European and Mediterranean Plant Protection Organization Organisation Européenne et Méditerranéenne pour la Protection des Plantes

Diagnostics

# PM 7/145 (1) Longidorus diadecturus

# Specific scope

This Standard describes a diagnostic protocol for *Longidorus diadecturus*.<sup>1</sup>

Terms used are those in the EPPO Pictorial Glossary of Morphological Terms in Nematology.<sup>2</sup>

This Standard should be used in conjunction with PM 7/76 Use *of EPPO diagnostic protocols*.

#### 1. Introduction

The genus *Longidorus* Micoletzky, 1922 is the second most diverse genus within the family Longidoridae (Thorne, 1935) Meyl, 1961, with over 180 species assigned to the genus considered valid (Decraemer, pers. comm.). These species, also named needle nematodes, have a very wide host range of herbaceous and woody plants within agriculture, horticulture and forestry and have been reported from all continents with the exception of Antarctica. *Longidorus diadecturus* Eveleigh & Allen, 1982 was described from southwestern Ontario (Essex County), Canada, and subsequently reported from the middle states of the United States (Robbins & Brown, 1991; Robbins *et al.*, 1995; Neilson *et al.*, 2004; Ye & Robbins, 2004); however, there remains some uncertainty regarding these records (EFSA, 2017).

Longidorus diadecturus has been recognised as a vector of Peach rosette mosaic virus (PRMV) in one field in Ontario (Eveleigh & Allen, 1982) and in transmission tests (Allen *et al.*, 1984). PRMV is also transmitted by certain species of the *Xiphinema americanum* group and is the only nepovirus that is transmitted by vectors belonging to two different nematode genera, *Xiphinema* and *Longidorus* (Brown *et al.*, 1988). Allen *et al.* (1984) reported that PRMV transmission efficiency was highest with *L. diadecturus*, where efficiency reached 53% compared to

<sup>1</sup>Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

<sup>2</sup>https://www.eppo.int/ACTIVITIES/plant\_quarantine/diagnostics#glossary

22% with X. americanum, using 2–10 nematodes in a single cucumber bait plant system. Nematode-borne viruses are transmitted by juveniles and adult specimens through the stylet when feeding but the virus does not multiply within the nematodes and may be lost during moulting (Taylor & Brown, 1997).

As free-living migratory ectoparasites, needle nematodes are found in soil or growing media, and as a consequence can be moved during trade with soil associated with plants for planting, plant products (such as potato tubers contaminated with soil), bulk soil and any other goods or machinery contaminated with soil.

# 2. Identity

Name: Longidorus diadecturus Eveleigh & Allen, 1982 Taxonomic position: Dorylaimida, Dorylaimina, Dorylaimoidea, Longidoridae, Longidorinae (after Decraemer & Hunt, 2013)

EPPO Code: LONGDI

**Phytosanitary categorization:** EU Annex IIA (as A1 Quarantine Pests)

#### 3. Detection

#### 3.1. Symptoms

*Longidorus diadecturus* is a migratory ectoparasitic nematode that, like other *Longidorus* species, feeds on root tips, causing small galls and stunting of roots (Taylor & Brown, 1997). In the absence of virus infection, the aerial parts of

ISSN 0250-8052. DOI: 10.1111/epp.12712

# Specific approval and amendment

Approved in 2020-10.

plants grown in soil infested with *Longidorus* spp. display few symptoms unless population levels are high, in which case roots exhibit swellings close to the root tips (terminal galling) and typical symptoms of root damage (such as reduction in vigour or signs similar to those that occur when a plant is under limited water conditions) may be observed.

#### 3.2. Test sample requirements

*Longidorus* species, as with most ectoparasitic plant-parasitic nematodes, can be detected by extraction from soil or growing media (EPPO, 2013). Sampling of the soil or growing media should not be performed using small diameter augers as these may damage longidorid nematodes. Further sampling guidelines can be found in EPPO PM 4/35 (1) *Soil test for virus–vector nematodes* (EPPO, 2009).

## 4. Identification

Identification of *L. diadecturus* is based on morphological features. To date, no molecular tool is available. However, the sequences of *L. diadecturus* rDNA are available at NCBI (18S partial: AY283166 & AY283167; ITS: AF511415 & AF511416; 28S partial: AY601584) and could be used for confirmation of identification. Identification of this species is extremely difficult and time-consuming, and should only be carried out by trained personnel (EFSA, 2017).

#### 4.1. Identification based on morphological features

It is recommended that a morphological study is carried out on as many nematode specimens as possible, mounted in water on microscopic slides. A key for the identification of the genus *Longidorus* is presented in Table 1.

