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MOLECULAR CHARACTERIZATION OF SIX *LONGIDORUS* SPECIES FROM SWITZERLAND WITH THE DESCRIPTION OF *LONGIDORUS HELVETICUS* SP.N. (NEMATODA, DORYLAIMIDA)

by

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Summary. Six species of *Longidorus* were found during a survey of longidorid nematodes carried out during 1996-2000 in fruit orchards in Switzerland. They are *L. elongatus* (De Man) Thorne *et* Swanger; *L. macrosoma* Hooper; *L. profundorum* Hooper; *L. raskii* Lamberti *et* Agostinelli; *L. arthensis* Brown, Grunder, Hooper, Klingler *et* Kunz and *L. helveticus* sp.n. *L. helveticus* is a bisexual species characterized by body length of ca. 8 mm, odontostyle of ca. 135 μm, hemi-elliptical lip region, continuous with the rest of the body, amphidial pouches not lobed, equally developed female genital branches and bluntly rounded tail. The first juvenile stage has a digitate tail. *L. helveticus* resembles *L. macrosoma, L. poessneckensis* Altherr, *L. picenus* Roca, Lamberti *et* Agostinelli and *L. nevesi* Macara. The six species are characterized by superoxide dismutase and esterase isozymes and by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of amplified ITS.

Several species of *Longidorus* Micoletzky occur in Switzerland (Klingler *et al.*, 1985b) and many of them are large and morphometrically similar (Klingler *et al.*, 1983; Lamberti and Agostinelli, 1993; Brown *et al.*, 1994) causing identification problems for non-specialists.

Isoelectrofocusing superoxide dismutase (SOD) profiles seem an appropriate and efficient method in separating species of *Xiphinema* (Lamberti *et al.*, 1999b), *Heterodera* (Molinari *et al.*, 1996) and *Meloidogyne* (Molinari, 2001). Recombinant DNA techniques are frequently being used to differentiate nematode species within a genus (Guirao *et al.* 1995; Braasch *et al.*, 1995; Irdani *et al.*, 1996; Schmitz *et al.*, 1998; Duncan *et al.*, 1999; Lamberti *et al.*, 1999a; 1999b).

Analysis of SOD and esterase isozymes and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were used to separate populations of *Longidorus* species collected in Switzerland during 1996-2000; some of the populations represent, to the best of our knowledge, a species new to science which is described here as *Longidorus helveticus* sp.n.

Materials and methods

Soil samples were collected from the rhizosphere of orchard trees at different localities. Nematodes were extracted by sieving and centrifugal flotation (Coolen and D'Herde, 1977 modified by Kunz). For taxonomic studies, specimens were killed in hot 5% formalin, mounted in anhydrous glycerol and measured with the aid of a camera lucida and with a microscope combined with a computer aided, pixel-based system from Zeiss AG (Brown *et al.*, 1994).

For isozyme activity analysis, hand-picked nematodes were placed in a small volume of distilled water and rinsed to cleanse them from debris. Aliquots of ten active specimens were then transferred to a plastic Eppendorf-shaped miniature homogenizer (Biomedix, U.K.). The distilled water was replaced with 10 µl of the extraction buffer, consisting of 20% sucrose 0.1 M Trizma-Base, 0.08 M boric acid, pH 8.4, 2.5 mMEDTA, 5 µg of bromophenol blue with the inhibitors of proteases PMSF (1 mM), pepstatin (1 µM) and leupeptin (1 µM). Samples kept in an ice bath were homogenized in the extraction tube, using a small plastic pestle, connected to a rotor and then centrifuged at 10,000 rpm for five mintues. The clarified supernatants were used immediately for electrophoresis.

SOD isozymes were separated by isoelectrofocusing (IEF) and esterase isozymes by native polyacrylamide gel electrophoresis (n-PAGE). Electrophoresis was carried out using Phast System equipment (Pharmacia Biotech, USA), which, associated with small size gels (4x4 cm) and allowing pre-programming of the chosen separation method, exhibited a high level of accuracy and reproducibility of band position. Procedures are described in Molinari *et al.*, (1996).

SOD activity was detected by its ability to inhibit the reduction of nitrobluetetrazolium (NBT) by riboflavin (Molinari *et al.* 1997). Esterase bands were visualized by staining gels for 20 min in the Phast System Developer set at 37 °C, using a solution containing 0.02% (w/v) of both α- and β-naphthyl acetate and 0.05% fast blue RR salt in 0.1 M potassium phosphate buffer, pH 6.0 (Soltis *et al.*, 1983). Stained esterase gels were rinsed in a preserving solution consisting of 13% glycerol and 10% acetic acid; all the gels were dried overnight. Mini-gels were scanned by means of a ScanJet II cx (Hewlett Packard). SOD gels were arranged by computer as negative images to strengthen band detection. Finally, gels were printed with a high-quality Laser Printer Jet 4, Hewlett Packard.

For PCR-RFLP, genomic DNA was isolated from single nematodes, as described by Molinari *et al.*, (1997). For these samples, a modified lysis buffer was used containing 50 mM KCL, 10 mM Tris-HC1 pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% NP40, 0.45% Tween 20, 10 mg/ml proteinase K. Sequences corresponding to ITS, 28 S and 18 S subunits of the rDNA cistron were amplified and analyzed for length polymorphism among nematode populations. PCR amplification conditions were: denaturation at 94 °C for 1 min, annealing at 55 °C for 50 sec and extension at 72 °C for 2 min, repeated for 35 cycles.

Following PCR, 1/10 of each amplification product was digested with the restriction enzymes: *Eco* RI, *Dde* I, *Rsa* I, *Alu* I, *Ava* II and *Hinf* I (Lamberti *et al.*, 1999a).

Results

The morphometric approach to the study of the populations revealed the presence of six species. They are *L. elongatus* (de Man, 1876) Thorne *et* Swanger, 1936; *L. macrosoma* Hooper, 1961; *L. profundorum* Hooper, 1966; *L. raskii* Lamberti *et* Agostinelli, 1993; *L. arthensis*, Brown, Grunder, Hooper, Klingler *et* Kunz, 1994 and an undescribed species which is here named *L. helveticus*.

Descriptions

LONGIDORUS ELONGATUS (De Man, 1876) Thorne *et* Swanger, 1936
(Tables I and VII; Figs 1, 2, 20-22)

A single population of *L. elongatus* was found mixed with *L. macrosoma, L. profundorum* and *L. arthensis* in the rhizosphere of a pear tree (*Pyrus communis* L.) at Horgen.

TABLE I - *Morphometrics of a population of* Longidorus elongatus *from Switzerland*

Locality	Horgen
Host	Pear
n	10 ♀♀
L (mm)	6 ±0.44 5.1 - 6.5
	107.5 ±7.01 92.2 - 116.3
b	13.2 ±1.50 10.5 - 15.3
	117.6 ±11.41 98.4 - 137.6
	1.2 ±0.06 1.1 - 1.3
V%	48.6 ±1.17 47 - 51
Odontostyle µm	87.3 ±3.52 80 - 94
Odontophore µm	65.1 ±3.69 60.6 - 71.8
Oral aperture to guide ring µm	32.1 ±1.29 29.4 - 33.5
Tail µm	51.6 ±4.33 46.5 - 61.8
J (hyaline portion of tail) µm	10.1 ±1.10 9 - 13
Body diam. at lip region µm	14 ±0.52 13.5 - 14.7
Body diam. at guide ring µm	23.3 ±0.83 21.8 - 24.7
Body diam. at base of oesophagus µm	46.1 ±2.32 41.8 - 48.8
Body diam. at vulva µm	56.3 ±3.18 51.2 - 60.6
Body diam. at anus µm	42.8 ±1.86 40.6 - 46
Body diam. at beginning of J µm	20.6 ±1.27 18 - 21.8

Female body of medium size, assuming a J or open C posture when killed; it tapers very gradually towards the extremities. Lip region cylindrical, continuous with or imperceptibly offset from the rest of the body, anteriorly flattened and laterally rounded. Amphidial pouches slightly but distinctly bilobed, with symmetrical lobes and obscure aperture. Vulva almost at mid-body; vagina occupying from 1/2 to 2/3 the body diameter; genital system amphidelphic with almost equally developed branches; uteri devoid of sperms; a large strongly muscularized sphincter separates the uterus from the oviduct; ovaries reflexed with large oocytes. Prerectum very long, 10 to 15 times anal body-width; rectum as long as or slightly longer then the anal body-width. Tail conoid with rounded terminus, convex dorsally and slightly concave ventrally, bearing two or three papillae on each side.

Male and juveniles not found.

The head and tail morphology of this population of *L. elongatus* is identical to that of topotypes (Hooper, 1961); biometrically the Swiss specimens are a little larger than Dutch and British populations (Hooper, 1961; 1973).

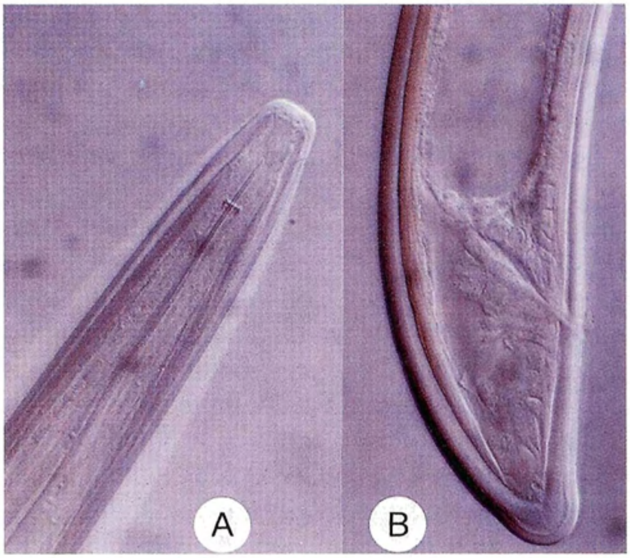

Fig. 1 - Photomicrographs of *Longidorus elongatus:* A, female anterior region; B, female posterior region.

According to the Chen *et al.*, (1997) polytomous key for the identification of *Longidorus* species the code for this Swiss population is: A3, B2, C2/3, D3, E2, F3, G2, H2, I1; which fits the code proposed for *L. elongatus.*

Electrofocusing of SOD isoforms of the Horgen population of *L. elongatus* (Fig. 20) showed a wide homology with the pattern of one population of *L. elongatus* from Scotland (Lamberti *et al.,* 1999a; Crozzoli *et al.*, 2000), although it lacks the acidic isoforms observed in the Scottish population. The esterase profile (Fig. 21) unequivocally descrimintes *L. elongatus* by two bands at Rm 0.45 and 0.5.

The PCR product of the ITS region of *L. elongatus* from Switzerland was about 1600 kb (Table VII) and it was digested by the six restriction enzymes giving the pattern indicated in Fig. 2.

LONGIDORUS MACROSOMA Hooper, 1961

(Tables II, and VII; Figs. 3-6, 20-22)

Populations of *L. macrosoma* were found at Liestal (Table II), Arth and Steinerberg, in the rhizosphere of cherry trees, *Prunus avium* L., and at Horgen in the rhizosphere of a pear tree.

Female with large size body, some specimens longer than 11 mm, assuming in the female a more or less open C posture, when killed; body tapering gradually towards the extremities. Lip region continuous with the rest of the body, frontally concave or flattened. Amphidial pouches pocket to funnel shaped, not lobed at the base, with obscure opening. Vulva almost at mid-body; vagina occupying slightly more than 1/2 of the corresponding body diameter. Genital system amphidelphic with equally developed branches; uteri filled with large sperms; a large and well muscularized sphincter separates the uterus from the oviduct; ovaries reflexed with large oocytes. Prerectum ca. 10 times as long as the anal body width, rectum as long as 2/3 of the anal body width. Tail short, conoid with broadly rounded terminus, dorsally convex and ventrally flat, bearing two caudal papillae on each side.

Males numerous, the body posterior region more coiled than in females; bow-shaped, robust spicules with a wedge-shaped gubernaculum; long testes with many sperms in the distal portion. The adanal pair of supplements is preceeded by a row of 13-15 ventromedian supplements. Tail short, bluntly rounded, dorsally convex and ventrally slightly concave, bearing three or four caudal papillae on each side.

Juveniles clearly separated in four groups (Fig. 5) the first development stage possessing a digitate tail, as illustrated by Hooper (1961); tail bluntly rounded in the second, third and fourth juvenile stages.

Morphometrics of the Swiss populations of *L. macrosoma* generally agree with the original description (Hooper, 1961); only the mean value of the c ratio (170-225 in the original description) and of the mean length of the odontostyle (125-137 μm in the original description) and the odontophore (62-84 μm in the original description) are bigger in the Swiss than in the British populations.

