Description of *Longidorus sturhani* sp. n. (Nematoda: Longidoridae) and molecular characterisation of several longidorid species from Western Europe

Tatjana V. Rubtsova*, Sergei A. Subbotin*, Derek J.F. Brown** and Maurice Moens***

*Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow 117071, Russia, **Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, U.K., ***Agricultural Research Centre, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium.

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Summary. During a nematological survey of orchards in Belgium and vineyards in Germany several populations of an undescribed *Longidorus* species were found. These populations are described as *L. sturhani* sp. n. The new species is characterised by a medium body length (4.4-6.4 mm), a slightly expanded and anteriorly flattened head region, symmetrically bilobed pocket-shaped amphids, conoid tail $35-49 \mu m$ long, and absence of males. The new *Longidorus* species is most similar to *L. seinhorsti* and *L. artemisiae*. The type population of *L. sturhani* sp. n. did not transmit virus in bait-tests. Analyses of sequences of D2-D3 regions of the large subunit of rDNA of *L. sturhani* sp. n. and its position on phylogenetic trees prepared with several other longidorids from Western Europe and New Zealand are presented.

Key words: Belgium, evolution, Germany, 28S gene, morphometrics, orchards, *Paralongidorus*, rDNA, vineyard.

In the summers of 1998 and 1999, soil samples from orchards and pastures in different parts of Belgium were collected and examined for the presence of longidorid nematodes. During this study an undescribed species of the genus Longidorus was found. Several populations of this species were also identified from soil samples collected at several localities in southern Germany. Morphological and morphometric studies confirmed that these populations represented an undescribed species of the genus Longidorus. A description of this new species, with analysis of its position on phylogenetic trees constructed from sequences obtained from the D2-D3 region of the 28S gene of rDNA of several Longidorus and one Paralongidorus species indigenous in Europe and New Zealand, are presented. This new Longidorus species extends the information available on the occurrence and distribution of longidorid nematode species reported from Belgium (D'Herde & Van den Brande, 1964; De Waele, 1980; De Waele & Coomans, 1983, 1990).

MATERIALS AND METHODS

Nematodes populations. Longidorid nematodes were extracted from soil samples by a modified decanting and sieving method (Flegg, 1967). For morphological studies nematodes were killed by heating, fixed in 4% formaldehyde, and processed and mounted in glycerine on glass slides by a modification of Seinhorst's (1959) method. Further specimens were fixed in 70% ethanol and used in dried condition for molecular study. Only the type population of the new species was used for molecular studies. The populations of longidorid nematode species used in the study are listed in Table 1. Specimens were measured using a Leica Orthoplan light microscope, and photographed under a Zeiss light microscope with Nomarski differential interference contrast. The mean and the standard error of the mean were calculated for each measured character.

Molecular analysis. DNA extraction and PCR

Species	Location	Source of material		
Longidorus sturhani sp. n.	Visé, Liège, Belgium	T. Rubtsova		
L. sturhani sp. n.	Auggen, Germany	D. Sturhan		
L. sturhani sp. n.	Bickensohl, Germany	D. Sturhan		
L. sturhani sp. n.	Heidelberg, Germany	D. Sturhan		
L. sturhani sp. n.	Jechtingen, Germany	D. Sturhan		
L. sturhani sp. n.	Nordweil, Germany	D. Sturhan		
L. sturhani sp. n.	Oberbergen, Germany	D. Sturhan		
L. sturhani sp. n.	Schriesheim, Germany	D. Sturhan		
L. macrosoma	Visé, Liège, Belgium	T. Rubtsova		
L. caespiticola	Vliermaal (V), Belgium	T. Rubtsova		
L. caespiticola	Brussegem (B), Belgium	T. Rubtsova		
L. caespiticola	Gandesbergen (G), Germany	D. Sturhan		
L. profundorum	Gandesbergen, Germany	D. Sturhan		
L. carpathicus	Kirchbichel, Germany	D. Sturhan		
L. intermedius	Planegg, Germany	D. Sturhan		
L. elongatus	Dundee (Du), Scotland	D.J.F. Brown		
L. elongatus	Grote Nete (GN), Belgium	T. Rubtsova		
L. elongatus	Merelbeke (Me), Belgium	T. Rubtsova		
L. elongatus	Lincoln, New Zealand (NZ)	D. Sturhan		
Paralongidorus maximus	Harrier Sand, Germany	D. Sturhan		