For identification of *Longidorus* species, morphological and morphometric characteristics are traditionally used (Chen *et al.*, 1997). The most important features for distinguishing and identifying species of the genus *Longidorus* are guiding ring position, lip region shape and width, odontostyle length, amphidial fovea shape, tail and body width and length (Ye & Robbins, 2004).

Longidorus diadecturus can be differentiated from morphologically similar species by the posterior position of the stylet guiding ring, shape of the lip region, stylet length of 168–187  $\mu$ m, odontostyle and odontophore length of 109–121  $\mu$ m and 55–66  $\mu$ m, respectively, and body length (Eveleigh & Allen, 1982; Robbins & Brown, 1991).

No details regarding the life cycle of *L. diadecturus* have been reported. As this nematode has been assigned to the genus *Longidorus*, it is assumed that the life cycle is similar to that of other species within the genus, having six stages: the egg, four juvenile stages and the female adult. Males have not been recorded and it is also assumed that *L. diadecturus* reproduces parthenogenetically (Robbins *et al.*, 1995). Morphological characters of *Longidorus* exhibited by the adult female, male and J1 (Fig. 1) are used in the polytomous key presented (Tables 2 and 3).

#### 4.1.1. Identification to genus

The key shown in Table 1 can be used to differentiate *Longidorus* from other soil-borne nematode genera.

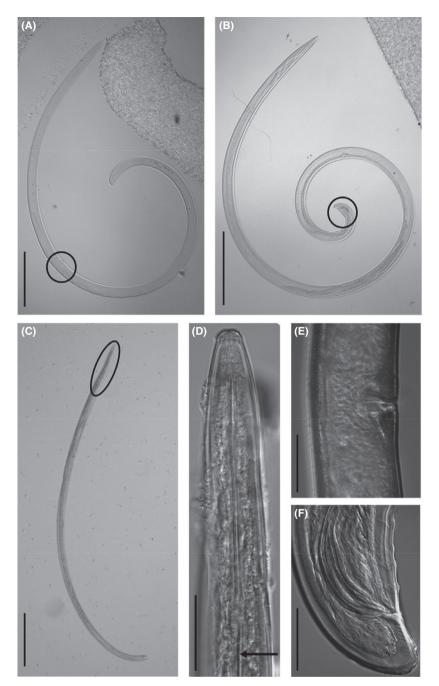
Longidorus Micoletzky, 1922

 Table 1. Key for the identification of Longidorus. Please refer to the EPPO Technical document No. 1056 (rev 6) Pictorial Glossary of

 Morphological Terms in Nematology for terminology and illustrations.

1.	Pharynx, excluding the pharyngostome, bipartite; the anterior a slender flexible tube expanding into posterior glands	2
	in the form of a cylindrus or fusiform bulb; pharyngeal glands usually 2/5–1/2 of pharyngeal region.	
-	Pharynx not bipartite; various forms such as tripartite, bulb with grinder or glands not clearly demarcated and pharynx appearing cylindrical.	NLS
2.	Stoma with protrusible odontostyle or a single ventrosublateral mural tooth. Adanal precloacal supplements in male paired.	3
_	Morphology of the stoma region variable. Stoma either unarmed, with denticles or teeth or with a complex protrusible tooth. No paired adanal supplement in male.	NLS
3.	Odontostyle elongated and attenuate (50-220 µm) with fine lumen and aperture; 3 pharyngeal glands (Longidoridae).	4
-	Typically, mural tooth or odontostyle comparatively much smaller with a wider aperture. If attenuate, odontostyle relatively short and with 5 pharyngeal glands.	NLS
4.	Dorsal nucleus (DN) smaller than the ventrosublateral nuclei (VSN) and situated some distance posterior to its orifice, located in anterior region of the glands; guide ring single when stylet retracted (Longidorinae).	5
-	DN larger than the VSN and situated adjacent to its orifice, located in the very anterior of the glands; guide ring typically appearing 'double' due to folding of guiding sheath.	NLS
5.	Odontostyle base non-furcate, odontophore base not swollen to slightly swollen; guide ring located within anterior third or two-thirds of odontostyle length from anterior (Longidorini).	6
-	Odontostyle base furcate, odontophore flanged; guide ring typically located more posteriorly.	NLS
6.	Amphidial aperture pore-like; amphidial fovea a pouch.	Longidorus
_	Amphidial aperture a minute to large transverse slit.	NLS

NLS, Not a Longidorus species.



