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Express Pest Risk Analysis
Meloidogyne ethiopica and *Meloidogyne luci*

Prepared by:

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Summary[1] of the Express Pest Risk Analysis for "***Meloidogyne ethiopica* and *Meloidogyne luci***"

Root knot nematodes *M. ethiopica* and *M. luci* are serious pests of several agricultural crops belonging to monocotyledons, dicotyledons, including herbaceous and woody plants. All previously reported populations of *M. ethiopica* in Europe and Turkey were reclassified as *M. luci.* In Brazil and Chile, *M. ethiopica* is considered to be a damaging species on kiwi and grapevine, as infestations lead to a reduction of plant growth, fruit size and quality. It is widely distributed in vineyards in Chile. In Brazil, *M. ethiopica* has also been reported to cause multiple galls on potato root systems, as well as protuberances on tubers. Similarly, *M. luci* has been reported on several vegetable crops and has also been shown as a potential threat to the potato production in Portugal.

M. ethiopica and *M. luci* can be introduced by several pathways but the highest risk for entry and spread in the PRA area is considered the pathway of host plants for planting with or without growing medium attached. In addition, plant parts like tubers may present also high risk of spreading in case of pest establishment outdoors. Human assisted spread of the pests is the most important one. The probability of establishment of *M. ethiopica* and *M. luci* in the protected area is high. Moreover, *M. luci* has already been detected in the open field production in Europe (Mediterranean climate), in corn and kiwi production in Greece, and potato production in Portugal. There is a high risk of *M. luci* establishment outdoors in the PRA area, as this species was proved to overwinter outdoors at the open field in continental and Mediterranean climate. The endangered areas in the PRA area for *M. luci* are therefore areas with continental and Mediterranean climate conditions. Once the root knot nematodes (RKNs) have been introduced, it is in general difficult to control or eradicate them, especially outdoors at the open field. On the other hand, the ability of *M. ethiopica* to overwinter at the open field in European continental and Mediterranean climate has not been tested yet. However, if the temperature requirements of *M. ethiopica* are similar to other tropical RKN species (*M. incognita*, *M. luci*, *M. arenaria*) the establishment in the warmer parts of the PRA area would be possible.

PRA area: whole territory of Slovenia

Describe the endangered area:

The endangered area for *M. luci* is area with continental and Mediterranean climate conditions. For *M. ethiopica* no data are available for its ability to survive outside in European climate condition. However, both species can establish under protected conditions.

Main conclusions

Root knot nematodes *M. ethiopica* and *M. luci* can be introduced by several pathways:

- High risk for entry in the PRA area: host plants for planting with roots, with or without growing medium attached (planting material of vegetables, ornamental plants, fruit trees and grapevine), non-host plants for planting with growing medium attached and in addition, host plant parts like tubers with or without growing medium may present also high risk of spreading in case of pest establishment outdoors.
- Moderate risk: growing medium (soil)
- Low risk: non-hosts plant parts contaminated with growing medium (such as roots, tubers, bulbs), for consumption or processing, soil attached to equipment and machinery, travellers (carrying infested plants, soil or plant products)

Human assisted spread of the pests is the most important.

M. ethiopica and *M. luci* have very wide host range including majority of vegetable crops which are cultivated in the protected area. The probability of establishment in the protected area is high. *M. luci* has already been detected outdoors in Europe (Mediterranean area), in corn and kiwi production in Greece, and potato production in Portugal. There is a high risk of *M. luci* establishment outdoors in the PRA area, as this species was proved to overwinter outdoors in European temperate regions and countries of Mediterranean area. Once the RKNs have been introduced, it is in general difficult

[1] The summary should be elaborated once the analysis is completed

to control or eradicate them, especially outdoors. On the other hand the ability of *M. ethiopica* overwintering in the open field in temperate and Mediterranean areas has not been tested yet.

The potential impact of both pest species in the PRA area is high especially in the light of climate change scenarios. The models of climate changes predict an increase of average temperatures and more frequent periods of drought, floods and heat waves. Higher temperatures would allow development of more generations of RKNs in a growing season and consequently higher yield losses. In addition, the findings of virulent populations of *M. luci* in Turkey that can reproduce on tomato plants bearing *Mi*-1 resistance gene adds to the concern about the damage potential of this pest.

Phytosanitary measures to prevent the spread of these pests within the PRA area in case of an outbreak are necessary. The measures should include prohibition of movement of plants for planting originating from infested area, ban of movement of soil form infested area, cleaning and disinfection of facilities, tools and machinery, destruction of nematode infested plant waste, survey, sampling scheme and laboratory testing (within infested area and the surroundings).

M. ethiopica* and *M. luci

Phytosanitary risk for the *endangered area* (*Individual ratings for likelihood of entry and establishment, and for magnitude of spread and impact are provided in the document*)	High ✓	Moderate ☐	Low ☐
Level of uncertainty of assessment (see Q 17 for the justification of the rating. Individual ratings of uncertainty of entry, establishment, spread and impact are provided in the document)	High ☐	Moderate ✓	Low ☐

Other recommendations:

- Inform EPPO and EU
- Surveys are recommended to confirm the pests status
- Additional research is needed to support phytosanitary decisions (needed research on the damage potential of both species to major crops (early, late potato cultivars, corn etc.); the ability of *M. ethiopica* to overwinter at the open field in continental and Mediterranean climate; assessment of the efficiency of biological and low risk control/management options)

Stage 1. Initiation

Reason for performing the PRA:
M. luci is a root knot nematode (RKN) which has recently been discovered in several European countries after its description in 2014 by Carneiro et al. (2014). Pest populations, previously found in Europe and Turkey have been identified as *M. ethiopica* but recent studies justified reclassification to *M. luci* (Janssen et al. 2016; Gerič Stare et al. 2017a). RKN *M. ethiopica* and *M. luci* can affect numerous plant species, including many important crops such as grapevine and potato. These two species are so closely related that there is no biological difference between the two species in terms of parasitizing different plant species and also morphometric characteristics of males, females and second stage juveniles are very similar. The pest risk assessment therefore comprises species, *M. ethiopica* and *M. luci*. However, less host plant species are to date confirmed for *M. luci*, since the description of the species was made only in 2014. RKN *M. luci* was detected for the second time in Slovenia, near Ljubljana in 2015 (Gerič Stare et al. 2017b), while the first found was in 2003.