Table 1. List of populations of Longidorus and Paralongidorus species used in the present study.

reaction were performed with a single specimen from each population following the protocols described by Subbotin et al. (2000). The D2-D3 expansion region of the 28S gene was amplified using the primers D2A (5'- ACAAGTACCGTGAG-GGAAAGTTG-3') and D3B (5'-TCGGAAGGA-ACCAGCTACTA-3'). The DNA-amplification was carried out in a GeneE DNA thermal cycler (New Brunswick Scientific, Wezembeek-Oppem, Belgium), and consisted of 4 minutes at 94 °C; 35 cycles of 1 min at 94 °C, 1.5 min at 55 °C, 2 min at 72 °C, and 10 min at 72 °C. DNA fragments were sequenced in both directions using two vectors, D2A, D3B or LonD3R2 (5'-CCTCTGGCT-TCGCTCTGCTC-3') primers, with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, UK) according to the manufacturer's instructions. Sequences were run on a 377 DNA sequencer (PE Applied Biosystems, UK). PCR products obtained for Belgian and Scottish longidorid populations were cloned and one clone for each sample was sequenced; PCR products of populations from Germany and New Zealand were sequenced directly. For cloning, amplified products were excised from 1% TBE buffered agarose gels using a OIAquick Gel Extraction Kit (Qiagen), cloned into a pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega Corporation, USA).

DNA sequences were edited with Chromas 1.45 and aligned using ClustalX 1.64 with default options (Thompson *et al.*, 1997). All longidorid sequences reported here are deposited in the Gene-Bank. The corresponding sequences of Xiphinema diversicaudatum (Y. He, unpublished data) were used as an outgroup taxon. Equally weighted maximum parsimony and maximum likelihood analyses were performed using PAUP (4.0 beta version) (Swofford, 1998). A heuristic search procedure was used with the following settings: tree bisection - reconnection (TBR) branch swapping, collapse ves, multrees ves, steepest no. The accelerated transformation was used to search for the shortest topologies. Gaps were treated as missing data. Bootstrap analysis with 100 replicates was conducted to assess the degree of support for each branch on the tree (Felsenstein, 1985). Trees were displayed using TreeView 1.6.1 (Page, 1996).

Nematode-virus association study. Specimens from the Belgian type population of L. sturhani sp. n. were examined for their association with plant viruses. One week-old cucumber bait seedlings (Cucumis sativus) were transferred into pots filled with 500 g field soil containing the nematodes, and grown for three weeks. Leaves with putative virus symptoms were comminuted in a mortar and pestle and the resultant suspension rubbed by finger onto leaves of 6 week-old Chenopodium amaranticolor virus indicator plants dusted with an abrasive (600 Carborundum). Also, the roots of the putative virus-infected cucumber bait-plants were harvested, washed free from adhering soil particles, and checked for the presence of virus as described for the leaves of the plants. Two bait-plants were

used from each of the three soil samples. The entire virus testing procedure was done in an insect proof chamber at 20 °C with 16 hours light for 14 days. Leaves of *C. amaranticolor* plants showing putative virus symptoms were tested using the ELISA technique for the presence of raspberry ringspot virus, genus *Nepovirus* (Duarte & Brown, 1997).

DESCRIPTION Longidorus sturhani sp. n. (Figs. 1-3)

Morphometrics of the holotype, paratype females and fourth-stage juveniles are given in Table 1.