**Fig. 1** Longidorus cheni paratype specimens (*L. jonesi* group); hot-formalin fixed and processed to glycerol. (A) Adult female with vulval region highlighted. (B) Adult male with spicular region highlighted. (C) First-stage juvenile with anterior region highlighted. (D) Replacement odontostyle within J1. (E) Vulval region of adult female. (F) Spicular region of adult male. Scale bar (A) and (B): 500  $\mu$ m; scale bar (C): 250  $\mu$ m; scale bar (D)–(F): 50  $\mu$ m). Male characters are included as they are referred to in the key in Table 1.

## Syn. Neolongidorus Khan, 1987

(Description after Hunt, 1993; Peña-Santiago, 2006; Decraemer & Geraert, 2013).

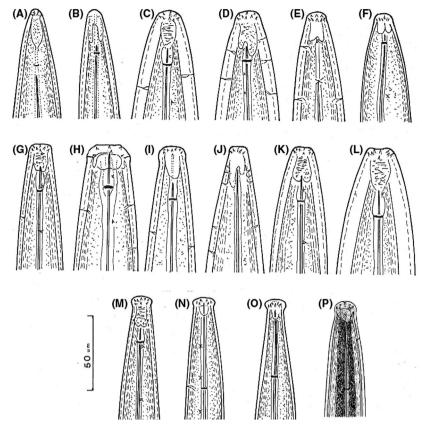
Longidorinae: Lip region rounded to more or less flattened, continuous or marked by a depression or constriction. Odontostyle non-furcate; base of odontophore without flanges. Cheilostome relatively long; guide ring single, anteriorly situated, compensation sacs present at level of the guide ring. Amphidial fovea a pouch, its aperture a pore. Male with an adanal pair of pre-cloacal supplements (2–3 adanal pairs in some species) in addition to a ventromedian series of up to 20 pre-cloacal supplements, without a hiatus between the adanal pair and the series; in some species the ventromedian row forms a double staggered

A: Length of odontostyle	1	Under 60 µm
	2	61–81 μm
	3	81–100 μm
	4	101–120 μm
	5	121–140 μm
	6	141–160 µm
	7	Over 160 µm
B: Diameter of lip region	1	Under 12 µm
	2	12–15 μm
	3	16–19 µm
	4	20–23 µm
C. Distance between the midling sing and entering	5	Over 23 μm
C: Distance between the guiding ring and anterior	1	17–20 µm
end (length of cheilostome)	2	21–30 µm
	3	31–40 µm
	4	41–50 µm
D. Shana of antarian ragion (Eig. 2)	5	51 µm or more
D: Shape of anterior region (Fig. 2)	1	Body tapering distinctly, lip region rounded, continuous (Fig. 2A–C) Body tapering distinctly, lip region rounded, off–set by depression (Fig. 2D–F, P)
	2 3	Body tapering distinctly, ip region flattened, continuous or slightly offset by depression
	5	(Fig. 2G–L)
	4	Body tapering distinctly or subcylindrical, lip region distinctly offset by constriction or expanded (Fig. 2M–O)
E: Shape of amphidial fovea (Fig. 2)	1	More or less pocket-shaped, posterior rounded without distinct basal lobes (Fig. 2G–I, N, P)
E. Shape of ampindial lovea (115. 2)	2	More or less pocket-shaped, shallowly or distinctly bilobed, symmetrical (lobes of about
	2	equal length) (Fig. 2B, E, F, K, M, O)
	3	More or less pocket-shaped, bilobed, asymmetrical, one lobe being shorter than the other
	5	or almost absent (Fig. 2D, J)
	4	Elongate pouch, not lobed (Fig. 2A, C, L)
F: Body length	1	Less than 3 mm
	2	3.1–5.0 mm
	3	5.1–7.0 mm
	4	7.1–9.0 mm
	5	9.1 mm or more
G: Index 'a'	1	80 or less
	2	81–120
	3	121–160
	4	More than 160
H: Tail shape (Fig. 3)	1	Hemispherical to bluntly conoid, $c' < 1$ (Fig. 3A–C, Q)
	2	Rounded to bluntly conoid, $c' 1.0-1.5$ (Fig. 3D-F)
	3	Rounded to bluntly conoid, c' 1.6-2.0 (Fig. 3G, H)
	4	Conoid, c' 1.0–1.5 (Fig. 3I–K)
	5	Conoid, c' 1.6–2.0 (Fig. 3L–M)
	6	Conoid, $c' > 2.0$ (Fig. 3N–P)
I: Presence/absence of males	1	Males absent
	2	Males present
	12	Males absent in some populations, present in others
J: Number of juvenile stages	1	4 juvenile stages*
	2	3 juvenile stages
K: Tail shape of 1st stage juveniles (Fig. 3)**	1	Hemispherical to bluntly conoid, $c' < 1$ (Fig. 3A–C)
	2	Rounded to bluntly conoid, c' 1.0-1.5 (Fig. 3D-F)
	3	Rounded to bluntly conoid, c' 1.6-2.0 (Fig. 3G, H)
	4	Conoid, c' 1.0-1.5 (Fig. 3I-K)
	5	Conoid, c' 1.6-2.0 (Fig. 3L, M)
	6	Conoid, c' >2.0 (Fig. 3 N–P)
	7	Tail digitate or with mucro