The identification code for this Swiss populations is: A 5/5, B 4/5, C 3/4, D 3, E 4, F 5, G 2/3, H 1, I 2, which fits the code proposed by Chen *et al.* (1997) for *L. macrosoma.*

The isozyme profiles of the Liestal population of *L. macrosoma* are characterized by a very active neutral isoform of SOD (Fig. 20), and a single marked band of esterase (Fig. 21).

The PCR product of the ITS region of *L. macrosoma* from Switzerland (specimens of the populations from Liestal, Steinerberg and Horgen were tested) was about 2000 kb (Table VII); it was digested by all the restriction enzymes tested, giving the pattern indicated in Fig. 6.

In Switzerland *L. macrosoma* has been reported as a vector of the raspberry ringspot virus (RRSV) to cherry trees (Klingler *et al.*, 1985a; Buser, 1990).

TABLE II - *Morphometrics of a population of* Longidorus macrosoma *from Switzerland.*

Locality	Liestal					
Host	Cherry					
n	10 ♀	5♂	10 J_1	11 J_2	10 J_3	10 J_4
L (mm)	10.3±0.53 9.6-11.5	10±1.11 8.4-11.1	2±0.13 1.8-2.2	3.1±0.29 2.6-3.6	4.7±0.67 4-5.7	6.8±0.49 6.1-7.7
a	114.±7.17 104.9-126	124±9.48 114.3-135.7	67.8±3.14 63.8-74.8	76±4.60 68.4-82.7	86.4±6.16 73-99.9	98.2±8.33 83.8-109
b	16.3±1.17 15-19	15.1±2.14 12.6-18.5	5.6±0.71 5-6.9	7.5±0.73 6.4-8.8	9.3±1.36 7.5-11.5	12.1±0.76 11-12.9
c	258.8±19.24 227.3-283	241.4±18.66 219.9-262.4	46±4.03 39.2-53.4	89.2±7.37 76.7-99	120.7±14.34 100-144.7	171.9±18.44 151.3-199.5
c'	0.6±0.06 0.5-0.7	0.7±0.04 0.6-0.7	1.9±0.13 1.8-2.2	1±0.08 0.9-1.1	0.9±0.07 0.8-1	0.6±0.05 0.6-0.7
V%	52±1.37 51-55	–	–	–	–	–
Odontostyle µm	140.6±5.42 131.8-148	140±6.34 130-147	74.7±5.14 64.7-80	83.8±2.50 80-88.2	102.4±8.12 88.2-111.8	121.2±6.31 111.8-129.4
Odontophore µm	90.9±2.98 85.3-94	83.5±4.45 80-88.2	50±2.02 47-51.8	62.2±2.17 58.8-66.5	70.4±3.56 67-76.5	79.2±2.32 76.5-82.3
Replacement odontostyle µm	–	–	85.8±3.82 79.4-92.3	101.6±4.59 94-111	119.7±6.26 111.8-129.4	138.6±7.20 123.5-146.5
Oral aperture to guide ring µm	41.6±2.04 38.2-44.7	44±2.57 40.4-47.6	21.9±1.13 20-23.5	25.6±2.12 22.9-29.4	31.5±1.65 28.2-34	37.4±2.57 34-41.2
Tail µm	40±2.71 37-44	41.3±2.44 38.2-44.7	42.6±2.60 38.8-47	34.8±2.12 32.3-38.2	38.7±2.35 34.7-42.3	39.6±3.03 35.3-44
J (hyaline portion of tail) µm	16±1.97 14-19.4	16.2±1.18 15-17.6	12.4±2.17 9.4-14.7	8.4±0.93 7-10.5	10.3±0.97 9-11.8	11.5±1.60 9-14.7
Body diam. at lip region µm	21.7±1.49 20-24.7	21.4±0.54 20.6-21.8	9.5±0.60 8.8-10.6	11.7±0.32 11-11.8	13.7±0.99 12-15	17.3±0.35 15.9-18.2
Body diam. at guide ring µm	40±2.16 36.5-44	40±2.14 37-42.3	15.3±0.62 14.7-16.5	21±1.50 19.4-23.5	26.6±2.69 21.2-29.4	32.8±2.39 28.8-36.5
Body diam. at base of oesophagus µm	78.8±3.26 73.5-82.9	71±3.89 64.7-74.7	26.3±1.01 24.7-28.3	38±3.60 32.3-44.7	49.8±6.48 38.8-61.8	63.3±4.13 56.5-71.8
Body diam. at mid-body or vulva µm	89.8±3.63 82.3-95.3	80.4±4.31 73.5-85.3	28.8±1.38 26.5-31.8	41.2±3.88 35.3-47.6	54±6.08 44-64.7	69.2±4.32 63-76.5
Body diam. at anus µm	64.4±2.24 61.2-68.2	61±7.81 52.3-70.6	22.2±1.13 20.6-24.7	35±2.51 31.8-38.8	44.7±4.63 37-51.2	58.7±4.44 53-67.6
Body diam. at beginning of J µm	44.9±3.19 40.6-51.8	46.4±4.94 41.2-51.2	10.3±1.18 9-11.8	20.4±2.49 17-24	30±4.29 23-35.3	39.3±4.20 34-46.5
Spicules µm		116.2±3.28 111.8-120.6				

LONGIDORUS PROFUNDORUM Hooper, 1966

(Tables III and VII; Figs 7-9, 20-22)

A population of *L. profundorum* was found in the rhizosphere of an apple, *Malus sylvestris* Mill, tree at Hinter Schlatt.

Female with medium size body, assuming a closed C posture, when killed. Body very gradually tapering towards the extremities. Lip region continuous with the rest of body, frontally flattened and laterally rounded. Amphidial pouches pocket-like, symmetrically bilobed at the base, with obscure aperture. Vulva slightly posterior to mid-body; vagina strongly muscularized, occupying ca. 2/3 of the corresponding body diameter. Genital system amphidelphic with equally developed branches; long uterus filled with large sperms; a robust, muscularized sphincter separates the uterus from the oviduct which starts with an enlarged portion like a chamber, possibly a vestigial spermatheca; ovaries reflexed with large sperms in the distal portion. Prerectum ca. ten times as long as the anal body width and rectum as long as anal body width. Tail conoid, dorsally rounded and ventrally flattened, with bluntly rounded terminus bearing two or three caudal papillae on each side.

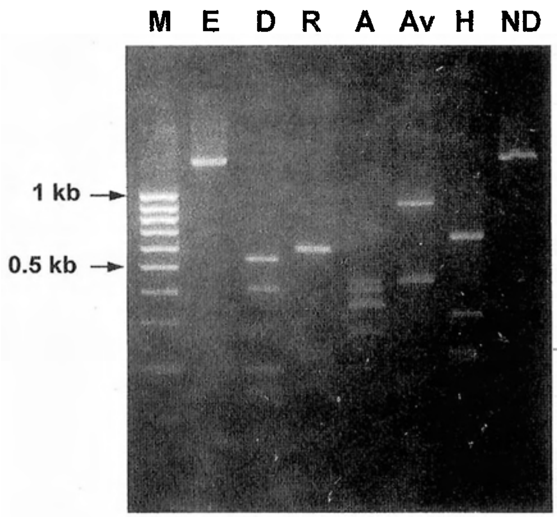

Fig. 2 - Patterns of the PCR amplified ITS region of *L. elongatus* on 2.5% agarose gel digested with *Eco* RI (E), *Dde* I (D), *Rsa* I (R), *Alu* I (A), *Ava* II (Av) and *Hinf* I (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

Males as numerous as females; more coiled than females in the posterior portion of the body. Spicules ventrally curved, more bent in their terminal part; gubernaculum well sclerotized. Long testes with large sperms in the distal portion. The adanal pair of supplements is preceeded by a row of 13-16 ventromedian supplements. Tail asymmetrical, convex dorsally and deeply concave ventrally, with hemi-elliptical terminus. It bears two or three caudal papillae on each side.

Juveniles clearly separated into four groups (Fig. 8). The first stage juveniles posses a pointed, subdigitate tail; tail is hemi-elliptical in the second, third and fourth juveniles stages.

Morphometrics of this Swiss population of *L. profundorum* fully agree with those of the original description (Hooper, 1966). The only consistent difference between the Swiss and the British populations occurs in the males because of the spicules which are longer in the Swiss population (64-79 μm in the British populations).

The identification code for the Swiss population of *L. profundorum* is: A 3/4, B 2, C 2/3, D 3, E 2, F 3/4, G 2/3, H 1/2, I 2, which fits the code proposed by Chen *et al.*, 1997.

The SOD electrofocusing profile of this population of *L. profundorum* is characterized by a neutral isoform (Fig. 20); esterase activity is detected by a slow-migrating band (Fig. 21).

The PCR product of the ITS region of *L. profundorum* from Switzerland was ca.> 1800 kb (Table VII) and it was digested by all the restriction enzymes tested, giving the pattern illustrated in Fig. 9.

LONGIDORUS RASKII Lamberti *et* Agostinelli, 1993

(Tables IV and VII, Figs 10-12, 20-22)

Topotypes were collected at Etoy from the rhizosphere of apple trees.

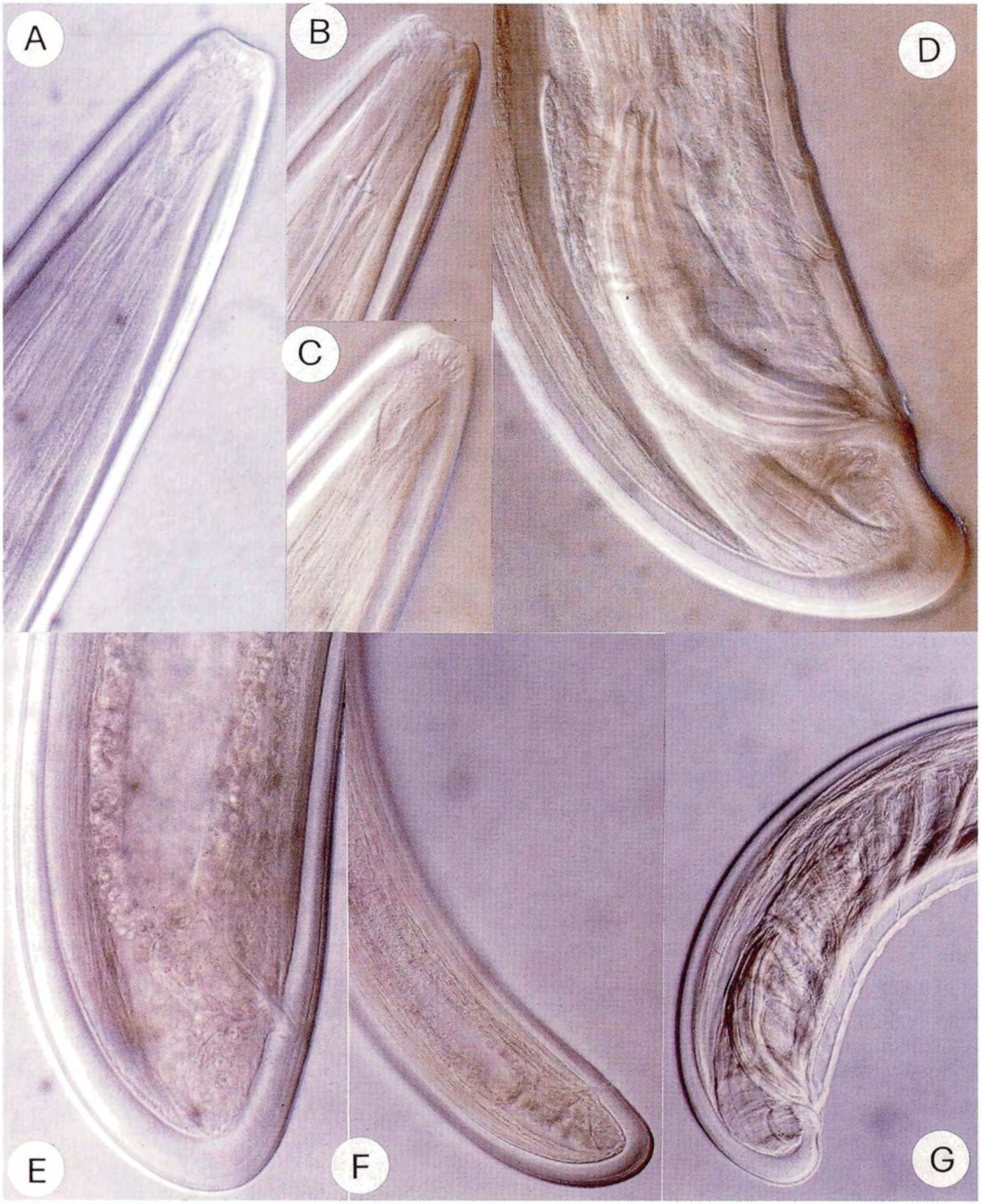

Fig. 3 - Photomicrographs of *L. macrosoma:* A-C, female anterior region; D and G male posterior region; E and F, fema posterior region.