Female. Body of heat-relaxed females assuming a J or C habitus. Cuticle along the body about 2.5 µm thick, 3-3.5 µm at postlabial region and considerably thickening towards posterior end. Lip region flattened anteriorly, set-off from the rest of the body by a slight depression. Amphids more or less pocket-shaped, symmetrically bilobed, extending more than half the distance between stomatal aperture and guide ring. Numerous lateral body pores along body, the first pore often anterior to guide ring; 4-7 ventral pores in anterior region between guide ring and oesophageal bulb, 2 dorsal pores at level of odontostyle. Nerve ring wide, single, situated at 175-190 µm from anterior end. Width of body at guide ring level 19-23 µm. Odontostyle long and slender, 1.1-1.4 µm wide at base. Guide ring two lip region diameters from anterior body end, 4.5 µm wide. Oesophageal bulb about five times longer than wide. Location of oesophageal gland nuclei as common for the genus, *i.e.* dorsal nucleus at 30-35% and subventral nuclei at 50-56% of the basal bulb (Fig. 3C). Nucleoli rounded, nucleolus of dorsal gland approximately 2 µm in diameter; nucleoli of subventral glands almost 2.5 µm. Oesophagointestinal valve (cardia) bluntly rounded. Vulva a transverse slit. Vagina extending to half of midbody width or slightly less. Reproductive system didelphic, amphidelphic; ovaries about 146 µm long; uteri 235-372 µm long, thick-walled. One female with eggs in uteri. Pre-rectum 240-445 µm long, rectum shorter than body width at anus (13-22 vs 31-40 μ m). Tail bluntly conoid, dorsally convex, ventrally straight or slightly concave, terminus rounded. At tail terminus striated cuticle layer about three times thicker than outer layers. Two lateral pores on each side of tail, occasionally a third pore close to anus level.

Male. Not found.

Juveniles. Five juveniles were found in samples from Belgium and were identified as being the preadult stage. Body J-shaped, smaller than females; lip shape similar to females; amphids not distinctly bilobed; reproductive system not developed; tail more conical than in female. Replacement odontostyle 89-94 μ m.

Other populations. The morphology of females from seven populations from Germany resemble in morphology with the type population, with their morphometric values extending the range of variation for the species (Table 1). The distance of the guide ring from the anterior end is slightly shorter in specimens from Germany than in the type females. Also the body of the German specimens is often G-shaped to an almost closed circle. The shape of the anterior end and other morphological characteristics are in accordance with the description of the type population. The symmetrically bilobed amphidial pouches mostly extend to about three-quarters of the distance between the anterior end and the guide ring. In the oesophagus region 18 to 22 lateral pores are present, with the first pore at the level of the guide ring, or immediately anterior or posterior, and 3 to 4 in the odontostyle region. Dorsal pores, 1 to 5, all posterior to the guide ring and all within the region of the odontostyle, only the posterior pore is occasionally slightly posterior to this region. Ventral pores, 4 to 6 in the anterior part of the oesophagus, all posterior to the region of the guide ring, and 2 to 3 in the region of the odontostyle. Tail mostly with two lateral pores; occasionally a third pore immediately posterior to the anus. Nucleolus of dorsal oesophageal gland nucleus always slightly smaller than the nucleoli of the subventral gland nuclei. Mucro not observed in oesophageal tissue and sperm not present in the uteri of the females. One female (population Brackenheim) with distinct supplements: one precloacal pair + six singular supplements anterior; ovaries in this female well developed.

Juveniles. A total of 15 juveniles were obtained from the German populations. Several Longidorus and Xiphinema species have only three juvenile development stages (Halbrendt, 1993; Robbins et al., 1995; Robbins et al., 1996), but measurements obtained of the functional and replacement odontostyles revealed the presence of four juvenile stages in L. sturhani sp. n. The odontostyle/replacement odontostyle lengths in the specimens



Fig. 1. Females of *Longidorus sturhani* sp. n. A: Anterior end; B: Head; C: Habitus of females; D: Vulva region; E-G: Tail.