PRA area: whole territory of Slovenia

Stage 2. Pest risk assessment

1. Taxonomy:

Meloidogyne ethiopica Whitehead, 1968
Domain: Eukaryota
Kingdom: Metazoa
Phylum: Nematoda
Family: Meloidogynidae
Genus: *Meloidogyne*
Species: *Meloidogyne ethiopica*

And

Meloidogyne luci Carneiro et al., 2014
Domain: Eukaryota
Kingdom: Metazoa
Phylum: Nematoda
Family: Meloidogynidae
Genus: *Meloidogyne*
Species: *Meloidogyne luci*

Common name (of the genus): root knot nematodes (RKN)

2. Pests overview

M. ethiopica was described by Whitehead in 1968 from the Mlalo region of Tanzania. It has been reported from Africa - Kenya, Ethiopia, Mozambique, Zimbabwe, Tanzania, South Africa (Whitehead 1968, 1969), South America - Chile, Brazil, Peru (Carneiro et al. 2003, 2004), Europe - Slovenia (Širca et al. 2004), Greece (Conceição et al. 2012), and Italy (Maleita et al. 2012) and Asia - Turkey (Aydinli et al. 2013). *M. ethiopica* can parasitize several crops including monocotyledons, dicotyledons, and herbaceous and woody plants (Carneiro et al. 2003, 2004; Lima et al. 2009; O'Bannon 1975; Strajnar et al. 2009, 2010; Whitehead 1968). It is widely distributed in vineyards in Chile (Aballay et al. 2009). In Brazil, *M. ethiopica* affects the root growth, yield and quality of grapevines and kiwi, causing serious crop damage and economic losses (Carneiro et al. 2003). In Chile, *M. ethiopica* was found to be the major root knot nematode parasite of grapevines (*Vitis vinifera*) and other crops (Carneiro et al.,

2007). Furthermore, Medina et al. (2014) reported *M. ethiopica* on potato plants from Parana state, Brazil, showing root systems with multiple galls and tubers with protuberances.

M. ethiopica is very difficult to distinguish from the sibling species *M. inornata* and the recently described *M. luci* (Carneiro et al. 2014) due to morphological and morphometrical similarity and intraspecific variation in the group. Besides, the perineal patterns of *M. ethiopica* are highly variable and similar to *M. luci* (Carneiro et al. 2014) and were characterized as varying from the *M. arenaria* type to the "*acrita*" type of *M. incognita* (Golden 1992). Analysing phenotypes of malate dehydrogenase (MDH) and esterase (EST) isozymes has proved to be a useful approach in RKN species identification (Esbenshade and Triantaphyllou 1985; 1990). Isozyme phenotyping is currently the most useful procedure for routine differentiation of *M. ethiopica* from other RKN species as the esterase E3 is species-specific (Carneiro et al. 2004). However, E3 is not the only esterase phenotype of *M. ethiopica*. Mandefro and Dagne (2000) observed phenotype E2 in a *M. ethiopica* population from Africa. In *M. ethiopica* populations reported from Slovenia, Italy, Greece and Turkey, esterase phenotype E3 has also been reported. However, a closer examination showed that all these populations have a three banded esterase pattern where the upper band is slightly lower than the one in *M. ethiopica* populations from Brazil, Chile and Peru. The pattern has recently been recognised as L3 and specific for a newly described species *M. luci* (Carneiro et al. 2014). The molecular approach also confirmed a very close relationship between *M. ethiopica* and *M. luci* and also *M. inornata*. Sequence of COII/lRNA, a region of mtDNA proved to be very useful for analysing the phylogenetic relationship of the very closely related RKN species and populations. Low levels of heteroplasmy in this region allowed the formation of a monophyletic clade for *M. luci* with a clear separation (Geric Stare et al., 2017a). Close relationship between these three species has also been shown on coding mtDNA regions by Janssen et al. (2016). Therefore all previously reported populations of *M. ethiopica* in Europe and Turkey were reclassified as *M. luci* (Geric Stare et al., 2017a).

Both *M. ethiopica* and *M. luci* cause severe damage to many host plants by affecting the development of the root systems which are distorted by multiple small and large galls. As a consequence, the water supply to the shoots is impaired. Affected plants can also show above ground symptoms such as stunting and wilting. Based on crop damage and economic losses that *M. ethiopica* has been causing on grapevines and kiwi (Carneiro et al. 2003, 2007) *M. ethiopica* has been included on the EPPO Alert list of harmful organisms in 2011. *M. ethiopica* is also reported to affect potato production (Medina et al. 2014) and *M. luci* has been shown as a potential threat to the potato production in Europe (Maleita et al. 2016). Further, *M. luci* has been reported on several vegetables, flowers and fruits species (Carneiro et al. 2014). In addition, the potential of *M. ethiopica* to survive outdoors under a continental climate even in areas where soil temperatures fall below zero during winter, as well as under a sub-Mediterranean climate (Strajnar et al. 2011) now applies to *M. luci* as the identity of Slovene *M. ethiopica* population has been reclassified into *M. luci* (Janssen et al. 2016; Geric Stare et al., 2017a). This indicates that *M. luci* could establish and spread in the PRA area.