TABLE III - *Morphometrics of a population of* Longidorus profundorum *from Switzerland.*

Locality	Hinter Schlatt					
Host	Apple					
n	10 ♀	10 ♂	11 J_1	12 J_2	6 J_3	16 J_4
L (mm)	6.9±0.51 6-7.7	6.9±0.61 6.2-8.2	1.6±0.13 1.4-1.8	2.4±0.26 2.1-3	3.7±0.29 3.3-4	5.2±0.48 4.4-5.9
a	112.3±5.83 102-121.7	118.2±6.69 106.9-131.2	69.4±5.64 62.8-76.6	77±3.34 72.3-84.9	86±3.83 80-90.2	104.5±5.49 96-114.9
b	13.4±1.26 11.6-15.2	13.5±1.15 11.5-15	5.3±0.58 4.6-6.7	7±0.63 6.2-8.5	9.6±1.20 8.4-11.2	11.6±1.21 10-13.8
c	145.7±10.29 124.5-161	151±10.65 139.170.8	33.7±3.15 30-40.3	56±3.70 52-64.6	80±4.48 72.8-86.2	112.1±8.80 97.2-124.7
c'	1±0.06 0.9-1.1	1±0.08 0.9-1.1	2.9±0.16 2.6-3.1	1.8±0.11 1.6-1.9	1.4±0.08 1.3-1.5	1.1±0.05 1-1.2
V%	52±1.09 50-54	–	–	–	–	–
Odontostyle µm	98.7±2.17 95.3-101.2	97.2±3.48 92-102.3	57.5±2.61 53.5-61.8	62±1.98 58.8-64.7	75.5±2.89 73.5-80	85.7±3.28 79-91.2
Odontophore µm	66.3±2.78 63.5-72.3	67.5±2.19 63.6-70.6	37.3±3.07 32.3-41.2	45.5±2.65 41.2-48.8	54.6±3.87 47-57	62±2.78 58-67.6
Replacement odontostyle µm	–	–	61.5±2.37 57.6-64.7	73±3.09 68.2-78.2	87.7±1.35 85.3-88.8	96.8±3.86 92-102.3
Oral aperture to guide ring µm	34±2.22 29.4-37	37.2±1.75 33-38.8	20.7±0.65 20-21.8	23±1.40 21.2-26	27±1.71 24-28.8	30.4±1.44 27.6-33.5
Tail µm	47.2±2.82 43.5-52.3	45.6±3.65 40-52	46.3±2.93 42.3-50	42.4±3.51 38.2-50	45.9±2.83 42.3-50	46.7±3.92 38.8-53
J (hyaline portion of (tail) µm	11.7±0.74 10.6-13	12.3±1.09 11.2-14.5	11.6±1.01 10-13.5	6.6±1.12 5.3-8.8	7.5±1.52 6.5-10.6	8.9±0.77 7.6-10
Body diam. at lip region µm	14±0.60 13-14.7	14.3±0.52 14-15.3	7.4±0.28 7-7.6	9±0.37 8.2-9.4	11±0.31 10.6-11.2	12.3±0.49 11.8-13
Body diam. at guide ring µm	26.6±0.89 25.3-28.2	27.2±1.39 25.3-30	13.3±0.44 13-14	16±0.66 15.3-17.6	20±0.80 18.8-20.6	22.7±0.80 21.2-23.5
Body diam. at base of oesophagus µm	51.2±2.20 47.6-55.3	49±3.94 40-54	21±0.66 20-22.3	28.7±1.82 26.5-31.8	39.8±4.09 35.3-47	45.2±2.51 41.2-50
Body diam. at mid-body or vulva µm	61.2±3.92 56.5-67.6	58.2±5.53 52.3-69	22.4±0.63 21.2-23.5	30.7±2.20 28.2-35.3	42.6±4.12 38.8-50	49.5±3.17 44-53.5
Body diam. at anus µm	48±2.03 44-51.2	46.4±2.18 43-51.2	16±0.70 14.7-17	24±2.41 21.2-30	34±2.67 30.6-38.2	41±2.06 37-44
Body diam. at beginning of J µm	31.6±1.94 29.4-35.3	22.7±1.87 20.26	7.8±0.54 7-8.8	14±1.30 12.3-17	21±1.42 19.4-23.5	25.3±1.78 22-28.8
Spicules µm	–	78.9±2.81 75-83.8	–	–	–	–

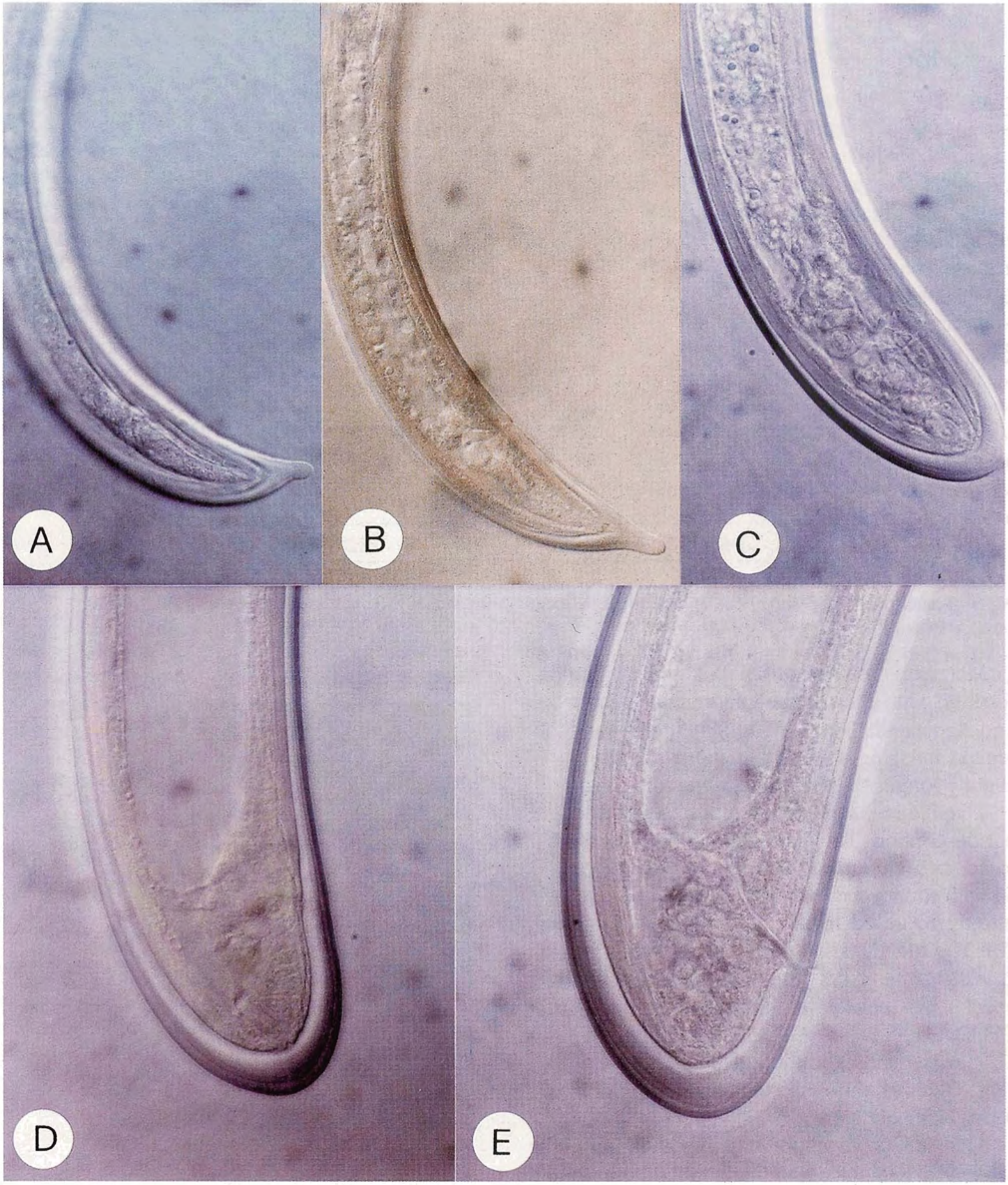

Fig. 4 - Photomicrographs of juveniles of *L. macrosoma* posterior region: A and B, first stage; C, second stage; D, third stage; E, fourth stage.

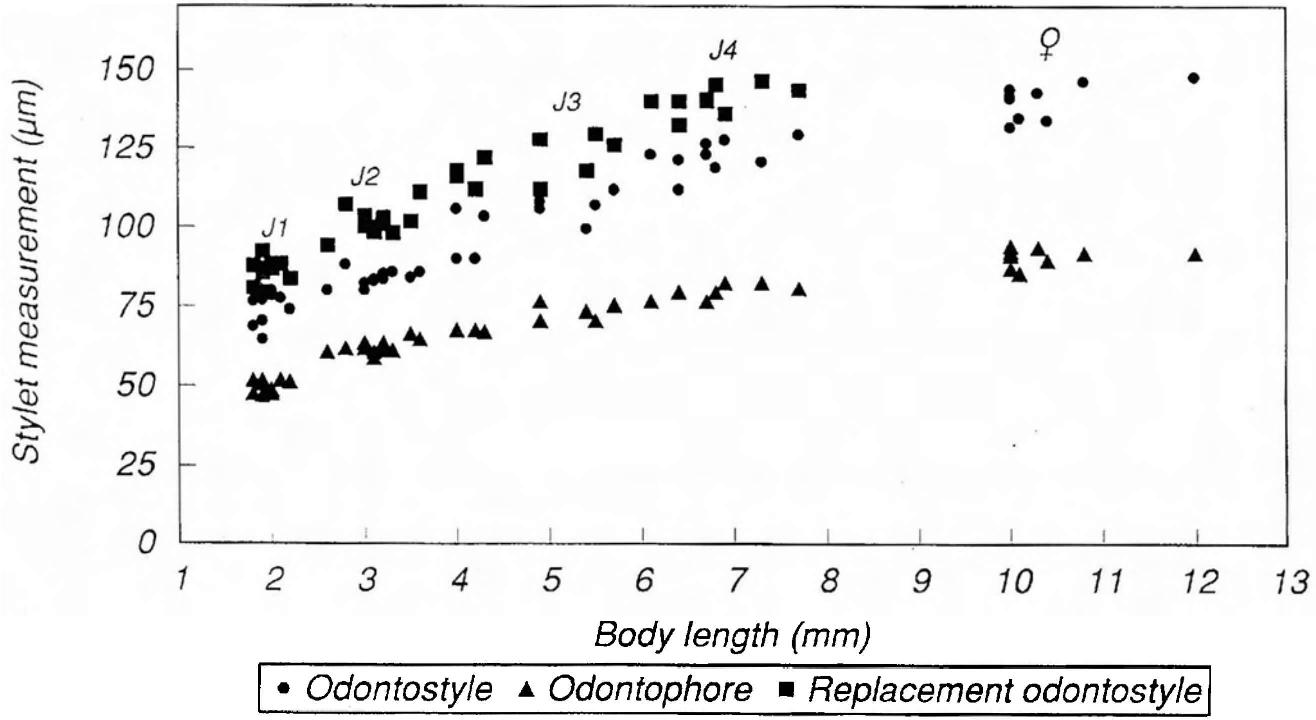

Fig. 5 - Scatter diagram plotting body and odontostyle length of individual juveniles and females of *L. macrosoma*

Morphobiometrics of females and males correspond with the original description (Lamberti and Agostinelli, 1993), with the exception of the female tail length which in these specimens is slightly longer than in paratypes.

Juvenile stages were not reported in the original description. They are morphologically similar to adults and separate into four groups (Fig. 11), all with rounded tail: conoid in the first stage and bluntly rounded in the next three stages (Fig. 10). The identification code of this population of *L. raskii* totally fits the formula given for this species by Chen *et al.* (1997), that is: A 3/4, B 3 C 3, D 1, E 2, F 3/4, G 1/2, H 1, I 2.

L. raskii shows a marked basic SOD isoform (Fig. 20) and various esterase high weight isoforms (Fig. 21).

The PCR product of ITS region was ca. 1600 kb (Table VII) and was digested by the restriction enzymes tested giving the pattern indicated in Fig. 12.

