

***Heterodera riparia* sp. n. (Tylenchida: Heteroderidae) from common nettle, *Urtica dioica* L., and rDNA-RFLP separation of species from the *H. humuli* group**

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Summary. *Heterodera riparia* sp. n. from roots of *Urtica dioica* L. is described based on materials collected from plants growing at the sides of rivers, ponds and lakes in Russia, Germany and Belgium. The new species is similar to *H. humuli*, but differs by its smaller average cyst size (415-468 μm vs 452-524 μm in *H. humuli*) and shorter average fenestra length (46-52 μm vs 56-61 μm). The second stage juveniles of *H. riparia* sp. n. have a lower average body length (350-373 μm vs generally >375 μm), a shorter tail (40-47 μm vs 50-57 μm) and a shorter hyaline part of tail (18-23 μm vs generally >27 μm). One generation of the nematode developed during the vegetative season. Restriction enzyme analysis of ribosomal DNA sequences was used to distinguish *H. riparia* sp. n. from the related species *H. humuli* and *H. fici*. The pattern of restriction bands obtained with *AluI* clearly distinguished all species from each other and the enzymes *CfoI* and *PstI* also distinguished the new species from the other species. The distribution of the new species in Europe is reported.

Key words: cyst nematodes, *Heterodera riparia* sp. n., *Heterodera humuli*, *Heterodera fici*, morphometrics, distribution, ITS, PCR, RFLP.

In 1982, a cyst-forming nematode was found on the roots of common nettle (*Urtica dioica* L.) growing in the Moscow region, Russia. This nematode was clearly different from *Heterodera urticae* Cooper, 1955 but similar to the hop cyst nematode *H. humuli* Filipjev, 1934. The different stages of this new nematode were described by Subbotin and Chizhov (1985) and further investigations revealed that it did not infect hop plants. Thus, this nematode was considered as a "nettle race" of the hop cyst nematode (Subbotin, 1986). Subsequent detailed morphological studies indicated that this nematode is representing a new species. Analysis of data published in the former Soviet Union and examination of permanent slides of cyst nematodes from several collections, and from our own survey, showed that the species is widely distributed throughout the European part of the former USSR. In Germany, a cyst nematode similar to *H. humuli* was recorded from *U. dioica* as early as 1976 and 1977 (Sturhan, 1976; Wouts & Weischer, 1977). Subsequently, this species

has been recovered from numerous sites throughout the country; it was also found in Belgium in 1997 during a survey of plants growing in riverbanks and woodlands.

Heterodera specimens collected from Russia, Germany and Belgium, are used to describe *Heterodera riparia* sp. n. The specific epithet indicates that the new species is commonly found associated with plants growing at the sides of rivers, ponds and lakes. Populations of the closely related species *H. humuli* and *H. fici* Kirjanova, 1954 are included in the comparative morphological, morphometric and molecular studies. The distribution of the new species in Europe is reported.

MATERIALS AND METHODS

Nematode populations. Populations of the new cyst nematode species collected from *Urtica dioica* growing at the following localities were used in our study:

- bank of the Jauza river, Mytishchi district, Moscow region, Russia (= type locality);
- bank of the stream, s. Lesnoi, Pushkin district, Moscow region, Russia;
- bank of Valdai lake, Novgorod region, Russia;
- bank of the Rybinsk reservoir, Borok, Jaroslavl region, Russia;
- bank of a brook at the experimental field of the Biologische Bundesanstalt, Münster, Germany, and preserved sampling material present in the German Nematode Collection at Münster originating from numerous other sites in Germany;
- bank of the Lesse river, near Han-sur-Lesse, Luxembourg province, Belgium;
- moist woodland near Knokke, West-Vlaanderen province, Belgium.

The following populations of *H. humuli* associated with *Humulus lupulus* L. were included:

- hop plantation near Cheboksary, Chuvashija, Russia;
- hop plantation near Tsvilsk, Chuvashija, Russia;
- hop plantation near Poperinge, West-Vlaanderen, Belgium;
- hop plants from an experimental plot, Institut für Nematologie und Wirbeltierkunde, Münster, Germany.

Additionally, slide material of *H. humuli* from several hop plantations in Bavaria and of *H. fici* from greenhouse cultures of *Ficus* spp. from Germany and of *Ficus carica* L. originating from Abkhazija were used for comparative morphological studies.