In addition, the findings of virulent populations of *M. luci* in Turkey that can reproduce on tomato plants bearing *Mi*-1 resistance gene (Aydinli & Mennan, 2016) adds to the concern about the damage potential of this pest. The *Mi*-1 resistance gene has been introgressed into many commercial tomato cultivars to control the root knot nematode species *M. incognita, M. javanica* and *M. arenaria*. It has also been shown that *Mi*-1 prevents the reproduction of *M. luci* (Strajnar & Širca, 2011; Conceição et al., 2012). *Mi*-1 tomato cultivars are widely used to control root knot nematode species including *M. luci.* However, the finding of *M. luci* virulent populations may present serious threat to vegetable production as there are very limited efficient management options available.

As both *M. ethiopica* and *M. luci* can damage a large number of economically important crops, the EPPO Secretariat decided to include both species in the EPPO Alert List.

3. Is the pest a vector? **Yes** ☐ **No** ✓

4. Is a vector needed for pest entry or spread? **Yes** ☐ **No** ✓

5. Regulatory status of the pest

Meloidogyne ethiopica: EPPO Alert List pest since 2011, no other status
Meloidogyne luci: EPPO Alert List pest since 2017. Official measures for eradication were adopted in Slovenia in 2015.

6. Distribution

Meloidogyne ethiopica distribution

Continent	***Distribution*** *(list countries, or provide a general indication , e.g. present in West Africa)*	***Provide comments on the pest status in the different countries where it occurs*** *(e.g. widespread, native, introduced….)*	***Reference***
Africa	Ethiopia, Kenya, Mozambique, South Africa, Tanzania, Zimbabwe	Native, Present, no details	Whitehead, 1969; Mandefro & Dagne, 2000 Carneiro et al., 2004;
America	Brazil, Chile, Peru	Brazil (present in 5 provinces); Chile (widespread: area of 1000 km distance, causing economical damage)	Carneiro et al., 2003; Gomes et al., 2005; Carneiro et al., 2007; Somavilla et al., 2011; Murga-Gutierrez et al., 2012;
Asia	Absent	Reclassified in *M. luci*	Gerič Stare et al. 2017a;
Europe	Absent	Reclassified in *M. luci*	Gerič Stare et al. 2017a;
Oceania	Absent	No records	

Meloidogyne luci distribution

Continent	***Distribution*** *(list countries, or provide a general indication , e.g. present in West Africa)*	***Provide comments on the pest status in the different countries where it occurs*** *(e.g. widespread, native, introduced….)*	***Reference***
Africa	Absent	No records	
America	Brazil, Chile, Guatemala	Brazil (Present, few occurrences) Chile (Present, one occurrence) Guatemala (no details)	Carneiro et al., 2014; Belle et al., 2016a; Janssen et al. 2016
Asia	Iran, Turkey	Iran (Present, no details)	Aydinli et al., 2013; Gerič

Continent	***Distribution*** *(list countries, or provide a general indication , e.g. present in West Africa)*	***Provide comments on the pest status in the different countries where it occurs*** *(e.g. widespread, native, introduced….)*	***Reference***
		Turkey (widespread in Samsun province)	Stare et al. 2017a;
Europe	Greece, Italy, Portugal, Slovenia	Greece (Present, few occurrences) Italy (Present, one occurrence) Portugal (Present, one occurrence) Slovenia (Present, two occurrences; first occurrence eradicated; second occurrence under eradication)	Sirca et al., 2004; Strajnar et al., 2009; Conceição et al., 2012; Maleita et al. 2012; Maleita et al. 2016; Gerič Stare et al. 2017b;
Oceania	Absent	No records	

7. Host plants /habitats* and their distribution in the PRA area

There is no dependable differential host plant between *M. ethiopica* and *M. luci* known so far. It seems that these two species are so closely related that there is no biological difference between them in terms of parasitizing different plant species. Host species lists for *M. ethiopica* and *M. luci* were prepared based on recorded infestation in the field and tests of host status for each species in literature. Lower numbers of host plant species are to date confirmed for *M. luci*, since the description of the species was done only in 2014.

In assessing whether the host is a major or minor crop two factors has been taken into account, the importance of crop in agricultural production and host suitability of plant to the root knot nematode *M. ethiopica* or *M. luci* (e.g. soybean is considered to be a major crop, since the agricultural area with soybean is increasing each year and the crop plants are a very good host for *M. luci* and *M. ethiopica*).

Host species list for *Meloidogyne ethiopica*

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Acacia mearnsii (Black wattle)	No	-	Whitehead 1968,1969
Actinidia deliciosa (Kiwi)	Yes	Minor crop for PRA area / Major crop for some PRA area regions	Carneiro et al. 2003, 2004
Agave sisalana (Sisal)	No	-	O'Bannon 1975.
Ageratum conyzoides (Billygoat-weed)	No	-	O'Bannon 1975.
Arachis hypogaea (Peanut)	No	-	Lima et al. 2009
Asparagus officinalis (Asparagus)	Yes	Minor crop	Murga-Gutierrez et al. 2012
Avena sativa (White oat)	Yes	Minor crop	Lima et al. 2009
Avena strigose (Black oat)	No	-	Lima et al. 2009
Brassica napus (Rapeseed)	Yes	Minor crop	Lima et al. 2009
Brassica oleracea (Cabbage)	Yes	Major crop	O'Bannon 1975; Whitehead, 1968, 1969 Carneiro et al., 2014