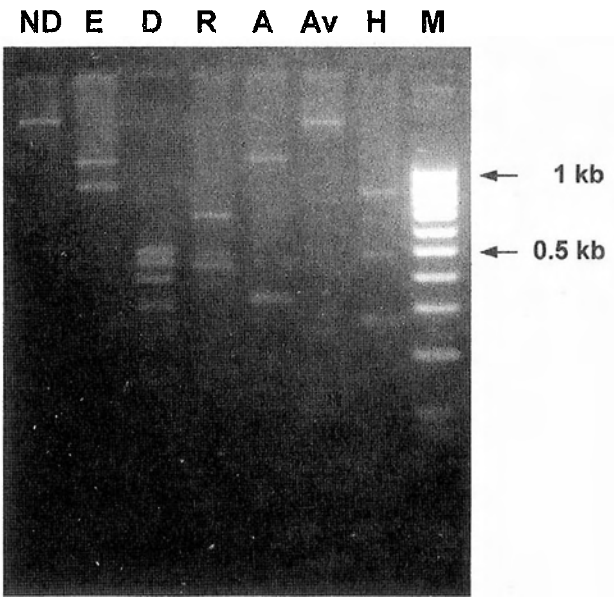

Fig. 6 - Patterns of the PCR amplified ITS region of *L. macrosoma* on 2.5% agarose gel digested with *Eco* RI (E), DdeI (D), *Rsa* I (R), *Alu* I (A), *Ava* II (Av) and *Hinf* I (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

TABLE IV - *Morphometrics of a population of* Longidorus raskii *from Switzerland.*

Locality	Etoy					
Host	Apple					
n	5 ♀	2 ♂	14 J_1	11 J_2	9 J_3	10 J_4
L (mm)	7.5±0.57 6.7-8	6.6-6.6	1.6±0.21 1.3-1.9	2.3±0.34 1.9-2.9	3.3±0.25 2.9-3.8	4.9±0.43 4.2-5.4
a	87.2±6.07 78.5-92.2	81.9-86.3	54?7±3.87 49-59.3	62.8±3.34 59.4-68.5	70.2±2.65 65.7-73.5	77±5.05 68.6-82.7
b	13.7±1.11 12.4-15.1	11.6-15	5.9±1.13 4.6-8.5	6.7±1.19 5.4-8.6	8.1±0.97 6.4-9.4	9.8±1.12 8.4-12
c	162±15.60 147.2-181.4	150-150	47.7±4.87 40.2-54.3	64.2±8.74 51.4-78.4	81±5.28 72.8-87.3	114.2±10.33 100-128.6
c'	0.8±0.06 0.7-0.8	0.8-0.8	1.6±0.19 1.3-1.8	1.2±0.10 1.1-1.4	1±0.05 1-1.1	0.8±0.05 0.8-0.9
V%	53±2.36 51-56	–	–	–	–	–
Odontostyle µm	99±2.87 95.3-102.3	106-100	54.3±1.28 51.8-56	58.7±1.52 56.5-61.8	72±3.89 64.7-78.2	86±3.80 80-91.2
Odontophore µm	66±1.19 64.7-67.6	68-67.6	39.2±2.67 35-42.3	44.4±2.22 41.8-48.8	54.4±1.53 52.3-56	66.6±3.55 62-70.6
Replacement odontostyle µm	–	–	59±2.90 53-64.7	70.3±2.11 67.6-73.5	86.6±3.48 80.6-90.6	101±3.66 94-106
Oral aperture to guide ring µm	35.6±1.43 34-37	34.7-35.3	19.4±1.51 17-23.5	22.3±1.13 20.6-24.7	26.3±0.99 24.7-27.6	30±1.38 27.6-32.3
Tail µm	46.6±3.92 44-52.3	44-44	32.5±3.19 27.6-38.8	36±3.42 30.6-41.2	40.3±2.62 36-44	43.3±3.25 38.2-47.6
J (hyaline portion of tail) µm	15.6±1.43 14-17	10.6-11	5.8±0.70 5-7	6.6±0.52 6-7.6	8.8±0.71 7.6-10	11±1.53 8.8-14
Body diam. at lip region µm	17±0.71 16-17.6	18.2-18.8	8.5±0.39 7.6-8.8	9.9±0.57 9-10.6	12±0.56 11.2-13	14.5±0.63 13-15.3
Body diam. at guide ring µm	31.2±1.87 28.8-33	30.6-32.4	14±0.92 13-16.5	17±0.93 15.3-18.2	21±0.83 19.4-22.3	26±1.71 22-27.6
Body diam. at base of oesophagus µm	68.4±5.27 60.6-72.3	70.6-67	25.6±2.74 23-31.8	33±3.56 28.8-39.4	43±2.41 40.6-48.8	57.2±2.98 53-61.8
Body diam. at mid-body or vulva µm	86.3±3.36 85.5-91.2	80.6-76.5	28.3±2.91 25.3-34	35.8±4.12 31.2-44	46.8±2.20 44-51.8	64.2±3.37 58.8-68.8
Body diam. at anus µm	62.4±2.14 60-64.7	53-54	20.2±2.63 17-24.7	28.7±3.38 25.3-35.3	38±2.00 35.3-41.8	51.4±3.57 46.5-57
Body diam. at beginning of J µm	44.4±2.22 41.2-46	30.6-34	13.3±1.11 12.3-15.3	17.8±1.36 16-20.6	25.8±1.21 23.5-27.6	34.6±4.40 26.5-41.2
Spicules µm		96-93.5				

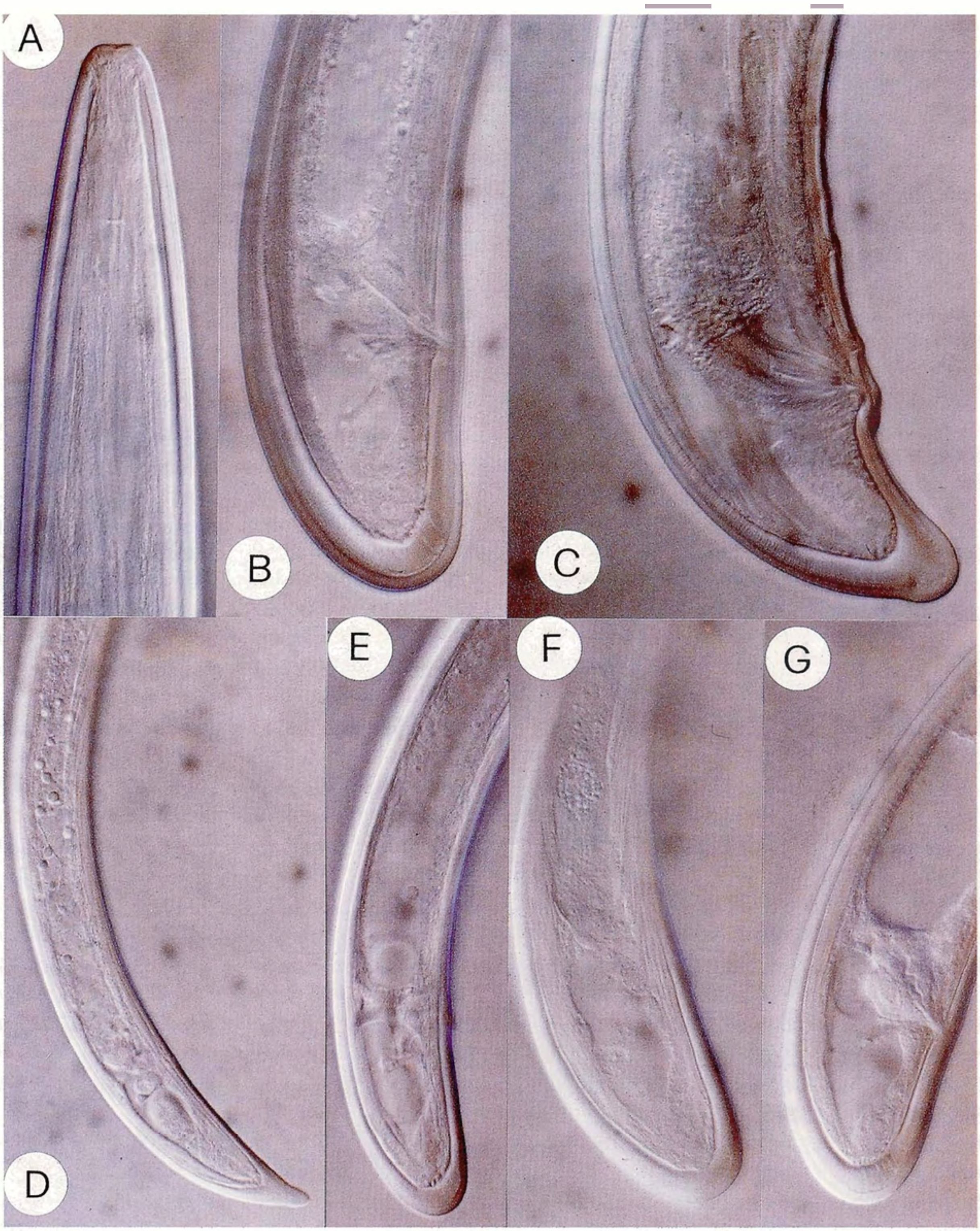

Fig. 7 - Photomicrographs of *L. profundorum*: A, female anterior region; B, female postrerior region; C, male posterior region; D-G, tail of first, second, third and fourth juvenile stages, respectively.

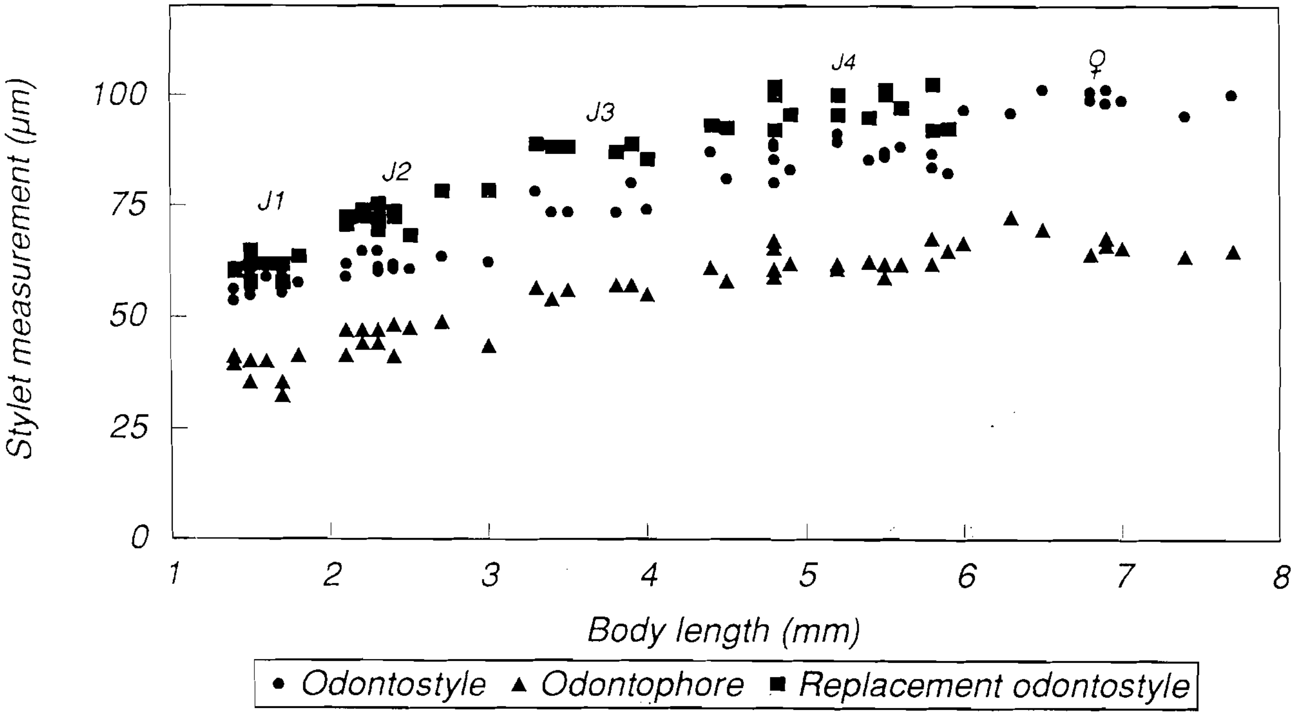

Fig. 8 - Scatter diagram plotting body and odontostyle length of individual juveniles and females of *L. profundorum*.

***LONGIDORUS ARTHENSIS* Brown, Grunder, Hooper, Klingler *et* Kunz, 1994**
(Tables V and VII; Fig 13, 14, 20-22)

Specimens of females and males *L. arthensis* were found at Arth (topotypes) in the rhizosphere of cherry trees and at Horgen, in the rhizosphere of a pear tree. Morphobiometrically they fit the original description (Brown *et al.*, 1994); the population from Horgen has specimens with the mean body length slighlty longer than that of the paratypes. Also, juveniles of the first stage have a mucronate tail, corresponding with the original description of the species. The identification code of the Horgen population is: A 3/4, B 3, C 3, D 1, E 2, F 3/4, G 2/3, H 1/2, I 2 (Chen *et al.*, 1997).