Cysts and females were isolated from soil and roots by a flotation and sieving methods. Second stage juveniles were isolated directly from cysts, or from soil samples, and males from nettle roots, using the Baermann funnel method.

Light microscopy. Juveniles, males and females were killed by gentle heat, fixed in TAF and mounted in anhydrous glycerol on permanent slides following Seinhorst's or a slow evaporation method. Cyst vulval cones were mounted in glycerine-gelatine. Specimens were examined and measured with JENAVAL and LEITZ Dialux light microscopes equipped with Nomarski optics. All measurements are presented as the mean and the standard error of the mean followed by the range in parenthesis.

Scanning electron microscopy. Cysts were used for examination of the vulval cones. Specimens were processed through critical point drying, coated with gold and examined with a Hitachi S 450 A scanning electron microscope at 15 kV.

DNA extraction. One or two cysts were hand-picked and placed in a 10 µl drop of double distilled water on a glass slide and crushed. Juveniles and eggs

were transferred into a sterile Eppendorf tube containing 8 µl worm lysis buffer (500 mM KCl, 100 mM Tris-Cl pH 8.3, 15 mM MgCl₂, 10 mM DTT, 4.5% Tween 20, 0.1% gelatin), homogenised and 2 µl of proteinase K (600 µg/ml) added. After freezing (-80 °C, at least 10 min) the tubes were incubated at 65 °C for 1 hour and then at 95 °C for 10 min.

PCR reaction. After centrifugation (1 min; 16 RCF) 10 µl of the DNA suspension were added to the PCR reaction mixture containing 10 µl 10X *Taq* incubation buffer with 25 mM MgCl₂ (Appligene, B & L Systems); 4 µl dNTP-mixture 5 mM each (Eurogentec), 1 µl (1.5 µM) of each primer (synthesised by Eurogentec), 0.8U *Taq* Polymerase (Appligene, B & L Systems) and double distilled water added to a final volume of 100 µl. Primers AB 28 and TW 81, as described by Joyce *et al.* (1994), were used in the PCR reaction. Amplification was carried out in a GeneE New Brunswick Scientific DNA thermal cycler. PCR amplification conditions were: denaturation at 94 °C for 1 min, annealing at 62 °C for 1.5 min, and extension at 72 °C for 2 min, repeated for 35 cycles. A 5 min incubation period at 72 °C followed the last cycle to complete any partially synthesised strands. After DNA amplification, 5 µl of the product were analysed on 1% agarose gel.

RFLP. Seven µl of each PCR-product were digested with one of the following twelve restriction enzymes: *AluI*, *BsuRI*, *Bsh1236I*, *Bsp143I*, *CfoI*, *HindIII*, *HinfI*, *MspI*, *MvaI*, *PstI*, *RsaI*, and *TaqI* in the corresponding buffer according to the manufacturer's instructions. The digested DNA was loaded on a 1.5% agarose gel, separated by electrophoresis, stained with ethidium bromide, visualised and photographed under UV light. Procedures for obtaining PCR amplified products and restriction endonuclease digestion of PCR products were repeated several times for consistency of results.

DESCRIPTION

Heterodera riparia sp. n. (Figs. 1-4 & Tables 1 & 2)

Holotype cyst: L (excluding neck) = 469 µm, width = 296 µm.

Paratype cysts: see Table 1.

Paratype females (n=10): L (including neck) = 491±24.0 (407-675) µm; width = 283±16.5 (225-425) µm; length of neck = 97±8.3 (60-150) µm; length of stylet = 25.4±0.5 (23.0-27.5) µm; distance of opening of dorsal oesophageal gland behind stylet base = 4.3±0.3 (3.0-5.0) µm; distance of median bulb from anterior end = 71±5.6 (40-98) µm; length of median bulb = 31.5±1.7 (25.0-35.0) µm; width of median bulb = 27.8±1.7 (22.5-32.5) µm.