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Cajanus cajan (Dwarf pigeon pea)	Yes	Minor crop	Lima et al. 2009
Capsicum annum (Pepper)	Yes	Major crop	Carneiro *et al*., 2004
Capsicum frutescens (Chili Pepper)	Yes	Minor crop	Whitehead 1968, 1969
Canavalia ensiformis (Jack-bean)	No	-	Lima et al. 2009
Carthamus tinctorius (Safflower)	Yes	Minor crop	Lima et al. 2009
Citrullus lantanus (Watermelon)	Yes	Minor crop	Carneiro *et al*., 2003; Carneiro *et al*., 2004
Clitoria ternatea (Butterfly pea)	Yes	Ornamental, minor crop	Lima et al. 2009
Crotalaria anaguroydes (Rattlepod)	No	-	Lima et al. 2009
Crotalaria apioclice (Rattlepod)	No	-	Lima et al. 2009
Crotalaria grantiana (Rattlepod)	No	-	Lima et al. 2009
Crotalaria juncea (Rattlepod)	No	-	Lima et al. 2009
Crotalaria lanceolata (Rattlepod)	No	-	Lima et al. 2009
Crotalaria okoelvka (Rattlepod)	No	-	Lima et al. 2009
Crotalaria spectabilis (Rattlepod)	No	-	Lima et al. 2009
Cucumis melo (Melon)	Yes	Major crop	Bellé et al. 2017b
Cucumis sativus (Cucumber)	Yes	Major crop	Carneiro et al., 2014
Cucurbita sp. (Pumpkin)	Yes	Major crop	Whitehead 1969;
Datura stramonium (Jimson weed)	Yes	weed	O'Bannon 1975.
Dahlstedtia pentaphylla (Timbó)	No	-	Lima et al. 2009
Dolichos lablab (Hyacinth bean)	Yes	Ornamental, minor crop	Lima et al., 2009
Eleusine coracana (Finger millet)	Yes	Minor crop	Lima et al. 2009
Ensete ventricosum (Ethiopian banana)	Yes	Ornamental, minor crop	Mandefro & Dagne 2000
Eruca sativa (Aragula)	Yes	Minor crop	Carneiro et al., 2014
Euchlaena mexicana (synonim *Zea mexicana*)	No	-	Lima et al. 2009
Fagopyrum esculentum (Buckwheat)	Yes	Major crop	Lima et al. 2009
Glycine max (Soybean)	Yes	Major crop	O'Bannon 1975. Castro et al., 2003, Carneiro et al., 2003
Glycine wightti (Cooper)	No	-	Lima et al. 2009

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Gossypium hirsutum (Cotton)	No	-	O'Bannon, 1975
Helianthus annuus (Sunflower)	Yes	Minor crop	Lima et al. 2009
Jatropha curcas (Barbados nut)	No	-	Lima et al. 2009
Lactuca sativa (Lettuce)	Yes	Major crop	O'Bannon 1975.
Lolium multiflorum (Italian ryegrass)	Yes	Minor crop	Lima et al. 2009
Lupinus albus (White lupin)	Yes	Minor crop	Lima et al. 2009
Lupinus angustifolius (Blue lupin)	Yes	Minor crop	Lima et al. 2009
Lycopersicon esculentum (Tomato)	Yes	Major crop	Carneiro et al., 2003 Mandefro i Dagne 2000
Medicago sativa (Alfalfa)	Yes	Major crop	Lima et al. 2009
Mucuna aterrima (Black velvet bean)	No	-	Lima et al. 2009
Mucuna cinerea (Grey velvet bean)	No	-	Lima et al. 2009
Mucuna deeringiana (Dwarf velvet bean)	No	-	Lima et al. 2009
Mucuna puriens (Green velvet bean)		-	Lima et al. 2009
Nicotiana tabacum (Tobacco)	No	-	Whitehead 1968, 1969
Ornithopus compressus (Yellow serradella)	No	-	Lima et al. 2009
Oxalis corniculata (Creeping woodsorrel)	Yes	weed	Belle et al. 2016b
Oryza sativa (Rice)	No	-	Lima et al. 2009
Pennisetum glaucum (Pearl millet)	Yes	Minor crop	Lima et al. 2009
Pisum sativum subsp. *arvense*) (Forage pea)	Yes	Major crop	Lima et al. 2009
Pisum sativum (Pea)	Yes	Minor crop	Lima et al. 2009
Phaseolus vulgaris (Bean)	Yes	Major crop	Mandefro & Dagne 2000 Bellé et al. 2017a
Polymnia sonchifolia (Yacon)	No	-	Carneiro & Almeida, 2005
Psidium guajava (Guava)	No	-	Dias et al. 2010
Prunus persica (Peach)	Yes	Major crop	Carneiro et al., 2003 Somavilla et al. 2009
Raphanus sativus (Radish)	Yes	Minor crop	Lima et al. 2009
Saccharum spp. (Sugarcane)	No	-	Bellé et al. 2017c
Setaria italica (Foxtail millet)	Yes	Minor crop	Lima et al. 2009
Secale cereale (Rye)	Yes	Major crop	Lima et al. 2009

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Sida rhombifolia (Arrowleaf sida)	No	-	Gomez et al. 2005; Lima-Medina et al. 2013
Solanum melongena (Eggplant)	Yes	Major crop	Carneiro et al. 2014
Solanum nigrum (Nightshade)	Yes	weed	O'Bannon 1975.
Solanum tuberosum (Potato)	Yes	Major crop	Whitehead 1968, 1969; Lima-Medina et al., 2011
Spinacia oleracea (Spinach)	Yes	Minor crop	Carneiro et al. 2014
Tephrosia candida (White hoarypea)	No	-	Lima et al. 2009
Triticum aestivum (Triticale)	Yes	Minor crop	Lima et al. 2009
Vicia faba (Broad bean)	Yes	Minor crop	Whitehead 1969
Vigna umbellata (Ricebean)	Yes	Minor crop	Lima et al. 2009
Vigna unguiculata (Cowpea)	Yes	Minor crop	Whitehead, 1968 Lima et al. 2009
Vigna radiata (Mungbean)	Yes	Minor crop	Lima et al. 2009
Vicia sativa (Common vetch)	Yes	Minor crop	Lima et al. 2009
Vicia villosa (Hairy vetch)	Yes	Minor crop	Lima et al. 2009
Vitis (Grapevine)	Yes	Major crop	Carneiro et al. 2003, 2004
Zea mays (Corn)	Yes	Major crop	Lima et al. 2009