Fig. 2. Photomicrographs of females of *Longidorus sturhani* sp. n. A: Head; B, C: Head with amphids; D: Vulva; E-G: Tail of females; G-J: Tail of juveniles. G: J1; H: J2; I: J3; J: J4. Scale bar $-14 \mu m$.

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Location	Belgium			Germany						
		Visé		Auggen	Bickensohl	Heidelberg	Jechtingen	Nordweil	Oberbergen	Schriesheim
n	Q Holotype	10 QQ paratypes	5 paratype pre- adult juveniles	8 QQ	7 ♀♀	6 QQ	12 ♀♀	2 QQ	5 QQ	4 QQ
L	5.5	5.4±0.2 (4.4-6.2)	4.2±0.7 (3.9-4.3)	4.9 ± 0.1 (4.4-5.4)	5.3±0.2 (4.7-6.1)	5.1±0.2 (4.7-5.7)	5.7±0.1 (5.3-6.4)	5.4, 5.6	5.2±0.1 (4.9-5.6)	5.1±0.2 (4.8-5.5)
а	111	114 ± 3.5 (100-130)	103 ± 1.6 (99-108)	112 ± 1.9 (103-117)	114 ± 1.9 (107-121)	121±2.6 (110-128)	124±2.6 (106-138)	116, 117	115±1.8 (110-121)	117 ± 1.6 (112-120)
b	18 `	13 ± 0.4	12 ± 1.8 (9.8-16)	13 ± 0.3 (12-14)	14 ± 0.3 (13-15)	12 ± 0.7 (11-15)	14 ± 0.4 (12-17)	15	13 ± 0.7 (12-16)	15 ± 0.6 (14-16)
с	152	136 ± 4.2 (108-153)	94 ± 4.8 (83-108)	133 ± 4.0 (119-155)	136 ± 3.7 (124-150)	126 ± 4.02 (116-141)	131 ± 3.1 (115-146)	115, 145	119 ± 5.3 (100-128)	139 ± 5.3 (133-150)
c'	1.1	1.1 ± 0.02	1.4 ± 0.04	1.2 ± 0.02 (1.1-1.3)	1.2 ± 0.02 (1.15-1.31)	1.2 ± 0.03 (1.15-1.31)	1.2 ± 0.03 (1.11-1.39)	1.11, 1.27	1.3 ± 0.07 (1.1-1.5)	1.1 ± 0.04 (1.05-1.18)
D	2.0	(1.0-1.2) 2.0±0.04 (1.7-2.2)	2.0 ± 0.08	1.7 ± 0.03	1.6 ± 0.03 (1.5-1.7)	1.9 ± 2.1 (1.7-2.1)	1.8 ± 0.03 (1.7-1.9)	1.7, 1.9	1.7 ± 0.05 (1.6-1.8)	1.7 ± 0.03 (1.6-1.8)
ď	1.4	(1.7-2.2) 1.5 ± 0.03 (1.4-1.7)	(1.3-2.3) 1.5 ± 0.06 (1.3-1.7)	(1.0-1.0) 1.3 ± 0.04 (1.1-1.4)	(1.3 ± 0.03) (1.25-1.5)	1.3 ± 0.03 (1.2-1.4)	1.2 ± 0.03 (1.15-1.4)	1.3, 1.4	1.3 ± 0.03 (1.25-1.4)	1.3 ± 0.05 (1.25-1.4)
V%	52	50 ± 0.6 (46-52)	-	49 ± 0.5 (47-51)	50±0.3 (49-51)	52±0.5 (51-54)	49±0.3 (47-51)	49	49±1.2 (45-51)	50±0.6 (48-51)