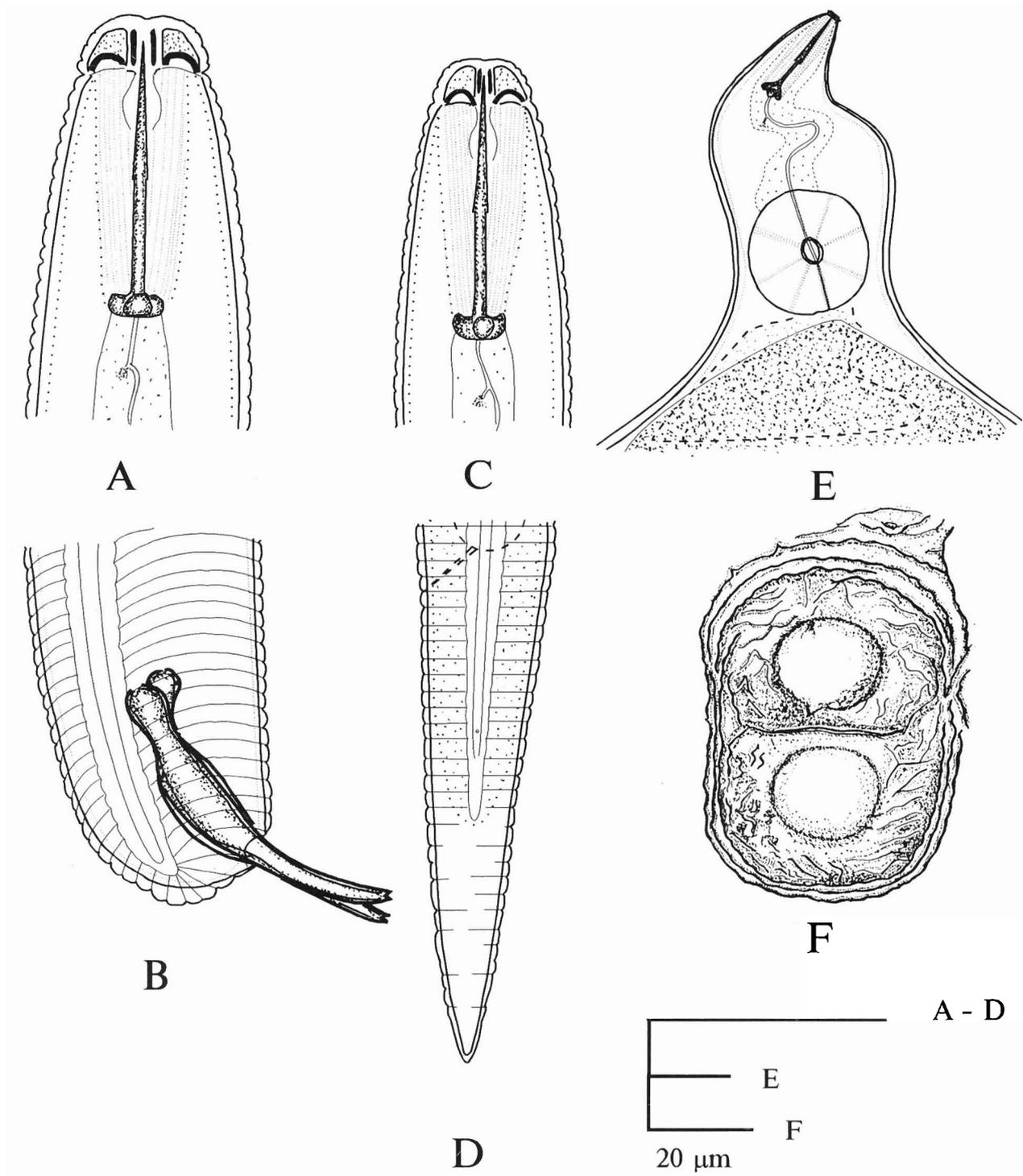


Fig. 1. *Heterodera riparia* sp. n. (paratypes). A: Head, male; B: Tail, male; C: Head, juvenile; D: Tail, juvenile; E: Anterior end of female; F: Vulval plate.