L. arthensis is characterized by active neutral and faint basic SOD isoform (Fig. 20) and by two central esterase isoforms (Fig. 21).

The PCR product of the ITS region was ca. 1700 kb (Table VII) and was digested by the restriction enzymes tested giving the pattern indicated in Fig. 14.

***LONGIDORUS HELVETICUS* sp.n.**
(Tables VI and VII; Figs 15-22)

Female *habitus* curved in an open C when killed. Body of large size, tapering towards the extremities. Cuticle smooth in appearance, ca. 2.5 µm thick along body, except at the vulva level where it is 3 µm thick. Lip region hemi-elliptical broadly rounded laterally, continuous with the rest of the body. Amphidial pouches pocketlike, large, extending backwards almost to the guide ring, not lobed at the base. Odontostyle, odontophore and guide ring typical of the genus. Muscular posterior bulb of the oe-

TABLE V - *Morphometrics of a population of* Longidorus arthensis *from Switzerland.*

Locality Host	Horgen Pear
n	10 ♀♀
L (mm)	6.3±0.63 5.8 - 7.6
	103.4±10.72 87 - 118.6
	14±1.49 11.9 - 15.7
	148.3±15.75 128 - 184
	1.0±0.09 0.9 - 1.1
V%	50±1.22 48 - 52
Odontostyle µm	106.5±4.77 98 - 111.8
Odontophore µm	68.4±2.38 64.5 - 70.6
Oral aperture to guide ring µm	34.9±2.13 32.3 - 38.2
Tail µm	42.9±2.26 40 - 46.5
J (hyaline portion of tail) µm	14.1±1.39 12 - 16
Body diam. at lip region µm	17.1±1.02 16 - 19.4
Body diam. at guide ring µm	27.4±1.91 23.5 - 30
Body diam. at base of oesophagus µm	51±4.01 45.9 - 59.4
Body diam. at vulva µm	61.8±4.95 57.6 - 73.5
Body diam. at anus µm	43.1±2.30 38.8 - 47
Body diam. at beginning of J µm	28.6±3.90 20.6 - 34

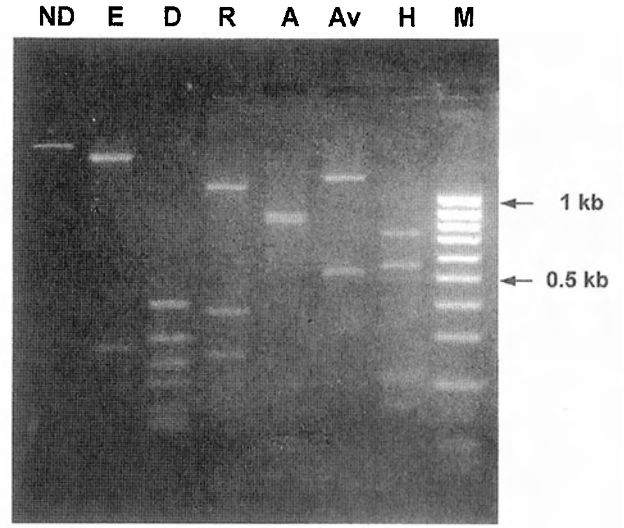

Fig. 9 - Patterns of the PCR amplified ITS region of *L. profundorum* on 2.5% agarose gel digested with EcoRI (E), DdeI (D), RsaI (R), AluI (A), AvaII (Av) and HinfI (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

sophagus representing 1/4 to 1/5 of the oesophagus total length; it measures 110-130 µm long and 30-40 µm wide and contains three glandular nuclei: the dorsal gland nucleus in the anterior third and the two subventral gland nuclei just behind the middle region of the bulb; oesophageal intestinal valve large, bluntly conoid. Vulva slightly posterior to mid-body; vagina thick-walled occupying from 1/2 to 2/3 of the corresponding body width; genital system amphidelphic with two equally developed branches, 700 to 950 µm long: uteri large, well muscularized, containing many oblong sperms measuring 5.5-6.5x2-3 µm; a sphincter separates the uterus from the oviduct, which starts with a chamber-like structure; ovaries reflexed, containing large oocytes. Prerectum 450-680 µm long; rectum 1/2 to 2/3 anal body width. Tail bluntly rounded with two caudal papillae on each side.

Males as numerous as females, with the posterior region more coiled than in female. Spicules robust, ventrally curved, gubernaculum wedge-shaped. Testes paired very long with sperms in the distal portion. Adanal pair of supplement preceeded by a row of 15-17 ventro-

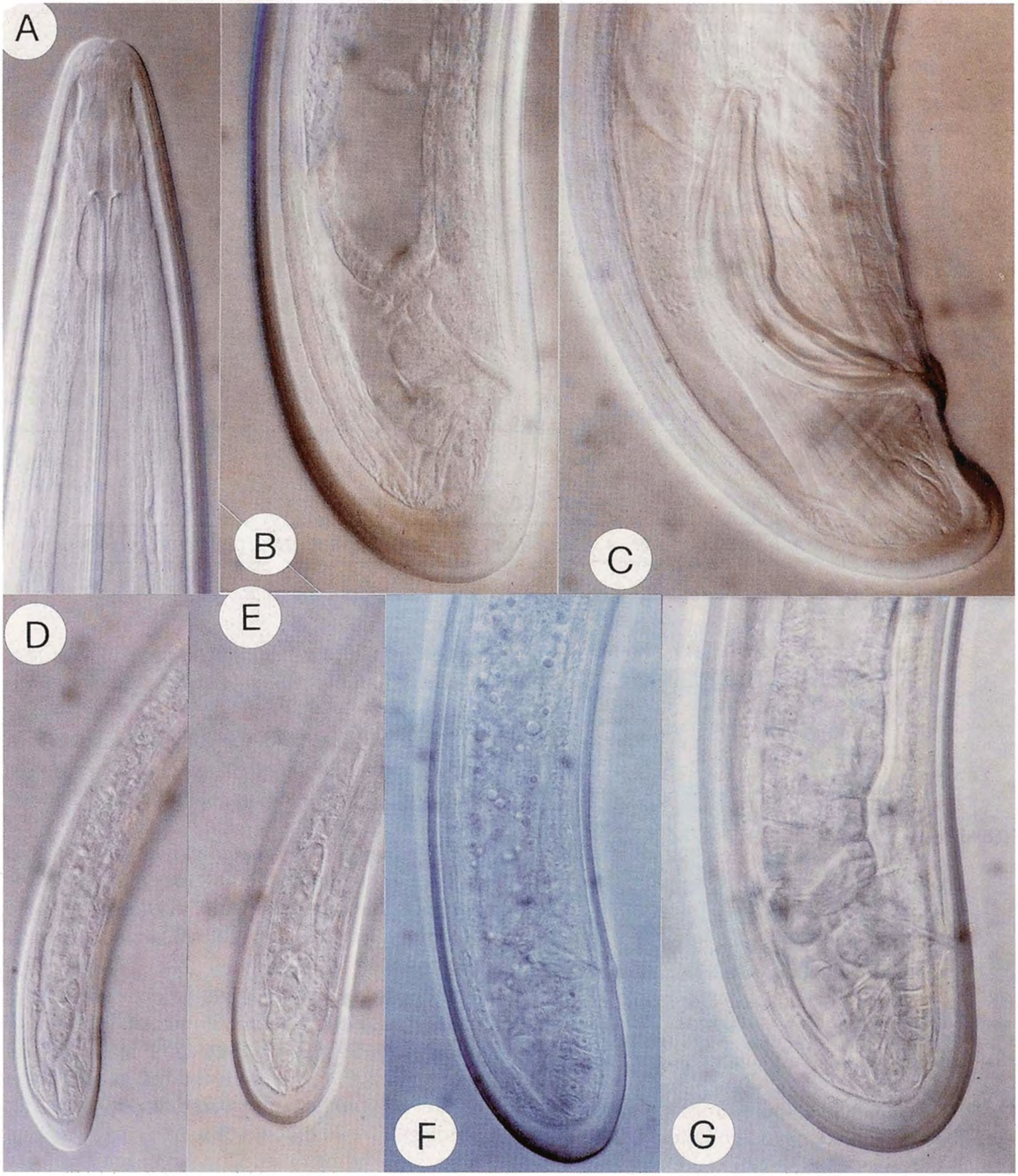

Fig. 10 - Photomicrographs of *L. raskii*: A, female anterior region; B, female posterior region; C, male posterior region; D-G, tail of first, second, third and fourth juvenile stages, respectively.

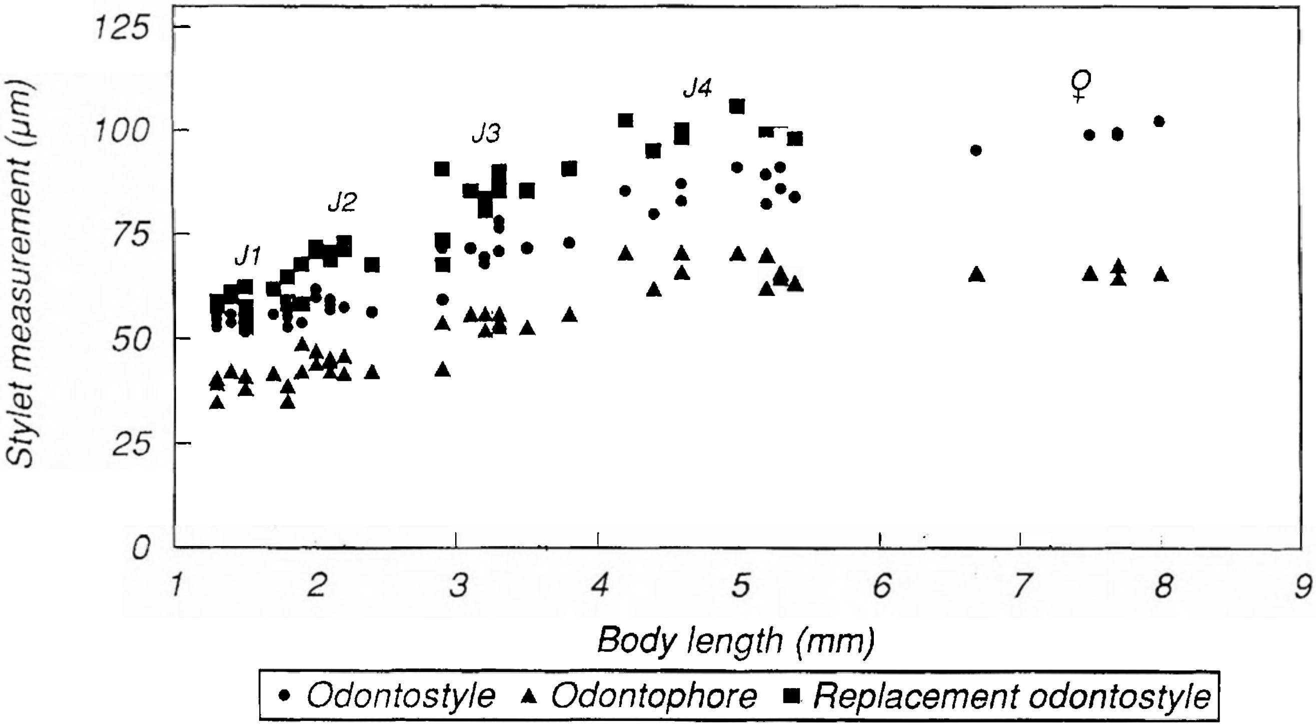

Fig. 11 - Scatter diagram plotting body and odontostyle length of individual juveniles and females of *L. raskii*.

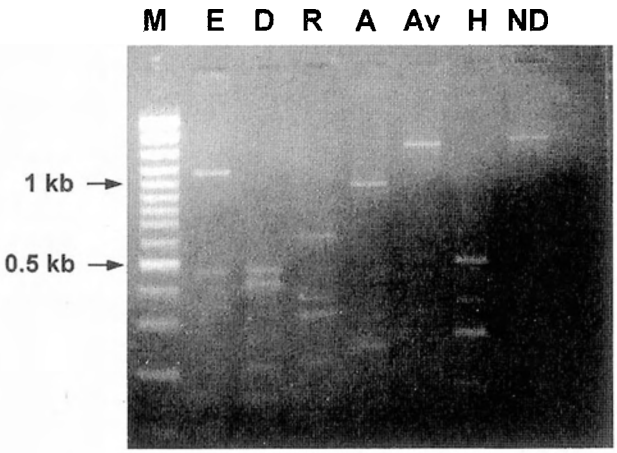

Fig. 12 - Patterns of the PCR amplified ITS region of *L. raskii* on 2.5% gel digested with *Eco* RI (E), *Dde* I (D), *Rsa* I (R), *Alu* I (A), *Ava* II (Av) and *Hinf* I (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

median supplements. Tail bluntly rounded, convex dorsally and concave ventrally, bearing two caudal pores on each side.