Host species list for *Meloidogyne luci*

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Abelmoschus esculentus (Okra)	No	-	Carneiro et al., 2014
Actinidia deliciosa (Kiwi)	Yes	Minor crop for PRA area / Major crop for some PRA area regions	Carneiro et al., 2014
Allium cepa var. cepa (Onion)	Yes	Minor crop	Strajnar et al., 2009
Antirrhinum majus (Snapdragon)	Yes	Ornamental Minor crop	Carneiro et al., 2014
Apium graveolens (Celery)	Yes	Minor crop	Strajnar et al., 2009
Brassica oleracea var. capitate (Cabbage)	Yes	Major crop	Strajnar et al., 2009
Brassica oleracea var. botrytis (Cauliflower)	Yes	Minor crop	Strajnar et al., 2009
Brassica oleracea var. gongylodes (Kohlrabi)	Yes	Minor crop	Strajnar et al., 2009
Brassica oleracea var. italic (Broccoli)	Yes	Minor crop	Strajnar et al., 2009 Carneiro et al., 2014

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Brassica oleracea var. subauda (Kale)	Yes	Minor crop	Strajnar et al., 2009
Beta vulgaris var. cicla (Chard)	Yes	Minor crop	Strajnar et al., 2011
Beta vulgaris var. conditiva (Beet)	Yes	Minor crop	Strajnar et al., 2009
Capsicum annuum (Pepper)	Yes	Major crop	Carneiro et al., 2014
Cichorium endivia (Endive)	Yes	Major crop	Strajnar et al., 2009
Cichorium intybus var. foliosum (Chicory)	Yes	Major crop	Strajnar et al., 2009
Cucumis melo (Melon)	Yes	Major crop	Strajnar et al., 2009
Citrullus lantanus (Watermelon)	Yes	Minor crop	Geric Stare et al., 2017a
Cucumis sativus (Cucumber)	Yes	Major crop	Strajnar et al., 2009 Carneiro et al., 2014
Daucus carota (Carrot)	Yes	Minor crop	Strajnar et al., 2009
Foeniculum vulgare var. azoricum (Florence fennel)	Yes	Minor crop	Strajnar et al., 2009
Glycine max L. (Soybean)	Yes	Major crop	Bellé et al., 2016a
Helianthus annuus L. (Sunflower)	Yes	Minor crop	Strajnar et al., 2009
Hordeum vulgare L. (Barley)	Yes	Major crop	Strajnar et al., 2009
Hylotelephinum spectabile L. (Sedum)	Yes	Ornamental minor crop	Carneiro et al., 2014
Lactuca sativa (Lettuce)	Yes	Major crop	Strajnar et al., 2009 Carneiro et al., 2014
Lavandula spica L (Lavender)	Yes	Ornamental minor crop	Carneiro et al., 2014
Lycopersicum esculentum (Tomato)	Yes	Major crop	Strajnar et al., 2009
Nicotiana tabacum L. (tobacco)	No	-	Carneiro et al., 2014
Phaseolus vulgaris (Common Bean)	Yes	Major crop	Strajnar et al., 2009 Carneiro et al., 2014
Pisum sativum (Pea)	Yes	Major crop	Strajnar et al., 2009
Polymnia sonchifolia Poepp. Endl (Yakon)	No	-	Carneiro et al., 2014
Raphanus sativus var. *radicula* (Radish)	Yes	Minor crop	Strajnar et al., 2009
Rosa sp. (Rose)	Yes	Ornamental minor crop	Carneiro et al., 2014
Rumex patientia L (Curled dock)	Yes	Weed	Strajnar et al., 2009
Solanum melongena (Eggplant)	Yes	Major crop	Strajnar et al., 2009
Solanum tuberosum (Potato)	Yes	Major crop	Maleita et al., 2016
Spinacia oleracea (Spinach)	Yes	Minor crop	Strajnar et al., 2009

Host Scientific name (common name) ***/ habitats****	**Presence in PRA area (Yes/No)**	**Comments (e.g. total area, major/minor crop in the PRA area, *major/minor habitats**)**	***Reference***
Vitis vinifera (Grapevine)	Yes	Major crop	Carneiro et al., 2014
Zea mays (Corn)	Yes	Major crop	Strajnar et al., 2011
Zea mays var. *saccharata* (Sweet corn)	Yes	Minor crop	Strajnar et al., 2009

8. Pathways for entry

Possible pathways *(in order of importance)*	**Short description explaining why it is considered as a pathway**	Pathway prohibited in the PRA area? Yes/No	Pest already intercepted on the pathway? Yes/No
Host plants for planting with roots, including bulbs, tubers, corms, rhizomes (except plants in tissue culture) with or without growing medium attached; excluding seeds	Infested plant roots, tubers (e.g. potato) etc., carry viable pest (early or later stage)	No for all hosts/countries Yes- for some hosts from some non-EU or non-European countries. See points 9., 10., 11., 13., 15., 18. of Annex III CD 2000/29/EC Specific requirements for all soil attached to plants originating in some non-EU countries and non-European countries. See point 34. of Annex IV CD 2000/29/EC	No to the species level. Only as *Meloidogyne* sp.
Non-host plants for planting (including tubers, rhizomes, bulbs and corms) with growing medium attached	Infested soil attached (pest in egg stage and J2)	No for all hosts/countries Yes- for some hosts from some non-EU or non-European countries. See points 1,2,3, 9.1, 16, 17,19. of Annex III CD 2000/29/EC. Specific requirements for all soil attached to plants originating in some non-EU countries and non-European countries. See point 34. of Annex IV CD 2000/29/EC	No
Host plant parts (such as roots, tubers, bulbs, corms, rhizomes), for	Infested consumption tubers, corms (e.g. infested	No for all hosts/countries	No