Table 2. Characters used in the polytomous key and their codes. For terminology refer to the EPPO Technical Document No. 1056 (rev 6) Pictorial Glossary of Morphological Terms in Nematology. Adapted from Chen et al. (1997), Loof & Chen (1999) and Peneva Peneva et al. (2013).

\*Assumed for Longidorus diadecturus.

\*\*Currently unknown for Longidorus diadecturus.



**Fig. 2** Shape of the anterior end (code D). (A)–(C): Code D1; (D)–(F), (P): Code D2; (G)–(L): Code D3; (M)–(O): Code D4. Shape of amphidial pouch (code E). (G)–(I), (N), (P): Code E1; (B), (E), (F), (K), (M), (O): Code E2; (D), (J): Code E3; (A), (C), (L): Code E4. (A) *L. belondiroides*; (B) *L. orientalis*; (C) *L. caespiticola*; (D) *L. goodeyi*; (E) *L. litchi*; (F) *L. juveniloides*; (G): *L. elongatus*; (H) *L. kuiperi*; (I) *L. proximus*; (J) *L. belloi*; (K) *L. profundorum*; (L) *L. macrosoma*; (M) *L. attenuatus*; (N) *L. fursti*; (O) *L. mobae*; (P) *L. diadecturus*. After Chen *et al.* (1997) and Eveleigh & Allen (1982), courtesy of *Fundamental and Applied Nematology* and *Canadian Journal of Zoology*.

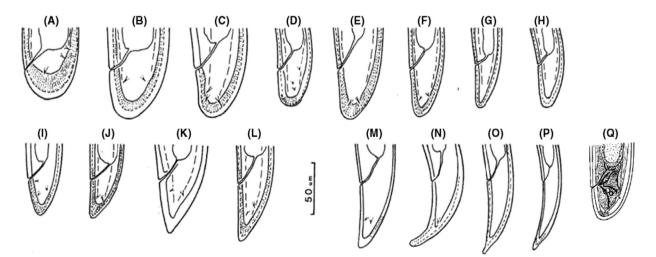


Fig. 3 Female tail shape (code H). (A)–(C), (Q): Code H1. (A) *L. belondiroides*; (B) *L. caespiticola*; (C) *L. profundorum*; (Q) *L. diadecturus*. (D)–(F) Code H2. (D) *L. protae*; (E) *L. elongatus*; (F) *L. closelongatus*. (G), (H) Code H3. (G) *L. mirus*; (H) *L. laevicapitatus*. (I)–(K) Code H4. (I) *L. arenosus*; (J) *L. leptocephalus*; (K) *L. indicus*. (L), (M) Code H5. (L) *L. attenuatus*; (M) *L. globulicauda*. (N)–(P) Code H6. (N) *L. nirulai*; (O) *L. pini*; (P) *L. longicaudatus*. After Chen *et al.* (1997) and Eveleigh & Allen (1982), courtesy of *Fundamental and Applied Nematology* and *Canadian Journal of Zoology*.

	Character codes (refer to Table 2)										
Species	A	В	С	D	Е	F	G	Н	Ι	J	K
martini	3	12	5	4	1	2	23	12	1	_	
jagerae	34	12	5	4	1	2	2	12	1	_	_
diadecturus	4	23	5	2	1	2	12	1	1	1*	_
fursti	4	23	5	4	1	2	23	12	1	1	6
waikouaitii	4	3	5	1	4	3	12	1	1	_	_
macromucronatus	45	3	5	3	1	2	2	1	1	1	56
himalayensis	45	2	5	2	2	2	2	1	1	_	_
jonesi	45	2	5	1	1	2	1	1	12	2	67
juglans	5	23	5	1	1	23	1	1	2	2	23
fangi	56	3	5	23	1	23	2	12	1	1	56
litchii	567	2	5	2	2	23	12	1	2	1	7
cheni	6	3	5	12	2	3	1	1	2	2	2
naganensis	6	3	5	2	2	2(3)	1	1	1	2	7
doonensis	67	2	5	2	2	2	12	1	1	_	_
orongorongensis	67	4	5	1	4	34	2	1	2	1	12
ishigakiensis	7	2	5	1	1	3	23	12	1	2	3
laricis	7	3	5	4	2	23	2	1	2	2	7

**Table 3.** Partial polytomous key for *Longidorus* species with guiding ring around mid-odontostyle when retracted, with cheilostome  $\geq$ 51 µm, group C5. Adapted from Peneva *et al.* (2013), Barsalote *et al.* (2018) and Cai *et al.* (2018).