Juveniles similar to adults, separated into four developmental stages (Fig. 18); the first stage has a digitate tail with a long mucro; second, third and fourth juvenile stages have bluntly rounded tails.

Type habitat and locality: rhizosphere of cherry trees, *Prunus avium* L., at Gersau, Camenzind, in the Province of Lucerne, Switzerland.

Specimens of *L. helveticus* were also found at Roch D'Or, Seewen and Randen in *Fagus sylvatica* L. forests and at Arth in the rhizosphere of cherry trees mixed with *L. arthensis* or *L. macrosoma*.

Diagnosis: *Longidorus helveticus* sp.n. is characterized by body length of ca. 8 mm; odontostyle length of ca. 135 µm; hemi-elliptical lip region continuous with the rest of the body; pocket-like not-lobed amphidial pouches; amphidelphic equally developed female genital branches; almost mid-body vulva; bluntly rounded tail; presence of males; and mucronated tail in the first juvenile stage.

TABLE VI - *Morphometrics of a population of* Longidorus helveticus sp.n. *from Switzerland.*

Locality Host	Gersau, Camenzind Cherry Paratypes						
n	holotype ♀	27 ♀	23 ♂	20 J_1	27 J_2	23 J_3	43 J_4
L (mm)	7.6	7.8±0.77 6.2-9.3	7.3±0.65 6.0-8.6	1.8±0.25 1.4-2.2	2.7±0.33 2.2-3;5	4.0±0.34 3.3-4.5	5.7±0.73 4.4-7.3
a	71	71.1±5.07 62.1-80.5	75.6±6.26 63.3-89.6	48.8±3.05 44-55.3	52±3.48 44.6-58.6	57.4±4.64 48-67.4	65.3±5.17 53.6-75.7
b	13.6	14.3±2.19 10.8-19.9	12.8±1.38 11-16.7	5.7±1.19 4-8.4	7.1±1.37 5.4-10.2	9±1.47 6.7-12.3	11±2.13 8.2-17.7
c	161.4	200.3±29.46 150.4-260	176.8±21.12 144.8-216.4	35.2±4.16 28.3-43.1	72.8±9.57 60.6-95.6	103.1±12.79 76.7-123	144.3±18.96 100.9-184
c'	0.7	0.6±0.05 0.5-0.7	0.7±0.05 0.6-0.8	1.8±0.17 1.5-2.1	0.9±0.09 0.8-1.1	0.7±0.08 0.6-0.9	0.7±0.05 0.6-0.8
V%	50	52±1.82 48.3-56.7	–	–	–	–	–
Odontostyle µm	145.3	135.4±5.34 127-145.5	136.5±5.88 125-146.7	80.2±3.18 73.7-87.5	87.7±3.19 81.1-94.4	108.2±3.71 99.5-115.2	123.2±4.92 111.5-134.8
Odontophore µm	82	89.5±5.10 76-98.2	90.5±3.20 85-97.5	52±2.37 48.4-55.6	65.3±3.91 59.1-72	75.5±3.14 70-81.6	80.7±3.53 72.5-86.4
Replacement odontostyle µm	–	–	–	86.8±2.52 82.2-92	108±3.84 100.8-116.7	123.1±2.98 116.5-129.5	136±4.16 128.3-146
Oral aperture to guide ring µm	44	42±1.54 39-46	42.8±2.16 39.4-46.7	22.4±1.01 20.5-23.8	27.4±1.32 25.3-31.1	32.5±1.73 29.7-36.1	36.8±1.85 33-40.1
Tail µm	47	39.2±3.94 31.7-46.6	41.8±3.70 37.2-51.5	51.3±3.50 43.8-58	37.5±2.82 32-43.5	39.3±3.34 34.1-45.6	39.7±3.33 33.7-47
J (hyaline portion of tail) µm	19.4	17.3±1.54 12.8-20.3	16.1±1.60 13.3-20.8	22.6±2.38 17.5-28.2	9.9±1.53 7.3-13.5	12.6±1.64 10.1-15.9	14.8±1.61 12.2-20.4
Body diam. at lip region µm	21.8	21.6±0.86 19.7-23.7	22.3±0.87 20.6-24	9.9±0.34 9.3-10.7	13.2±0.70 11.5-14.3	16.4±0.99 14.6-18	19±0.88 17.3-21
Body diam. at guide ring µm	42.4	44±2.05 38.1-49.1	44.1±1.88 40-49.7	19±0.79 17.3-20.5	25.7±1.01 23.3-27.5	32.4±1.88 29-36	38.4±1.97 34.1-42.7
Body diam. at base of oesophagus µm	79.5	88.6±7.52 73.3-105.6	84.1±8.04 74.2-108	35±3.25 28.5-40.4	47.2±4.41 38.3-54.7	63±6.04 50.8-70.7	75.8±5.93 61.4-89.1
Body diam. at mid-body or vulva µm	107	109.1±6.74 97.1-123.5	97.2±5.71 87.8-112	37.2±5.13 27.7-46.8	52.5±7.07 41.8-67.7	70.5±8.32 54-85.7	87.4±9.31 66.7-103.7
Body diam. at anus µm	67.6	64.8±3.76 58-72.1	60.1±2.95 55.7-66.1	28.2±3.24 23.5-35.8	40.7±3.21 35.1-47.2	52.8±3.85 44.6-58.7	60.4±3.64 53.4-68.8
Body diam. at beginning of J µm	47.6	46.7±3.35 40.7-53.3	38.7±1.52 35.6-41.5	18±1.24 15.5-20.8	26.3±2.56 21.7-31.8	35.7±3.72 29.3-41.4	41.8±2.92 36.2-48.2
Spicules µm			111.6±4.38 104-118				

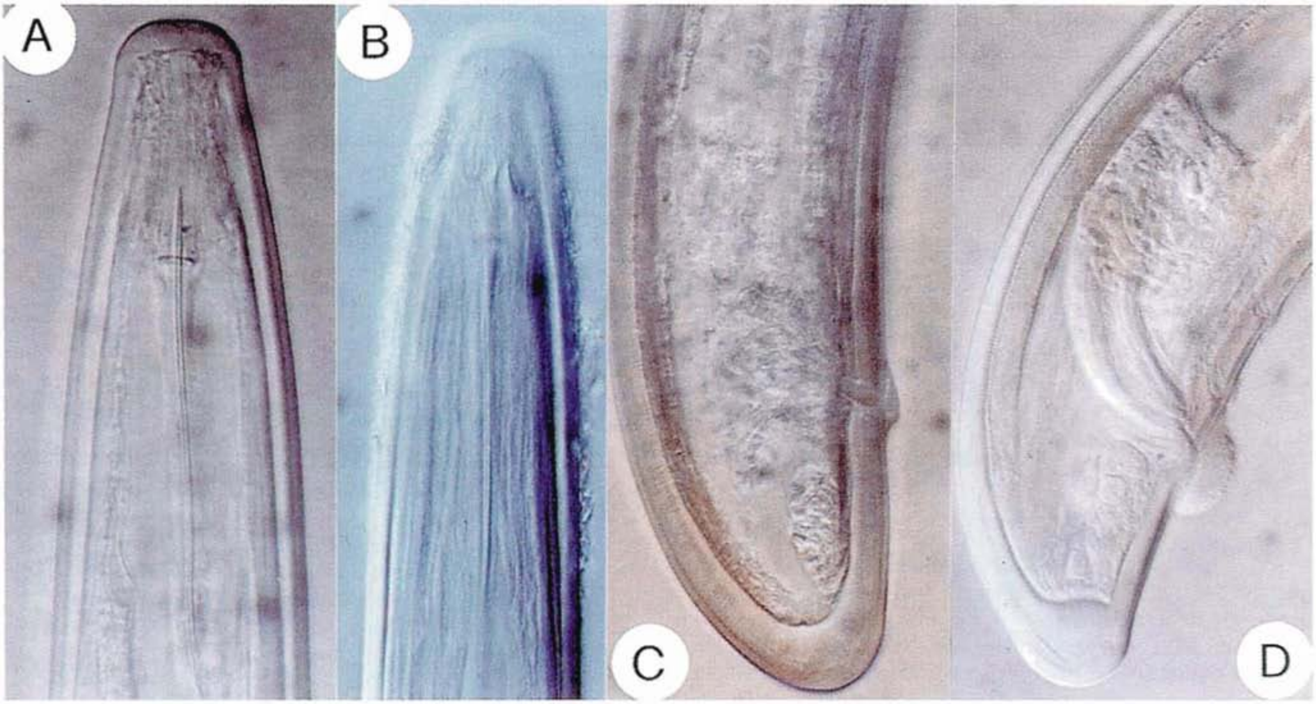

Fig. 13 - Photomicrographs of *L. arthensis*: A and B, female anterior region; C, female posterior region; D, male posterior region.

Relationships: *L. helveticus* sp.n. is very similar to *L. macrosoma* Hooper, 1961 from which it differs in its shorter body (*L. macrosoma* generally longer than 9 mm), much lower a ratio value (105-115 in *L. macrosoma*) and frontally rounded lip region (flat/slightly depressed in *L. macrosoma*). Moreveor, the hyaline portion of the tail of the first stage juveniles of *L. helveticus* is much longer in the corresponding juveniles of *L. macrosoma* (ca. 12 in *L. macrosoma* from Liestal).

L. helveticus also resembles *L. poessneckensis* Altherr, 1974, *L. picenus* Roca, Lamberti *et* Agostinelli, 1985 and *L. nevesi* Macara, 1986.

However, compared with *L. poessneckensis* (Sturhan and Loof, 2001), *L. helveticus* has lower a ratio value (a = 104 in *L. poessneckensis*), higher c ratio value (c = 179 in *L. poessneckensis*), frontally rounded lip region (flat or depressed in *L. poessneckensis*), anterior vulva (V = 55 in *L. poessneckensis*) and first stage ju-

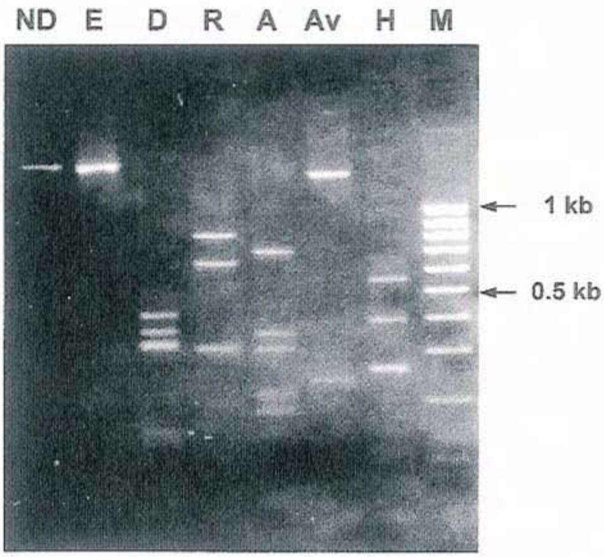

Fig. 14 - Patterns of the PCR amplified ITS region of *L. arthensis* on 2.5% agarose gel digested with *Eco* RI (E), *Dde* I (D), *Rsa* I (R), *Alu* I (A), *Ava* II (Av) and *Hinf* I (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

veniles with digitate tail (rounded in *L. poessneckensis*).

Compared to *L. picenus* (Roca *et al.*, 1985), *L. helveticus* has a longer body (6.8 mm in *L. picenus*), higher c ratio value (c = 179 in *L. picenus*), and amphidial pouches not lobed (bilobed in *L. picenus*).

Finally, *L. helveticus* differs from *L. nevesi* (Macara, 1986) in its less pointed lip region (anteriorly tapering abruptly in *L. nevesi*), amphidial pouches not lobed (deeply bilobed in *L. nevesi*), lower value of the a ratio (a = 85 in *L. nevesi*), higher value of the c ratio (c = 180 in *L. nevesi*) and digitate first stage juvenile tail (conoid in *L. nevesi*).