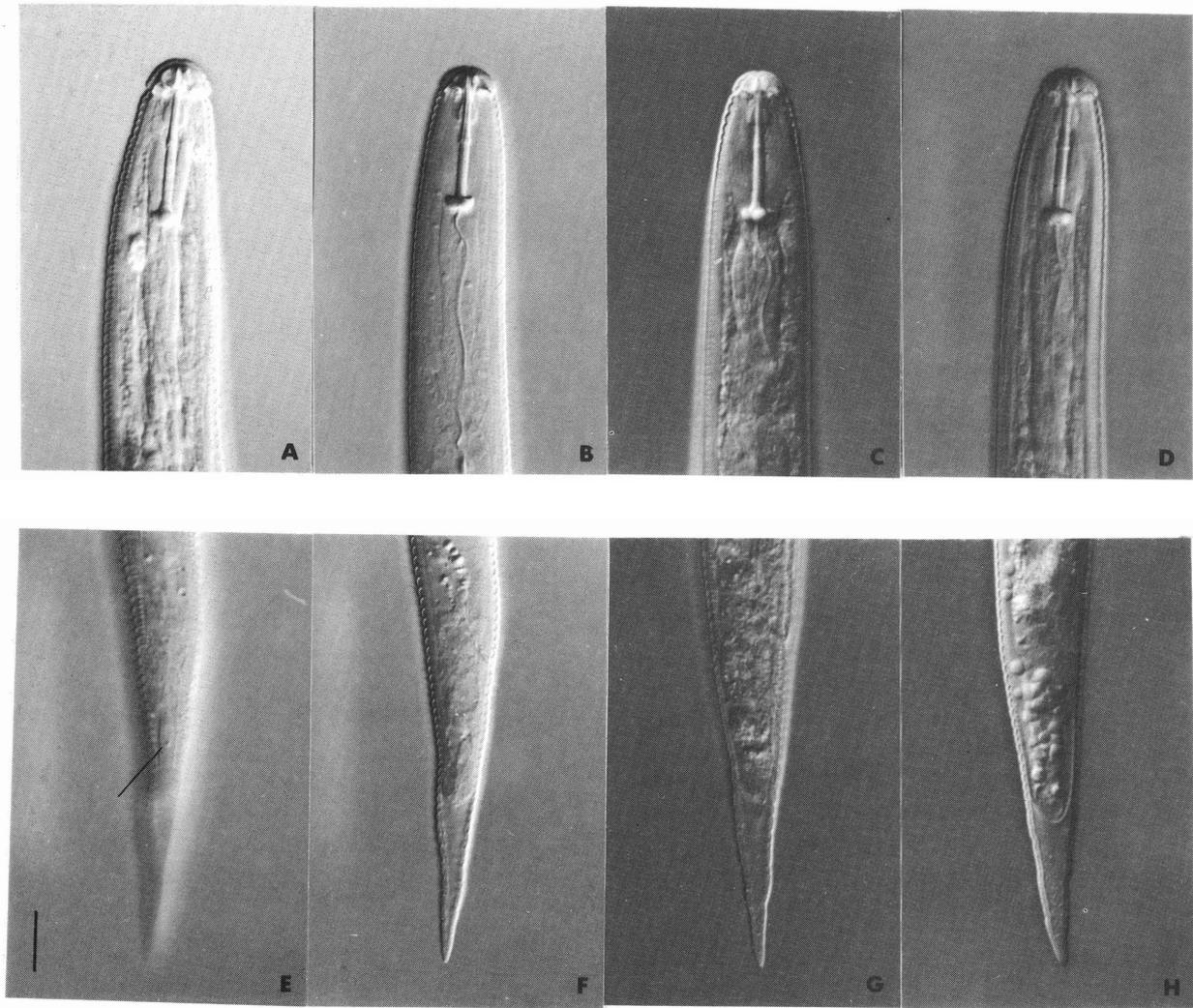


Fig. 2. *Heterodera riparia* sp. n. (paratypes). A: Anterior end of male; B: Anterior end of second stage juvenile; E: Posterior end of second stage juvenile, showing position of phasid (arrowed); F: The same specimen, at median level; *H. humuli* (Bavaria, Germany); C & G: Anterior and posterior end of second stage juvenile; *H. fici* (Münster, Germany). D & H: Anterior and posterior end of second stage juvenile. Scale bar - 10 μ m.

Paratype males (n=21): L = 825 ± 12.2 (723-940) μ m; height of lip region = 5.9 ± 0.1 (4.8-6.4) μ m; width of lip region = 9.7 ± 0.1 (8.5-10.7) μ m; length of stylet = 23.7 ± 0.3 (22.5-26.5) μ m; height of stylet knobs = 2.7 ± 0.1 (2.0-3.2) μ m; width of stylet base = 4.4 ± 0.1 (3.8-4.8) μ m; distance of opening of dorsal oesophageal gland from stylet base = 4.5 ± 0.1 (3.8-5.1) μ m; distance of median bulb from anterior end = 82 ± 0.9 (76-92) μ m; length of oesophagus (from anterior end to oesophago-intestinal junction) = 125 ± 1.5 (110-137) μ m; distance of excretory pore from anterior end = 137 ± 2.1 (126-160) μ m; length of genital tract (n=4) = 423 ± 32.7 (330-474) μ m; length of spicules (n=13) = 31.1 ± 0.4 (27.4-33.8) μ m; a = 32.4 ± 0.5 (29.9-36.5); b = 6.4 ± 0.2 (5.7-7.5).