consumption or processing.	consumption potato, celery, carrots etc.);	Yes- for some hosts from non-EU or non-European countries. See points 12.of Annex III CD 2000/29/EC.	
Non-host plant parts contaminated with soil (such as roots, tubers, bulbs, corms, rhizomes), for consumption or processing.	Infested soil attached (pest in egg stage and J2)	No for all hosts/countries Yes- from some non-EU countries and non-European countries. See point 14. of Annex III CD 2000/29/EC	No
Soil and growing medium as such from areas where the nematode occurs	There is a possibility to introduce the pest in egg stage and J2.	No for all hosts/countries Yes- from some non-EU countries and non-European countries. See point 14. of Annex III CD 2000/29/EC	No
Machinery, tools, packaging materials carrying soil and growing medium	There is possibility to introduce the pest in egg stage and J2.	No	No
Travelers	There is possibility to introduce infested plants, plant products or soil on shoes	No	No

M. ethiopica

Rating of the likelihood of entry	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

M. luci

Rating of the likelihood of entry	*Low*☐	*Moderate*☐	*High*✓
Rating of uncertainty	*Low*☐	*Moderate*✓	*High*☐

Pathways for entry of *M. luci* from EU countries, where *M. luci* is present, are not regulated (Italy, Greece, Portugal). It is not known how RKN *M. luci* was introduced in Slovenia. However, *M. luci* was detected in Slovenia twice. The location of infestation from 2015 is situated more than 100 km away from location found in 2003 and no direct connections between these farms could be found. This indicates that infestation at the new location might be a result of a new entry into the county. Therefore rating of the likelihood of entry for *M. luci* is high and uncertainty was assessed as moderate. For *M. ethiopica* the rating of the likelihood of entry is moderate, since its presence has been reported only from non EU countries.

9. Likelihood of establishment outdoors in the PRA area

M. luci has already been detected in the open field production in Europe (Mediterranean climate) in corn and kiwi production in Greece, and potato production in Portugal. In addition, the potential of *M. luci* to survive outdoors under a continental climate even in areas where soil temperatures fall below zero during winter, as well as under a sub-Mediterranean climate (Strajnar et al. 2011) indicates high rating of the likelihood that *M. luci* could establish in most parts of the PRA area. For *M. ethiopica* the rating of the likelihood of establishment outdoors as well as rating of uncertainty is moderate. The ability of species to overwintering in the open field in temperate and Mediterranean areas has not been tested yet, but it is possible that *M. ethiopica* has similar temperature requirements as other tropical RKN species (M. incognita, M. luci, M. arenaria) which can establish in European climate conditions. Host plants of *M. luci* and *M. ethiopica* are present throughout the PRA area.

M. luci

Rating of the likelihood of establishment outdoors	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ✓	*Moderate* ☐	*High* ☐

M. ethiopica

Rating of the likelihood of establishment outdoors	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

10. Likelihood of establishment in protected conditions in the PRA area

Both pest species have very wide host range including majority of vegetable crops which are cultivated in the protected areas. The probability of establishment in the protected area is high.

M. ethiopica & *M. luci*

Rating of the likelihood of establishment in protected conditions	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ✓	*Moderate* ☐	*High* ☐

11. Spread in the PRA area

- Natural spread

Natural spreading of RKNs is limited to short distances (in the range of ca. 1m).

- Human assisted spread

Human assisted spread is much more important. The spreading over longer distances, within fields or regions, may occur by agricultural machinery (tractor wheals, tillage and cultivator equipment, any equipment that have direct soil contact).
Water for irrigation is considered to have a moderate role for spreading of nematodes in new area.
Host plants for planting (vegetables, ornamental plants, fruit trees and grapevine) with or without growing medium attached are considered the main pathway for spread of *M. ethiopica* and *M. luci* within the PRA area. In addition, plant parts like tubers may present also high risk of spreading in case of pest establishment outdoors as well as infested growing medium (soil) as such.

The rating of the magnitude of spread for *M. luci* is high, because of high number of host plants, its ability to survive outdoors; it was detected in the open field in Europe. For *M. ethiopica* the rating of the magnitude of spread is moderate, since no record of the species presence in the PRA area or other EU country has been reported yet.

M. ethiopica

Rating of the magnitude of spread	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

M. luci

Rating of the magnitude of spread	*Low*☐	*Moderate*☐	*High*✓
Rating of uncertainty	*Low*☐	*Moderate*✓	*High*☐

12. Impact in the current area of distribution

M. ethiopica is widely distributed in vineyards in Chile (Aballay et al. 2009) and was found to be the major root knot nematode parasite of grapevines (*Vitis vinifera*) and other crops in Chile (Carneiro et al., 2007). In Brazil, *M. ethiopica* affects the root growth, yield and quality of grapevines and kiwi, and cause serious economic losses (Carneiro et al. 2003). Furthermore, Medina et al. (2014) reported *M. ethiopica* on potato plants from Parana state, Brazil, showing root systems with multiple galls and tubers with protuberances. *M. luci* has been reported on several vegetables but there are no reports of economic damage caused by *M. luci* except from Slovenia.
In Slovenia, *M. luci* was found at two locations. In the greenhouse in Primorska from 2003 the pest was eradicated due to omission of the agricultural production. Second location was detected in 2015 in a greenhouse in the village near Ljubljana. Infested tomato plants showed symptoms of decline, leaf chlorosis and large galls were present on the roots. Nematode infestation was relatively high as more than 80 % of plants were severely damaged. Phytosanitary measures were imposed in the infested greenhouse including the use of dazomet, a soil fumigant. All infested plant material from the affected greenhouse was destroyed. However, the eradication program implemented in the past two years has not been successful. Therefore only resistant tomato cultivars carrying *Mi* gene and poor- to non-host crops in combination with registered chemical nematicide were allowed to be grown in the infested greenhouse. The success of this management program for nematode control will be assessed in the future. The infested area measures approximately 1100 m2.