The following codes are proposed for *L. helveticus* sp.n., according to the polytomous key for *Longidorus* (Chen et al., 1997; Loof and

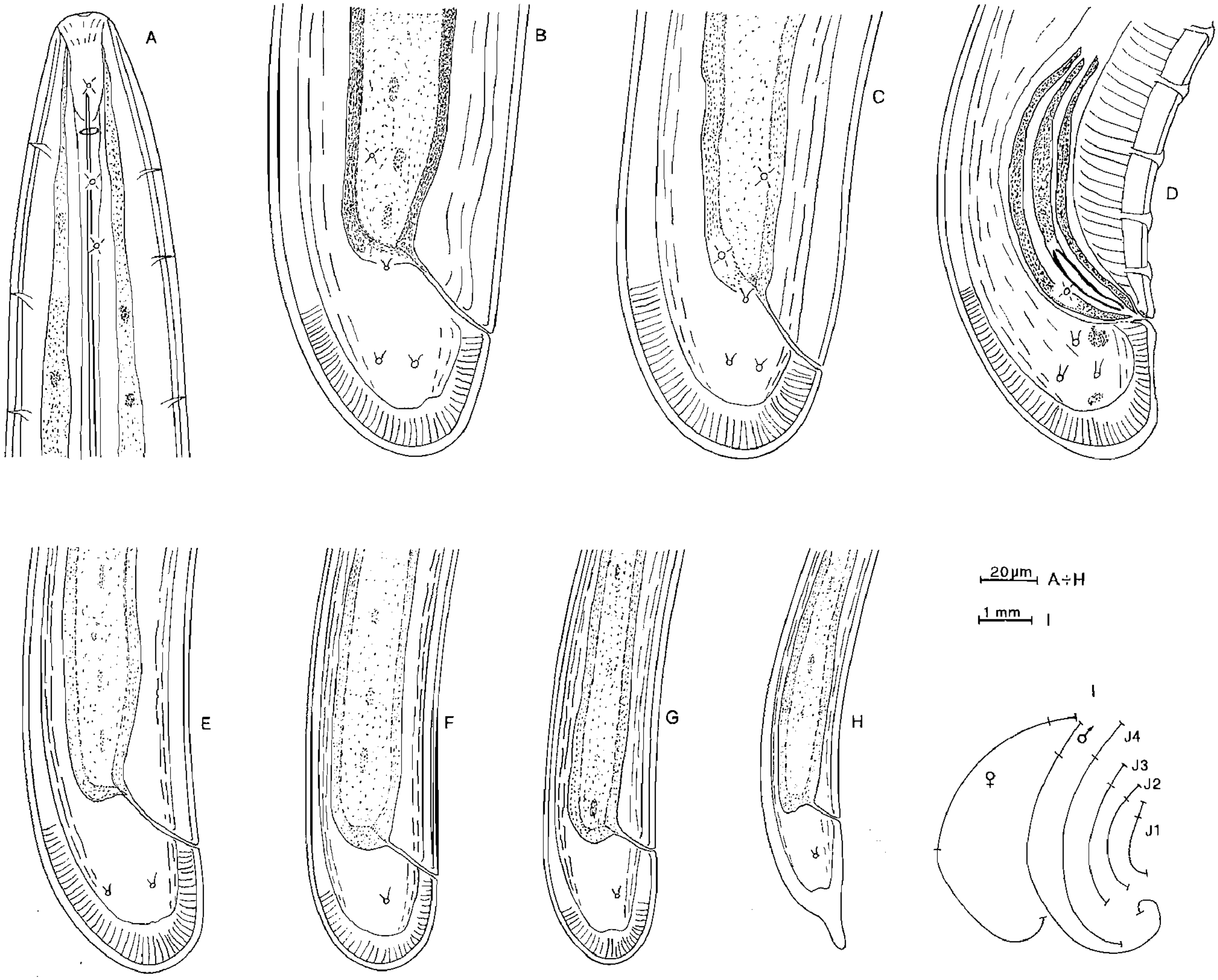

Fig. 15 - *Longidorus helveticus* sp.n.: A, female anterior region; B and C, female posterior region; D, male posterior region; E-H, fourth, third, second and first juvenile stages respectively; I, *habitus*.

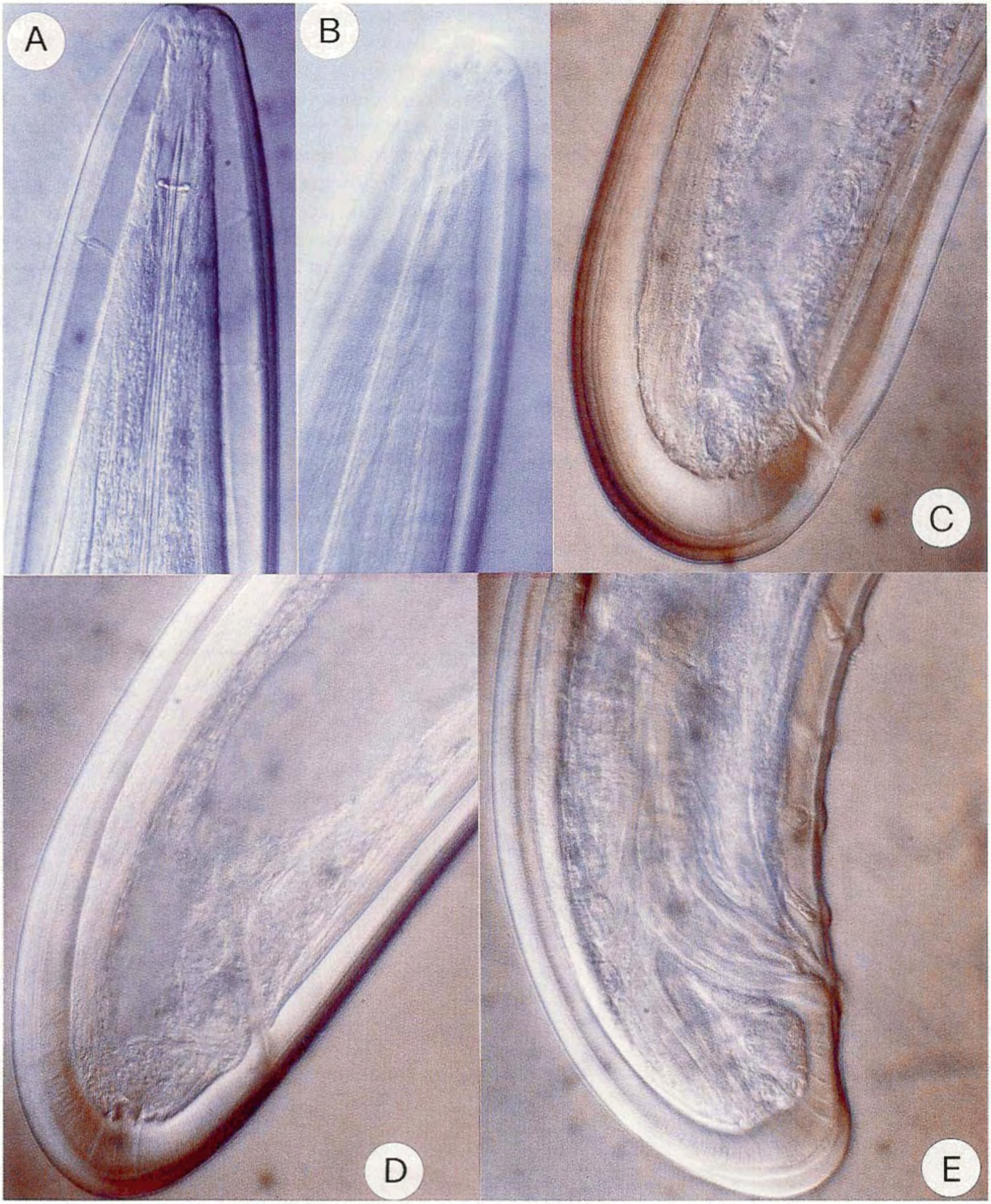

Fig. 16 - Photomicrographs of *L. helveticus* sp.n.: A and B, female anterior region; C and D, female posterior region; E, male posterior region.

Chen, 1999): A 5/6, B 4/5, C 3/4, D 1, E 4, F 3/4, G 1 H 1, I 2.

The SOD electrofocusing profile and the esterase stain of *L. helveticus* sp.n. is characterized by three typical major bands (Figs 20 and 21).

The PCR product of the ITS region of *L. helveticus* sp.n. was 2000 kb (Table VII) and it was digested by all the restriction enzymes tested, giving the pattern indicated in Fig. 19.

Type material: holotype female, 10 female, 10 male and juveniles paratypes in the collection of the Istituto di Nematologia Agraria del Consiglio Nazionale delle Ricerche, Bari, Italy; 10 female, 10 male and juveniles paratypes in the collection of the Swiss Federal Research Station, Wädenswil, Switzerland; 5 female and 5 male paratypes in the collection of the CABI Bioscience Centre, Egham, United Kingdom; 5 female and 5 male paratypes in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland, United States of America.

Discussion

From previous and more recent nematode surveys undertaken in Switzerland it appears that these six species of *Longidorus* are common and widespread in orchards and forests and often occur in mixed populations.

Some of them are more or less active vectors of plant viruses i.e. *L. elongatus, L. macrosoma* and *L. arthensis* (Taylor and Brown, 1997), or might cause damage to fruit trees e.g. *L. raskii, L. macrosoma, L. helveticus*. To help identifica-

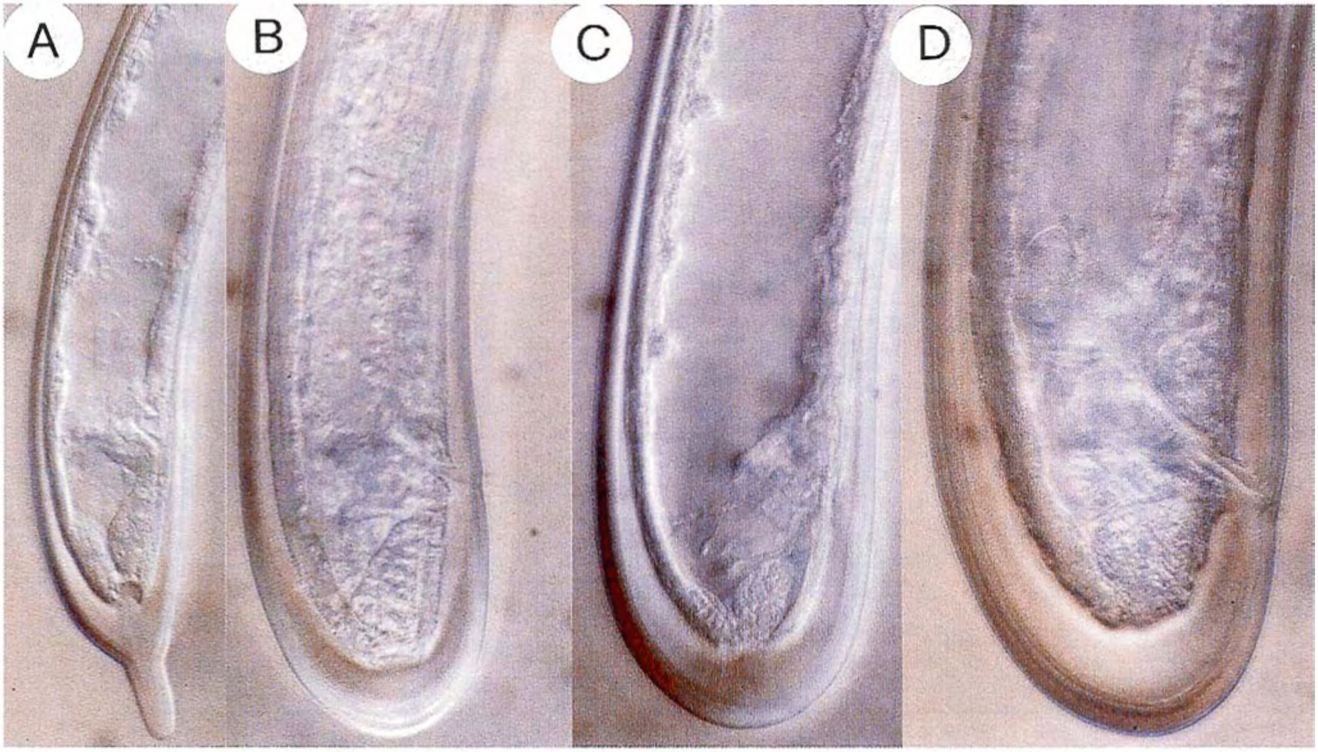

Fig. 17 - Photomicrographs of juveniles of *L. helveticus* sp. n. posterior region: A, first stage; B, second stage; C, third stage; D, fourth stage.

tion the codes from the polytomous key of Chen *et al.*, (1997) are compared:

	A	B	C	D	E	F	G	H	I
L. elongatus	3	2	2/3	3	2	3	2	2	1/2
L. macrosoma	5/6	4/5	3/4	3	4	5	2/3	1	2
L. profundor.	3/4	2	2/3	3	2	3/4	2/3	1/2	2
L. raskii	3/4	3	3	1	2	3/4	1/2	1	2
L. arthensis	3/4	3	3	1	2	3/4	2/3	1/2	2
L. helveticus	5/6	4/5	3/4	1	4	3/4	1	1	2

The main discriminants among them are: *L. elongatus* is the only species in which males are not common: they were never found during our survey; *L. elongatus* and *L. profundorum* have a diameter at lip region less than 16 µm (B2), but the former has cylindrical lip region, ca 90 µm long odontostyle and 1.2 c' ratio and the latter tapering lip region, ca 100 µm long odontostyle and 1.0 c' ratio; *L. helveticus* and *L. macrosoma* are the only two species with a non-lobed amphidial pouch (E4) and with an odontostyle longer than 120 µm (A 5/6); but the first has a body length of ca. 7.8 mm (F 3/4) and the second more than 9 mm (F5); finally, *L. arthensis* differs from *L. raskii* in its body length (6.5 vs 7.5 mm), c' value (1.0 vs 0.8) and vulva position (V = 50 vs 53).