Paratype juveniles: see Table 2.

Paratype eggs (n=30): L = 90 ± 0.5 (84-96) μ m; width = 37 ± 0.4 (33-41) μ m; length/width ratio = 2.4 ± 0.03 (2.1-2.4).

Cysts. Lemon shaped, with distinct and rather wide vulval cone, colour varying from yellow to pale brown, darkening with age (contents usually visible through cyst wall). Cyst wall with ridges forming an irregular zig-zag pattern. Young cysts covered by subcrystalline layer and with a small empty egg-sac. Neck distinct, often at an angle to body axis. Vulval cone bifenestrate, vulval bridge mostly broad (in particular, in recently developed cysts), semifenestrate circular or subcircular, vulva set in a transverse groove (Figs. 1F & 4). Bullae absent (small bullae-

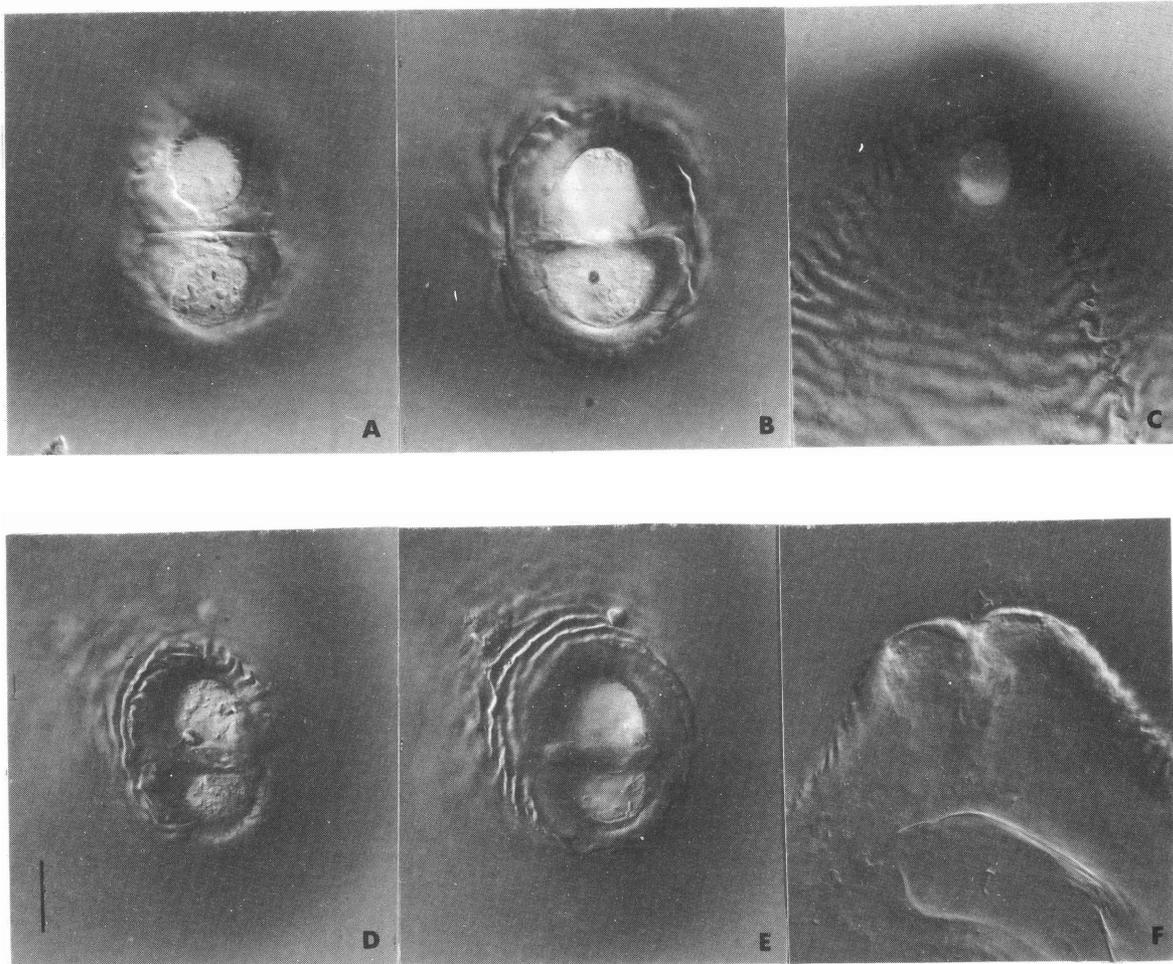


Fig. 3. *Heterodera riparia* sp. n. A: Fenestration in vulval cone, with vulval groove; B: The same specimen at lower level (Pulling, Bavaria, Germany); C: Semifenestra and vulval ridge (Münster, Germany); D: Fenestrae in upper view; E: The same specimen, at lower level (type population); F: Vulval cone in lateral view (Münster, Germany). Scale bar - 20 μ m.

like structures only occasionally present), under-bridge weak, slightly pigmented (Fig. 3). Recently formed cysts contained 40 to 255 eggs.

Females. Swollen, lemon shaped. Cuticle colourless or pale yellow. Cephalic region with wide anterior lip annule and distinct labial disc. Stylet slender, with backwardly raked basal knobs. Median bulb massive, with prominent valve. Excretory pore indistinct (Fig. 1E).

Males. General morphology typical for genus. Body twisted about longitudinal axis. Lip region dome-shaped, distinctly set-off, with 3-4 (rarely 5) lip annules (often interrupted) and a labial disc. Stylet strong, with rather small knobs flattened or slightly sloping anteriorly. Median bulb slender oval, valve posterior to centre. Hemizonid about 3 μ m long (= 2 body annules), situated several body annules (2-12