Odontostyle	89	88 ± 1.2 (82-94)	80 ± 2.4 (77-82)	86±1.0 (82-89)	89±0.7 (85-92)	86±2.5 (78-93)	92±0.6 (88-96)	93	86±1.8 (82-89)	87±2.2 (82-92)
Odontophore	72	63 ± 2.7 (57-78)	45 ± 3.6 (40-48)	62 ± 1.4 (57-66)	64 ± 1.7 (60-71)	73 ± 2.2 (70-78)	63±0.8 (60-68)	78	67±3.4 (62-74)	65±1.0 (62-66)
Ant. end to guide	30	30 ± 0.4	26 ± 0.4 (25-27)	25 ± 0.3 (24-26)	25 ± 0.4 (24-27)	29 ± 0.6 (27-31)	27 ± 0.3 (26-30)	29, 30	27±0.5 (25-28)	26±0.5 (25-28)
Oesophagus length	301	404 ± 4.4 (337-425)	360 ± 4.5 (272-415)	364 ± 4.5 (352-389)	382 ± 8.2 (362-418)	401±13.0 (365-427)	397 ± 11.0 (358-490)	372	394±13 (349-427)	349±5.7 (338-365)
Oes. bulbus length	94	94±1.5 (86-101)	80±1.4 (77-82)	87±1.4 (82-93)	83±1.0 (80-85)	94±2.5 (89-101)	85±1.8 (76-91)	93	93±2.5 (85-97)	85±2.7 (81-93)
Oes. bulbus width	17	19 ± 0.4 (16-19)	16 ± 1.6 (14-19)	17 ± 0.9 (14-19)	17 ± 0.8 (15-19)	16 ± 1.1 (14-19)	18 ± 0.6 (15-19)	17.5	16 ± 0.8 (15-19)	16 ± 0.6 (15-17)
Tail length	36	40 ± 1.0 (37-44)	45 ± 1.9 (39-50)	37 ± 0.8 (35-41)	39 ± 0.7 (37-43)	41 ± 0.9 (38-45)	44 ± 0.8 (40-48)	39, 47	44±1.9 (39-49)	38±0.5 (37-39)
Hyaline part of tail	10	12 ± 0.3 (10-13)	7.8 ± 0.6 (5.8-9.7)	12 ± 0.4 (12-14)	14 ± 0.6 (12-16)	14 ± 0.5 (12-16)	13 ± 0.5 (12-16)	12, 16	14 ± 0.6 (12-16)	13 ± 0.7 (12-14)
Width at level:		1510.2	1210.50	15+0.2	15+0.2	15+0.2	16+0.2	15 17	15 5+0 2	15 4+0 1
lips	15	(14-16)	(12-14)	(14-16)	(14-16)	(14-16)	(14-17)	-	(15-16)	(15-16)
guide ring	22	22±0.3 (19-23)	19±0.61 (17-21)	19 ± 0.6 (16-21)	20 ± 0.3 (19-22)	(19-22)	(18-21)	21, 23	(19-22)	(19-22)
mid-body	49	47±0.9 (44-50)	40±0.7 (38-42)	44±0.8 (41-47)	47±0.84 (44-50)	42±0.8 (39-45)	46±0.7 (43-51)	.48, 46	45-0.7 (43-47)	44±1.0 (42-47)
anus	36	37 ± 0.2 (36-37)	30±0.5 (29-32)	32±0.5 (31-35)	33±0.8 (31-37)	32±0.6 (31-35)	35±0.6 (33-40)	35, 37	34±1.2 (31-37)	33±0.6 (32-35)