\*Four juvenile stages are assumed for Longidorus diadecturus. Figures in brackets refer to rare cases.

 Table 4. Morphometric comparisons of Longidorus spp. included in the partial polytomous key to species (Table 3). Adapted from Peneva et al. (2013), Barsalote et al. (2018) and Cai et al. (2018).

Species	<i>L</i> (mm)	<i>c</i> ′	Odontostyle length $\left(\mu m\right)$	Lip region width ( $\mu m$ )	Length of cheilostome $\left(\mu m\right)$	V	
martini	2.9–4.5	1.3	83–96	11–13	51-66	52–56	
jagerae	3.10-3.87	0.8 - 1.02	95-109	11.5-12.5	62-81	51.5-56.3	
diadecturus	3.32-4.02	0.77-0.94	109–121	15-16	50-64	44-48	
fursti	3.93-5.08	0.9-1.14	99.5-108	14.5–16	64–73	51.5-53.6	
waikouaitii	6.44-7.17	0.51-0.74	113–117	16.5–17	56.5–59.5	48.6-53.1	
macromucronatus	4-4.9	0.63-0.8	117-128	14*	58–68	43-47.8	
himalayensis	3.42-3.9	0.7 - 0.8	115-125	15	55–60	47.4-50.1	
jonesi	3.17-3.8	0.6-0.87	107-120	23*	57–66	50.0-52.4	
juglans	3.90-5.25	0.6-0.9	125-140	14–18	69–78	47.1-50.7	
fangi	4.6-5.52	0.75-1.12	124–144	16-18	69.5–87	48-55	
litchii	4.14-5.29	0.61-0.79	138–171	12.5-14	82.5–96.5	49–54	
cheni	4.12-6.64	0.62-0.86	142-173	16–23	70–94	40-49.4	
naganensis	3.83-5.18	0.69-0.89	141–160	16-18	77–89	47.1-54.3	
doonensis	3.47-4.31	0.63**	100-122	12-12.5**	62–76	45.2-50.4	
orongorongensis	6.03-7.99	0.61-0.73	152–166	22–23	63–73	49–54	
ishigakiensis	5.31-6.85	1.0-1.2	158-181	13–14	83–95	45.4-51	
laricis	4.65-5.97	0.64-0.9	160-183	16-18	84–100.5	45.8-51.2	

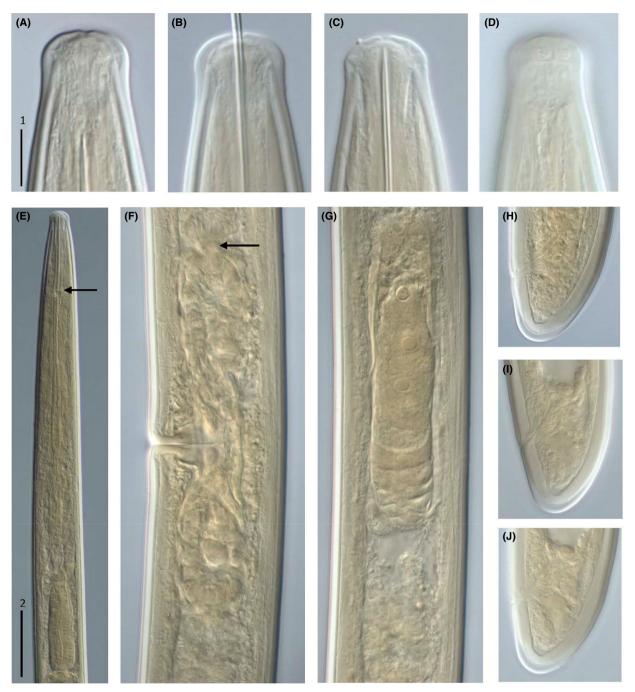
\*Calculated from the original description by Peneva et al. (2013).

\*\*Calculated from the original description.

row. Female reproductive system didelphic–amphidelphic reflexed; uterus without sclerotizations. Tail short, dorsally convex-conical with a finely or broadly rounded terminus. Mostly four juvenile stages present, but some species with three. The latter are mainly restricted to Asia and occur less frequently in Africa and Europe.