M. ethiopica (America)

Rating of the magnitude of impact in the current area of distribution	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

M. luci (America and Europe)

Rating of the magnitude of impact in the current area of distribution	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

Rating of the magnitude of impact in the current area of distribution was assessed as high because of high impact of *M. luci* at current area of distribution in PRA area and high impact of *M. ethiopica* in America.
Moderate rating of uncertainty was assessed due to lack of available data on economic damage caused by both species at current area of distribution.

13. Potential impact in the PRA area

M. ethiopica and *M. luci* could survive under protected cultivation (glasshouses) across the PRA area. Climatic conditions for establishment of *M. luci* in the PRA area outdoors are suitable since this species was proved to overwinter in continental and Mediterranean climate. Besides it was already detected

outdoors in Europe (Mediterranean climate), in corn and kiwi production in Greece, and potato production in Portugal. Once the RKNs are introduced into the PRA area, it is in general difficult to control or eradicate them, especially outdoors at the open field. The ability of *M. ethiopica* to overwinter at the open field in continental and Mediterranean climate conditions has not been confirmed yet. However, the models of climate changes predict an increase of average temperatures and more frequent periods of drought, floods and heat waves. Higher temperatures will allow development of more generations of RKNs in a growing season and consequently higher yield losses. It is therefore very likely that the potential impact of both pest species in the PRA area will be higher.

Will impacts be largely the same as in the current area of distribution? No

The potential impact of pests on outdoor crops in the PRA area is difficult to estimate. Epidemiological studies such as the assessment of the damage potential of both pest species for major crops such as potatoes, corn, soybean, grapevine, fruit trees etc. in the PRA area climate are not available. We can expect losses in the protected cultivation (with soil as natural growing medium) and the same impact as in the current area of distribution.

If No
Outdoors
M. ethiopica

Rating of the magnitude of spread	*Low* ☐	*Moderate* ✓	*High* ☐
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

M. luci

Rating of the magnitude of impact in the area of potential establishment	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

Indoors
M. luci, M. ethiopica

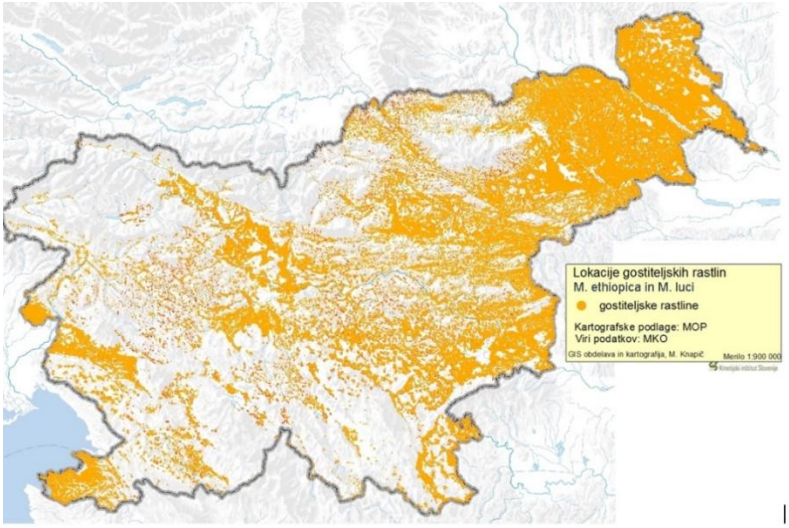
Rating of the magnitude of impact in the area of potential establishment	*Low* ☐	*Moderate* ☐	*High* ✓
Rating of uncertainty	*Low* ☐	*Moderate* ✓	*High* ☐

14. Identification of the endangered area

For the both species protected cultivation (glasshouses) across the PRA area. *M. luci* has already been found in a protected cultivation in Slovenia.

Climatic conditions are not considered to be a limiting factor for the establishment of the *M. luci* in PRA area. It may establish and cause damage in area with continental and Mediterranean climate conditions, wherever there are hosts, i.e. throughout Slovenia, except in the areas of alpine climatic conditions. On the other hand there are some uncertainties if *M. ethiopica* can establish in European continental and Mediterranean climate conditions, since the ability of *M. ethiopica* to overwinter at the open field in continental and Mediterranean climate has not been tested yet.

Figure: Distribution of the most important major crops in Slovenia (covers almost all national agricultural production land).



15. Overall assessment of risk

Root knot nematodes *M. ethiopica* and *M. luci* can be introduced by several pathways but the highest risk for entry and spread in the PRA area is considered the pathway of host plants for planting with roots with or without growing medium attached, like the planting material of vegetables, ornamental plants, fruit trees and grapevine. Furthermore, plant parts like tubers may present also high risk of spread in case of pest establishment outdoors, especially in potato tuber seed production where latent infestations may also occur (tubers with no visible symptoms of infestation). Human assisted spread of the pests is the most important.

M. ethiopica and *M. luci* have very wide host range including majority of vegetable crops which are cultivated indoors. The probability of establishment in the protected area is high. *M. luci* has already been detected in the open field production in Europe (Mediterranean climate), in corn and kiwi production in Greece, and potato production in Portugal. There is a high risk of *M. luci* establishment outdoors in the PRA area, as this species was proved to overwinter in open field in continental and Mediterranean climate. Once the RKNs have been introduced, it is in general difficult to control or eradicate them, especially outdoors. The ability of *M. ethiopica* to overwinter outdoors in continental and Mediterranean climate has not been tested yet.