Table 2. Morphometrics of Longidorus sturhani sp. n. from populations from Belgium and Germany
(all measurements in µm, except for L in mm).



Fig. 3. Photomicrographs of females of *Longidorus sturhani* sp. n. A: Head; B: Odontophore region; C: Oesophageal bulbus. Scale bar $-20 \ \mu m$.

were: J1 (n=1): $51/57 \mu m$; J2 (n=4): $56-59/65-69 \mu m$; J3 (n=4): $66-71/77-79 \mu m$; J4 (n=6) 75-81/88-95 μm . The tail is rather slender, conoid in the J1, becoming progressively more blunt in each subsequent development stage, with a resultant decrease in ratio c' from 2.7 to 1.4.

Morphological diagnosis and relationships. Longidorus sturhani sp. n. is characterised by its medium body length (4.4 to 6.4 mm), anteriorly flattened and slightly expanded head region, odontostyle length of 78 to 96 µm, symmetrically bilobed amphids extending from half to three quarters the distance between the anterior end and the guide ring, *i.e.*, about two lip widths, narrow body (39 to 51 µm diameter at mid-body), conoid tail 35 to 50 μ m in length and absence of males. The code for identifying the new species when using the polytomous identification key of Chen et al. (1997) including Supplement 1 (Loof & Chen, 1999) is: A3-B2(3)-C2(3)-D3-E2-F23-G23-H24-I1.

The new species is most similar to *L. seinhorsti* Peneva, Loof & Brown, 1998, from which it differs by having a shorter odontostyle (78-96 vs 113-128 μ m), narrower lip region width (14-17 vs 21-

22 µm), and males absent vs males present; from L. attenuatus Hooper, 1961 by a thicker and shorter body (4.4-6.4 vs 5.2-7.3 mm), smaller index a (100-138 vs 120-210), less distinctly expanded lip region, and longer odontostyle (78-96 vs 73-84 µm); from L. elongatus (de Man) Micoletzky, 1922, it differs by having pocketshaped amphids with a distinctly bilobed base vs large, pouch-like amphids with a less distinctly bilobed base, and larger index c (108-155 vs 75-125); from L. artemisiae Rubtsova, Chizhov & Subbotin, 1999, by having pocket-shaped amphids with a distinctly bilobed base vs large, not bilobed amphids, and males absent vs males present (Rubtsova et al., 1999); from L. julandicola Liskova, Robbins & Brown, 1997, by having a narrower body (lips, 14-17 vs 19.8-21 µm; midbody, 39-51 vs 51-64 µm; anus, 31-40 vs 41-49 μ m), more anterior vulva (45-54 vs 52-57%), and more anterior guide ring (24-31 vs 31-37 µm) (Lišková et al., 1997).

Type locality and host. Type specimens collected in July 1999 from the rhizosphere of an apple tree growing in an orchard with stony loam soil at Visé (Rue porte de Lorette 92), Liège province,



Fig. 4. Phylogenetic relationships within Longidoridae. A: Maximum likelihood tree (model: GTR+I+G, lnL = -3232.967); B: Strict consensus of four maximum parsimonious tree (tree length = 454). Bootstrap numbers are given on appropriate clades.

Belgium. Map Ref: 50°44'-N, 5°42'-E.

Other localities. Specimens collected from the following nine localities in south-west Germany: Heidelberg and Schriesheim near Heidelberg; Nordweil north of Freiburg; Bickensohl, Jechtingen, Oberbergen, Blankenhornsberg and Lilienthal, all the Kaiserstuhl area; Auggen south of Müllheim. All samples originated from loess soil vineyards with grasses and weed species as undergrowth. The samples were collected from these locations between 1988 and 2001, with most samples collected by G. Bleyer, **Staatliches** Weinbauinstitut. Freiburg, and others by D. Sturhan who processed and identified all populations.

Type material. Holotype, paratype females, and juveniles deposited in the Nematode Collection of the Institute of Parasitology, Moscow, Russia; paratype females deposited in the German Nematode Collection, Biologische Bundesanstalt, Münster, Germany and the Nematode collection of Gent University, Gent, Belgium.

Etymology. Longidorus sturhani sp. n. is named after Dr. Dieter Sturhan in recognition of the significant contribution he has made to the science of Nematology, and particularly to the taxonomy of the Longidoridae.