#### 4.1.2. Identification to species level

*Longidorus diadecturus* can be morphologically grouped with 17 other species assigned to the genus in having a guiding ring around mid-odontostyle when in retracted position (*L. jonesi*-group) with cheilostome  $\geq$ 51 µm (group C5 according to Chen *et al.*, 1997; Loof & Chen, 1999).



**Fig. 4** Longidorus diadecturus (paratype females, slides T261). (A), (B) Lip region, lateral view. (C) Lip region, dorso-ventral view. (D) Amphidial fovea, lateral view. (E) Anterior region (guiding ring indicated with an arrow). (F) Vagina, uteri and sphincters (indicated with arrows). (G) Reflexed anterior ovary showing ovarial sac at tip and widened part of oviduct at tip of ovary. (H)–(J) Tail. Scale bar 1: 10 μm (A)–(D); scale bar 2: 50 μm (E), 25 μm (F)–(J). Courtesy *EPPO Bulletin*.

The L. jonesi group also includes L. cheni Barsalote, Pham, Lazarova, Peneva & Zheng, 2018; L. doonensis (Singh & Khan, 1996) Ye & Robbins, 2004; L. fursti Heyns, Coomans, Hutsebaut & Swart, 1987; L. himalayensis (Khan, 1986) Xu & Hooper, 1990; L. ishigakiensis Hirata, 2002; L. jagerae Heyns & Swart, 1998; L. jonesi Siddiqi, 1962; L. juglans Xu, Guo, Ye, Wang, Zheng, & Zhao, 2017; L. laricis Hirata, 1995; L. litchi Xu & Cheng, 1992; L. macromucronatus Siddiqi, 1962; L. martini Merny, 1966; L. naganensis Hirata, 1995; L. orongorongensis Yeates, Van Etteger & Hooper, 1992; and L. waikouaitii Yeates, Boag & Brown, 1997. Three additional species assigned to the genus are reported to have a cheilostome length of  $\geq$ 51 µm (group C5 according to Chen *et al.*, 1997; note *L. mobae* Jacobs &

Heyns, 1987 should be placed in C4): *L. heynsi* Andrassy, 1970; *L. macrodorus* Archidona-Yuste, Navas-Cortés, Cantalapiedra-Navarrete, Palomares-Rius & Castillo, 2016; and

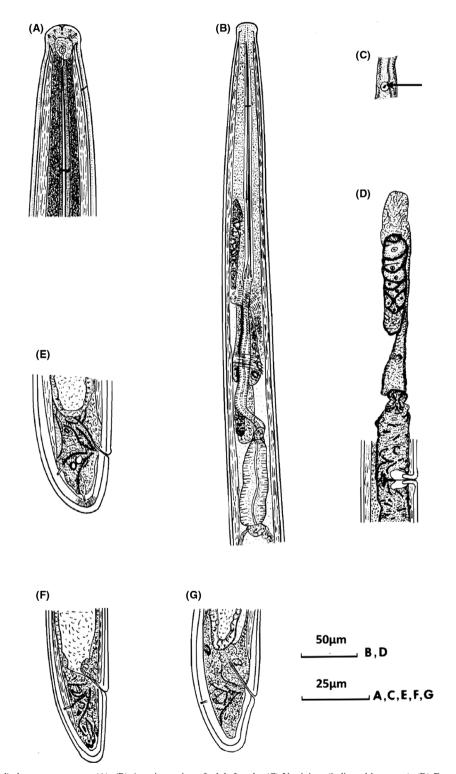


Fig. 5 Longidorus diadecturus paratypes. (A), (B) Anterior region of adult female. (C) Vestigium (indicated by arrow). (D) Female reproductive system, anterior branch. (E) Female tail. (F) Third-stage juvenile tail. (G) Fourth-stage juvenile tail. After Eveleigh & Allen (1982), courtesy of *Canadian Journal of Zoology*.

*L. taniwah* Clark, 1963. However, relative to the odontostyle in its retracted position, the guiding ring in these three species is situated within the anterior third, and not around mid-odontostyle as typical of the *L. jonesi* group.

The following diagnostic keys facilitate the morphological identification of *L. diadecturus*. Characters are described in Table 2 and Figures 1–3, with supplementary characters and morphometric data provided in Tables 3 and 4. Drawings and photomicrographs are included in Figures 4 and 5.

Longidorus diadecturus Eveleigh & Allen, 1982 (Figs 4 and 5).