Phytosanitary measures to prevent the spread of these pests into non-infested area in case of an outbreak are necessary. The measures should include prohibition of movement of plants for planting outside infested area, movement of soil outside infested area, disinfection of equipment and footwear, destruction of nematode infested plant waste, and intensive monitoring of infested area and the surroundings.

The potential impact of both pest species in the PRA area could be higher especially in the light of climate change scenarios. The models of climate changes predict an increase of average temperatures and more frequent periods of drought, floods and heat waves. Higher temperatures would allow development of more generations of RKNs in a growing season and consequently higher yield losses.

Stage 3. Pest risk management

16. Phytosanitary measures

16.1 Measures on individual pathways

Measures for host plants for planting with roots, bulbs, tubers, corms, rhizomes are listed in the table below as the high risk pathway, in order to prevent entry of *M. ethiopica and M. luci* into host production systems.

For soil (growing medium), the risk of entry relates mostly to soil from where RKNs are present, but many EPPO countries prohibit import of soil from third countries. Internal movement of soil within the EU is not regulated, but the likelihood of entry from Italy, Greece and Portugal is considered as low (due to the types of environments in which the nematode is currently present). Measures for soil were not studied, but are likely to be similar to those for other nematodes.

Possible pathways *(in order of importance))*	**Measures identified**
Host plants for planting with roots (including rooted cuttings, bulbs, tubers, corms, rhizomes; except plants in tissue culture), with or without growing medium	Pest free area Or Pest-free production site or pest-free place of production (with all production sites pest-free) Or Growing under complete physical isolation (EPPO Standard PM5/8) (with requirements appropriate for RKN)
Host plant parts (bulbs, tubers, corms, rhizomes) with or without growing medium	Pest free area Or Pest-free production site or pest-free place of production (with all production sites pest-free)
Non-host plants for planting with growing medium attached	Pest free area Or Pest-free production site or pest-free place of production (with all production sites pest-free) Or Growing under complete physical isolation (EPPO Standard PM5/8) (with requirements appropriate for RKN) Or Growing medium has been completely removed

16.2 Eradication and containment

- Prevention of spread

The measures include prohibition of movement of plants for planting and soil from infested area, disinfection of equipment and footwear, destruction of nematode infested plant waste, and intensive survey of the infested area and surroundings.
The irrigation system may also be a way of spreading nematodes to a new location, but more likely in protected production areas.

- Eradication/control

Once RKN have been introduced, it is in general difficult to control or eradicate them mainly because of their polyphagous nature and limited management options. At the place of production RKNs can be controlled with a proper crop rotation of poor and non-host crops, resistant varieties and the usage of nematocides. Efficient crop rotation is very difficult to set up in vegetable production as there are many vegetable crops that are good hosts for both species. Several commercial tomato and pepper varieties exhibit resistance and tolerance against RKN. However, the resistance breaking populations of *M. luci* have already been reported from Turkey. No other host crop exhibit resistance.

There are two registered nematocides in Slovenia, a soil fumigant dazomet for protected areas and open field, and fluopyram for protected areas only. Dazomet was used in *M. luci* eradication program at 2015 outbreak but did not reach successful effect as the RKN population rendered again in next growing season, maybe due to unsuitable application. The efficiency of fluopyram at the same location is currently under investigation. A product which employs a biological mode of action with *Bacillus firmus* bacteria strain that prevents nematodes from causing damage is currently in the registration process (Terefe et al., 2009).

17. Uncertainty

The main sources of uncertainty within the risk assessment:
- the likelihood of pest entry,
- the magnitude of spread in the PRA area,
- the magnitude of impact in the area of potential establishment.

It is presumed that *M. luci* was introduced in Slovenia twice, in separate entries into the county. The pathways of entry in both cases have not been identified. At the same time origin of the pest is difficult to suspect because of very limited known pest distribution in European and at the world scale.
The uncertainty for the magnitude of spread and potential impact in the PRA was also assessed as "moderate" mainly because of uncertain entry pathways and the lack of epidemiological data for the PRA area climate conditions. Both pest species might colonise entire PRA area. It is difficult to estimate the influence of pests on outdoor crops, but we can observe losses in the protected cultivation (with soil as natural growing medium). Epidemiological studies such as the assessment of the damage potential of both pest species for major crops such as potatoes, corn, soybean, grapevine, fruit trees etc. in the PRA area climate would enable informative phytosanitary decisions. Several tomato and pepper varieties exhibit resistance and tolerance against RKN however the resistance breaking populations of *M. luci* have already been reported from Turkey.

The main source of uncertainty within the risk of management is lack of efficient pests control measures. Because of limiting chemical pesticide usage trend, more environmentally friendly approaches will be use in the future. Performing pest management studies of genetic resistance sources and applications of soil amendments formulation like essential oils or biomasses from plants with biocidal properties, as well as microbial bio-nematicides based on nematode-parasitic fungi or bacteria are therefore of the utmost importance.

18. Remarks

The biggest concern for *M. luci* is the spread at the open field agricultural production area (see 9. likelihood of establishment outdoors in the PRA area). The ability of *M. ethiopica* to overwinter in the open field in continental and Mediterranean climate has not been tested yet. Because of the polyphagous nature of both pests and limited management options the pests (in case of a wide spread) would be very difficult to control. In addition, the climate changes are in favour of such tropical pests which complete more reproduction cycles at higher summer temperatures and thus cause greater damage.

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Appendix 1. Symptoms of *M. luci* infestation.

M. luci infestation of tomatoes in Slovenia in 2015. (Foto: KIS)	*M. luci* infested tomato root system. (Foto: KIS)