Molecular characterisation of *L. sturhani* sp. n. and its relationships to other longidorid species. The phylogenetic trees obtained from maximum parsimony and maximum likelihood analyses of D2-D3 sequences, obtained from specimens from thirteen populations of longidorids, representing six species, had identical topology and are shown in Fig. 4. Both trees distinguished two distinct groups. One group contained *Longidorus macrosoma* and *L. caespiticola*, the second group, in which *L. sturhani* sp. n. formed a clade with *L. carpathicus, L. intermedius,* and *L. elongatus,* included *Paralongidorus maximus.* The analysis revealed that *P. maximus* clustered with this group with a high statistical significance. Coomans (1996) concluded that *Paralongidorus*, Longidoroides and Longidorus formed a complex, with the primitive forms in the group, viz. Paralongidorus including P. maximus, having offset lip regions and stirrup shaped amphids with wide slit-like openings. Longidorus species are quite different in having non-stirrup shaped amphids with pore-like openings. Consequently, it appears surprising that analyses of D2-D3 region sequences revealed close relationships of the Paralongidorus species used in the present study with one of the Longidorus groups.

Only a few nucleotide differences were observed within the sequences obtained from the *L. elongatus* populations, and there were no significant differences in sequences from a population having males present (Me) with those obtained from three populations in which males were absent. Two to nine nucleotide differences in the sequences of the *L. elongatus* populations, and 4 to 15 nucleotides between three populations of *L. caespiticola* are probably the result of natural variation. Also, variation within the D2-D3 sequences may be a consequence that only one D2-D3 clone was sequenced for each population and do not represent an average variant.

The molecular analysis reported here, using sequence data obtained from the D2-D3 rDNA region, apparently does not provide support for validity of the genus *Paralongidorus*. The two molecular grouping of longidorids obtained in the study appear to have some congruence with the general structure of amphids: elongate-funnel shaped, and not bilobed, amphids and more or less pocket-shaped amphids.

The D2-D3 region sequence of the large subunit rDNA gene provides a useful diagnostic tool for *Acrobeloides* (De Ley *et al.*, 1999) and *Pratylenchus* species (Duncan *et al.*, 1999). The present study suggests that this region may prove useful for characterisation and examining phylogenetic relationships within longidorids.

Virus-transmission test. ELISA tests did not reveal the presence of raspberry ringspot virus in any of the bait-plants used in soil tests to detect the presence of the virus in soil samples containing L. *sturhani* sp. n.

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REFERENCES

- Chen, Q., Hooper, D.J., Loof, P.A.A. & 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.
- Coomans, A. 1996. Phylogeny of the Longidoridae. Russian Journal of Nematology 4: 51-60.
- D'Herde, J. & Van den Brande, J. 1964. Distribution of *Xiphinema* and *Longidorus* spp. in strawberry fields in Belgium and a method for their quantitative extraction. *Nematologica* 10: 454-458.
- De Ley, P., Félix, M.-A., Frisse, L.M., Nadler, S.A., Sternberg, P.W. & Thomas, W.K. 1999. Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1: 591-612.
- De Waele, D. 1980. Objectives, methods and preliminary results concerning a survey of virus-vector nematodes in Belgium. *Mededeling van de Faculteit Landbouwetenschappen Gent* 45: 807-813.
- De Waele, D. & Coomans, A. 1983. Distribution of Longidoridae and Trichodoridae. In: Atlas of Plant Parasitic Nematodes of Belgium (T.J.W. Alphey. Ed.).
 42 pp. Dundee, Scotland, Scottish Crop Research Institute..
- De Waele, D. & Coomans, A. 1990. Occurrence and ecology of longidorid nematodes in Belgium. *Revue de Nématology* 13: 197-202.
- Duarte, I.M.N.da R.D. & Brown, D.J.F. 1997. Sampling, detection and identification of nematode transmitted viruses. In: An Introduction to virus vector nematodes and their associated viruses (M.S.N de A. Santos, I.M. de O. Abrantes, D.J.F. Brown, R.M. Lemos, Eds.). pp. 313-353. Coimbra, Portugal, Instituto do Ambiente e Vida, Depart. Zoolg., Universidade de Coimbra.
- Duncan, L.W., Inserra, R.N., Thomas, W.K., Dunn, D., Mustika, I., Frisse, L.M., Mendes, M.L., Morris, K. & Kaplan, D.T. 1999. Molecular and morphological analyses of isolates of *Pratylenchus coffeae* and closely related species. *Nematropica* 29: 61-80.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- Flegg, J.J. 1967. Extraction of Xiphinema and Longidorus species from soil by a modification of Cobb's decanting sieving technique. Annals of Applied Biology 60: 429-437.
- Halbrendt, J.M. 1993. Virus-vector Longidoridae and their associated viruses in the Americas. *Russian Journal of Nematology* 1: 55-68