Additional measurements taken from formalin fixed paratype specimens on microscope slides T261 and from line drawings presented in the original description (note: scale bars in Fig. 5 as presented by Eveleigh & Allen (1982) were incorrectly labelled and have been amended here). A more detailed morphological description can be found in Prior *et al.*, 2020).

Female

Holotype: L = 3.79 mm; a = 92; b = 10; c = 126; c' = 1.0; V = 47.

Paratypes: L = 3.71 (3.32–4.02) mm; a = 84 (74–92); b = 10.2 (8–13); c = 139 (122–177); c' = 0.87 (0.77–0.94); length of cheilostome = 50–64 µm; length of Stomodeum (excl. cardia) = 350–380 µm; pharyngeal glands = 62– 83 µm long, 17–22 µm wide; glandularium = 45–60 µm; DN = 23–25%; VSN 39–52%; V = 46 (44–48); pars distalis vaginae = ca. 19 µm; pars proximalis vaginae = ca.19 µm; length of uterus = 65–75 µm; length of ovary = 115–130 µm; length of genital branch = 220– 250 µm; G<sup>1</sup> = 5.8 (4.2–7.1); G<sub>2</sub> = 5.8 (3.5–7.1).

Habitus ventrally arcuate, main curvature in posterior region when heat relaxed. Lip region anteriorly rounded and expanded from the body contour, expansion from contour not marked by a constriction. Amphidial fovea pouch-like, approximately as wide as it is long, posterior end rounded. Stylet length 168–187  $\mu$ m, odontostyle 109–121  $\mu$ m long, odontophore 55–66  $\mu$ m long. Length of anterior end to guide ring 50–64  $\mu$ m. Tail dorsally convex-conoid, broadly rounded, 27 (25–29)  $\mu$ m long, bearing two pairs of lateral caudal pores.

Male

Not recorded.

J4

(n = 6) L = 2.69 (2.35–3.02) mm; a = 72 (62–81); b = 7.7 (6.5–8.2); c = 89 (74–108); c' = 1.4 (1.0–1.2); total stylet length = 150 (148–153) µm; odontostyle = 97 (95– 99) µm; odontophore = 53 (50–58) µm; replacement odontostyle = 109 (107–112) µm; length of cheilostome = 49 (47–50) µm.

J3

(n = 2) L = 1.99, 2.04 mm; a = 65, 68; b = 6.8, 7.4; c = 65, 66; c' = 1.3, 1.4; total stylet length 133, 139 µm; odontostyle = 83, 86 µm; odontophore = 50, 53 µm; replacement odontostyle = 93, 97  $\mu$ m; length of cheilostome = 44, 45  $\mu$ m.

# 5. Reference material

Holotype and paratype reference material of *L. diadecturus* can be loaned from the Canadian National Collection of Nematodes, Ottawa Research and Development Centre, Agriculture and Agri-Food, Canada (contact Dr Qing Yu Qing.Yu@AGR.GC.CA). Paratype specimens are also available for loan from the USDA Nematode Collection, US Department of Agriculture, Agricultural Research Service, Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD, USA (contact Dr Zafar Handoo zafar.handoo@ars.usda.gov). Reference slides (non-types) are available at Wageningen Nematode Collection, The Netherlands (contact Dr Gerrit Karssen g.karssen@nvwa.nl).

# 6. Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 *Documentation and reporting on a diagnosis.* 

# 7. Performance characteristics

When performance characteristics are available, these are provided with the description of the test. Validation data are also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended to consult this database as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

# 8. Further information

Further information on this organism can be obtained from: T. Prior Fera Science Ltd, York, GB

S. Sirca, Agricultural Institute of Slovenia (KIS), Ljubljana SI M. Groza, B-dul Voluntari 11, 077190, Voluntari, Ilfov RO

# 9. Feedback on this Diagnostic Protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this protocol that you wish to share please contact diagnostics@eppo.int.

# 10. Protocol revision

An annual review process is in place to identify the need for revision of diagnostic protocols. Protocols identified as needing revision are marked as such on the EPPO website.

When errata and corrigenda are in press, this will also be marked on the website.

#### Acknowledgements

This protocol was originally drafted by T. Prior, Fera Science Ltd, York, GB, S. Sirca, Agricultural Institute of Slovenia (KIS), Ljubljana SI and M. Groza, B-dul Voluntari 11, 077190, Voluntari, Ilfov RO. It has been reviewed by the EPPO Panel on Diagnostics in Nematology with additional support from W. Decraemer (BE) and G. Karssen (NL). Photomicrographs of *L. cheni* were provided by E. Barsalote (AU) and S. Lazarova (BG).