L. arthensis, L. raskii, L. macrosoma, L. profundorum, L. elongatus, and *L. helveticus* are clearly distinguished by either IEF SOD isozyme profiles (Fig. 20) or n-PAGE esterase profile (Fig. 21). *L. arthensis* and *L. profundorum* show major analogies although they could be identified by a finer analysis. *L. raskii* displays electrophoresis profiles very different from the other five species tested, thus suggesting a more marked phylogenetical distance with respect to those species. *L. arthensis, L. macrosoma,* and *L. elongatus* are generally characterized by very active neutral SOD isoforms and faint basic

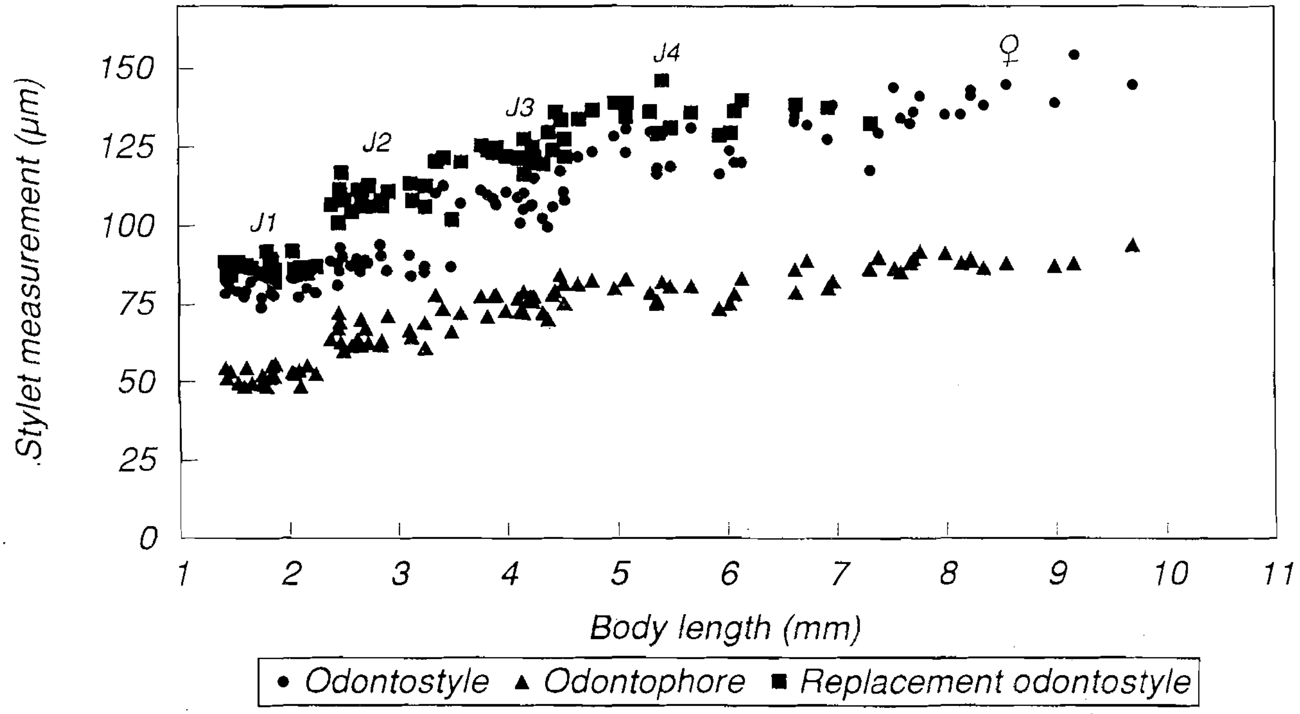

Fig. 18 - Scatter diagram plotting body and odontostyle length of individual juveniles and females of *L. helveticus* sp.n.

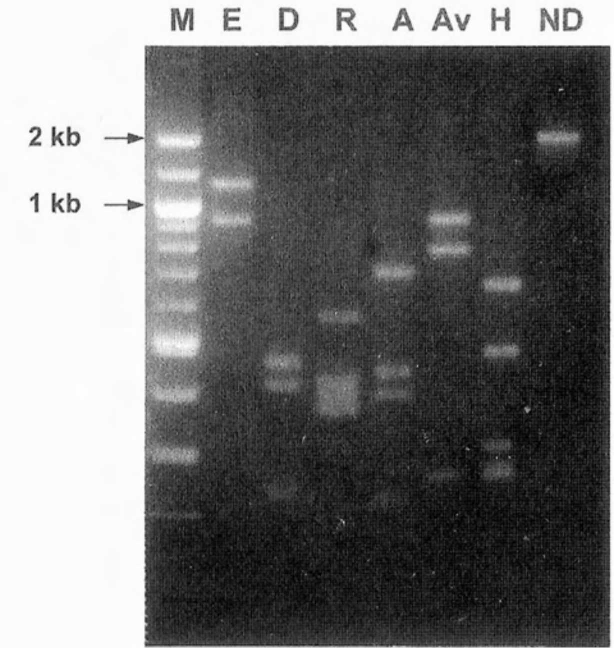

Fig. 19 - Patterns of the PCR amplified ITS region of *L. helveticus* sp.n. on 2.5% agarose gel digested with *Eco* RI (E), *Dde* I (D), *Rsa* I (R), *Alu* I (A), *Ava* II (Av) and *Hinf* I (H). The size in base pairs (bp) was estimated from 100 bp DNA ladder.

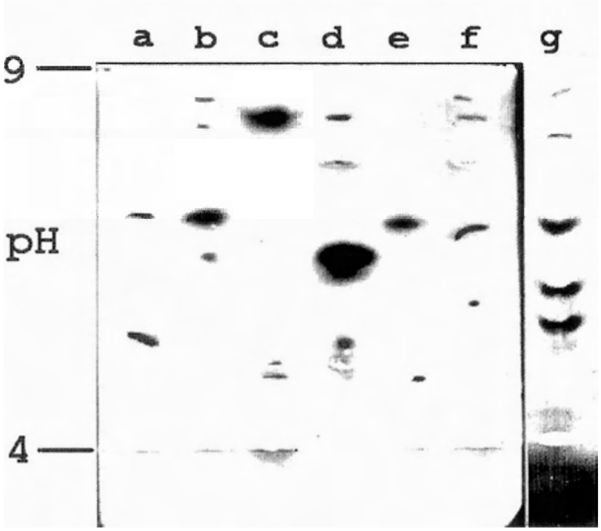

Fig. 20 - Isoelectrofocusing of *Longidorus* spp. extracts carried out by Phast System equipment. a) mixed population of *L. elongatus* and *L. helveticus*; b) *L. arthensis*; c) *L. raskii*; d) *L. macrosoma;* e) *L. profundorum*; f) *L. elongatus*; g) *L. helveticus* sp.n. Mini-gels (5x4.2 cm) were stained for SOD activity, then, gels were dried and scanned. Their digital images were turned into negatives and printed on high quality photo paper. SOD bands appear black over a white background.

bands, whilst *L. raskii* shows only a marked basic isoform. This latter species is specifically characterzied by consistent esterase isoforms with high molecular weight. The new species *L. helveticus*, is unequivocally identified by a typical three major band profile either by SOD or by esterase staining. A mixed population sample from Horgen shares bands of *L. elongatus* and *L. helveticus*.

The amplified fragments of the ITS region of the six *Longidorus* species indicate (Fig. 22) that their sizes are approximately 1.6 kb for *L.*

TABLE VII - *Estimated restriction fragment sizes (bp) of* PCR *amplified ITS of six species of* Longidorus *from Switzerland.*

Enzymes	*L. helveticus*	*L. profundorum*	*L. macrosoma*	*L. arthensis*	*L. elongatus*	*L. raskii*
ND	2000	1800	2000	1700	1600	1600
Alu I	720, 450, 400, 230	850, 880	1300, 350	750, 380, 320, 220, 180	450, 430, 380, 280	1000, 260, 150
Ava II	950, 800, 260	1350, 550	2000	1500, 250	950, 450	1600
Dde I	480, 430, 250	400, 300, 250, 220, 180, 150	530, 500, 420, 330	450, 390, 350, 180, 150	550, 430, 200, 180	490, 420, 200, 180
Eco RI	1100, 950	1500, 280	1200, 950	1700	1600	1100, 480
Rsa I	570, 440, 400, 380, 250	1200, 380, 260	750, 500, 380	870, 680, 320	650, 640, 250	620, 380, 320, 230
Hinf I	680, 500, 320, 290, 280	730, 560, 230, 180	900, 500, 280	570, 400, 280	660, 320, 230, 220	500, 380, 280, 180

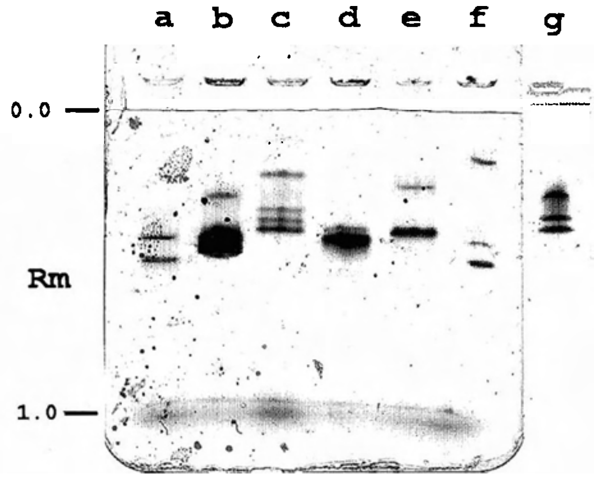

Fig. 21 - Native - PAGE of *Longidorus* spp. extracts carried out by Phast System equipment. a) mixed population of *L. elongatus* and *L. helveticus*; b) *L. arthensis*; c) *L. raskii*; d) *L. macrosoma*; e) *L. profundorum*; f) *L. elongatus*; g) *L. helveticus* sp.n. Mini-gels (5x4.2 cm) were stained for esterase activity, then dried and scanned. Esterase bands appear black on a white background.

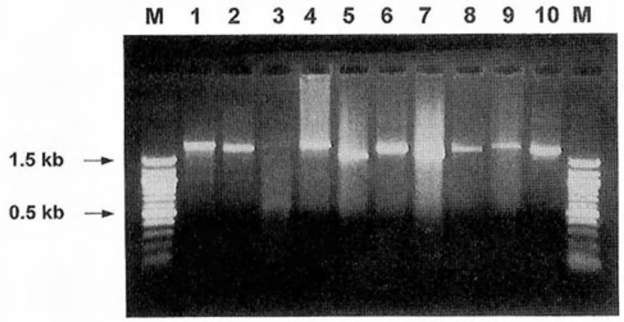

Fig. 22 - Agarose gel of amplified products obtained from *L. helveticus* (1), *L. profundorum* (2), *L. macrosoma* from Liestal (3), *L. macrosoma* from Horgen (4), *L. raskii* (5), *L. arthensis* (6), *L. elongatus* (7), *L. macrosoma* from Arth (8), *L. macrosoma* from Arth, Wiget (9) and *L. arthensis* (10) from Arth, Wiget DNAs. The size in base pairs (bp) was estimated from 100 bp DNA ladder.

raskii and *L. elongatus*, 1.7 kb for *L. arthensis*, 1.8 for *L. profundorum* and 2.0 kb for *L. macrosoma* and *L. helveticus*. However, *L. elongatus* and *L. raskii*; and *L. macrosoma* and *L. helveticus* are distinguished by specific differences in the restriction patterns (Table VII). There was no restriction site for *Eco*RI in *L. arthensis* and *L. elongatus* and for *Ava II* in *L. macrosoma* and *L. raskii*.

Specimens collected at Arth clearly indicated that they constituted a mixed population of *L. macrosoma* and *L. arthensis*.

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