- Hooper, D.J. 1961. A redescription of Longidorus elongatus (de Man, 1876) Thorne & Swanger, 1936, (Nematoda, Dorylaimidae) and descriptions of five species of Longidorus from Great Britain. Nematologica 6: 237-257.
- Lišková, M., Robbins, R.T. & Brown, D.J.F. 1997. Descriptions of three new *Longidorus* species from Slovakia (Nemata: Longidoridae). *Journal of Nematology* 29: 336-348.
- Loof, P.A.A. & Chen, Q. 1999. A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Dorylaimoidea). Supplement 1. *Nematology* 1: 55-59.
- Page, R.D.M. 1996. TREEVIEW: An application to view phylogenetic trees on personal computer. *CABIOS* 12: 357-358.
- Robbins, R.T. Brown, D.J.F., Halbrendt, J.M. & Vrain, T.C. 1995. Compendium of Longidorus juvenile stages with observations on L. pisi, L. taniwha, and L. diadecturus (Nematoda: Longidoridae). Systematic Parasitology 32: 33-52.
- Robbins, R.T. Brown, D.J.F., Halbrendt, J.M. & Vrain, T.C. 1996. Compendium of juvenile stages of

Xiphinema species (Nematoda: Longidoridae). Russian Journal of Nematology 4: 163-171.

- Rubtsova, T.V., Chizhov, V.N. & Subbotin, S.A. 1999. Longidorus artemisiae sp. n. (Nematoda: Longidoridae) from roots of Artemisia sp., Rostov region, Russia. Russian Journal of Nematology 7: 33-38.
- Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica* 4: 67-69.
- Subbotin, S.A, Waeyenberge, L. & Moens, M. 2000. Identification of cyst forming nematodes of the genus *Heterodera* (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLPs. *Nematology* 2: 153-164.
- Swofford, D.L. 1998. PAUP*. Phylogenetic analysis using parsimony. Version 4. Sunderland, Massachusetts, Sinauer Associates. 128 pp.
- Thompson, J.D., Gibson T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

Rubtsova T.V., Subbotin S.A., Brown D.J.F., Moens M. Описание Longidorus sturhani sp. n. (Nematoda: Longidoridae) и молекулярная характеризация нескольких видов лонгидорид из Западной Европы.

Резюме. В результате нематологического обследования садов в Бельгии и виноградников в Германии было обнаружено несколько популяций неописанного вида из рода Longidorus. Эти популяции описываются как новый вид Longidorus sturhani sp. п. Новый вид характеризуется средними размерами длины тела (4,4-6,2 мм), слегка расширенной и плоской головной областью, симметричными билобчатыми и кармановидными амфидами, коноидным хвостом длиной 35-49 мкм и отсутствием самцов. Новый вид наиболее близок к L. seinhorsti и L. artemisiae. Типовая популяция не переносила вирусы. Проанализирован секвенс D2-D3 участка 28S гена рибосомальной ДНК для Longidorus sturhani sp. п. и представлено филогенетическое древо с положением этого вида относительно других лонгидорид из Западной Европы и Новой Зеландии.