# References

- Allen WR, Van Schagen JG & Ebsary BA (1984) Comparative transmission of the peach rosette mosaic virus by Ontario populations of *Longidorus diadecturus* and *Xiphinema americanum* (Nematoda: Longidoridae). *Canadian Journal of Plant Pathology* 6, 29–32.
- Barsalote EM, Pham HT, Lazarova S, Peneva V & Zheng J (2018) Description of *Longidorus cheni* sp. n. (Nematoda, Longidoridae) from China. *ZooKeys* 744, 1.
- Brown DJF, Lamberti F, Taylor CE & Trudgill DL (1988) Nematodevirus plant interactions. *Nematologia Mediterranea* 16, 153–158.
- Cai R, Maria M, Qu N, Castillo P & Zheng JW (2018) Morphological and molecular characterization of *Paralongidorus sali* Siddiqi, Hooper, and Khan, 1963 with a description of the first-stage juvenile and male of *Longidorus jonesi* Siddiqi, 1962 from China. *Journal of Nematology* 53, 419–436.
- Chen Q, Hooper DJ, Loof PAA & Xu J (1997) A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). *Fundamental and Applied Nematology* **20**, 15–28.
- Decraemer W & Geraert E (2013) Ectoparasitic nematodes. Plant Nematology (Ed. 2), 179–216.
- Decraemer W & Hunt DJ (2013) Structure and classification. *Plant Nematology* (Ed. 2), 3–39.
- EFSA PLH Panel (EFSA Panel on Plant Health), Jeger M, Bragard C, Caffier D, Candresse T, Chatzivassiliou E *et al.* (2017) Scientific Opinion on the pest categorisation of Longidorus diadecturus. *EFSA Journal* **15** 5112, 19 pp.
- EPPO (2009) PM 4/35(1): Soil test for virus-vector nematodes in the framework of EPPO Standard PM4 Schemes for the production of

healthy plants for planting of fruit crops, grapevine, *Populus* and *Salix. EPPO Bulletin* **39**, 284–288. https://doi.org/10.1111/j.1365-2338.2009.02314.x.

- EPPO (2013) PM 7/119 (1) Nematode extraction. EPPO Bulletin 43, 471–495.
- EPPO Technical document (2020) No. 1056 (rev6) Pictorial Glossary of Morphological Terms in Nematology.
- Eveleigh ES & Allen WR (1982) Description of *Longidorus diadecturus* n. sp. (Nematoda: Longidoridae), a vector of the peach rosette mosaic virus in peach orchards in southwestern Ontario, Canada. *Canadian Journal of Zoology* **60**, 112–115.
- Hunt DJ (1993) Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics. CAB International.
- Loof P & Chen QW (1999) A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). Supplement 1. *Nematology* 1, 55– 59.
- Neilson R, Ye W, Oliveira CMG, Hübschen J, Robbins RT, Brown DJF et al. (2004) Phylogenetic relationships of Longidoridae species (Nematoda: Dorylamida) from North America inferred from 18S rDNAsequence data. *Helminthologia* 41, 209–215.
- Peña-Santiago R (2006) Dorylaimida Part I: Superfamilies Belondiroidea, Nygolaimoidea and Tylencholaimoidea. Freshwater nematodes, pp. 326–391. CAB International, Cambridge (UK).
- Peneva VK, Lazarova SS, De Luca F & Brown DJ. (2013) Description of Longidorus cholevae sp. n. (Nematoda, Dorylaimida) from a riparian habitat in the Rila Mountains, Bulgaria. *ZooKeys*, 330, 1.
- Prior T, Sirca S, Groza M & Karssen G (2020) A supplementary description of *Longidorus diadecturus* (Nematoda: Longidoridae) based on paratype specimens. *EPPO Bulletin*, **50**, 327–329.
- Robbins RT & Brown DJF (1991) Comments on the taxonomy, occurrence and distribution of Longidoridae (Nematoda) in North America. *Nematologica* 37, 395–419.
- Robbins RT, Brown DJF, Halbrendt JM & Vrain TC (1995) Compendium of *Longidorus* juvenile stages with observations on *L. pisi*, *L. taniwha* and *L. diadecturus* (Nematoda: Longidoridae). *Systematic Parasitology* **32**, 33–52.
- Taylor CE & Brown DJF (1997) Nematode vectors of plant viruses. CAB International, Cambridge (UK).
- Ye W & Robbins RT (2004) Cluster analysis of *Longidorus* species (Nematoda: Longidoridae), a new approach in species identification. *Journal of Nematology* **36**, 207.