and don't offer a high resolution to allow a good identification. Please consider if it is possible to replace them.	European Union
93.	*286*	Substantive	**Figure 11.** Distiphallus of *Liriomyza* spp. (×400 magnification): (a) *L. sativae*, anterior view; (b) *L. trifolii*, anterior view; (c) *L. sativae*, lateral view; (d) *L. trifolii*, lateral view; (e) *L. sativae*, dorso-ventral view; and (f) *L. trifolii*, dorso-ventral view.	The pictures are old and don't offer a high resolution to allow a good identification. Please consider if it is possible to replace them	European Union