μ m) anterior to the excretory pore which is located posterior to the oesophago-intestinal junction. Lateral field with 4 lines at more or less even distances, outer bands usually distinctly areolated. Annules at mid-body 1.8-2.1 μ m wide. Spicules ventrally curved, with distinctly bidentate tips. Gubernaculum simple, almost straight, 9-9.5 μ m long. Tail short (Figs. 1A, B & 2A).

Second stage juveniles. Body slightly curved ventrally. Lip region rounded, about twice as wide as high, set-off from rest of body, with 3-4 lip annules and a labial disc. Stylet strong, knobs rather wide and slightly projecting anteriorly; height of stylet knobs = 2.3 ± 0.1 (2.1-2.8) μ m, width of stylet base = 4.1 ± 0.04 (4.0-4.3) μ m. Median bulb oval; oesophageal glands well developed. Excretory pore slightly anterior to level of oesophago-intestinal junction, immediately

Table 1. Morphometrics of cysts of *Heterodera riparia* sp. n. and *H. humuli* populations (measurements in $\mu\text{m} \pm$ standard error).

Population	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Jauza river Moscow region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Valdai Novgorod region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Borok Jaroslavl region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Münster Germany	<i>H. humuli</i> <i>Humulus lupulus</i> Cheboksary Chuvashija Russia	<i>H. humuli</i> <i>Humulus lupulus</i> Tsivilsk Chuvashija Russia	<i>H. humuli</i> <i>Humulus lupulus</i> Münster Germany
Cysts	paratypes						
n	50	10	23	25	29	25	30
Length (excluding neck)	462 \pm 7.9 (348-580)	468 \pm 20.5 (344-560)	427 \pm 8.9 (336-496)	415 \pm 9.6 (289-497)	515 \pm 15.3 (336-664)	463 \pm 11.2 (360-576)	524 \pm 12.0 (338-632)
Width	327 \pm 9.0 (212-454)	348 \pm 16.1 (248-400)	324 \pm 9.8 (240-416)	307 \pm 9.4 (214-383)	367 \pm 11.4 (248-504)	336 \pm 10.9 (240-472)	367 \pm 12.8 (214-498)
Length/width	1.4 \pm 0.03 (1.1-1.8)	1.4 \pm 0.04 (1.1-1.6)	1.3 \pm 0.03 (1.1-1.7)	1.4 \pm 0.02 (1.1-1.7)	1.4 \pm 0.02 (1.2-1.7)	1.4 \pm 0.02 (1.1-1.6)	1.5 \pm 0.04 (1.2-2.1)
Vulval areas							
n	35	8	7	15	20	8	10
Fenestra length	46 \pm 0.8 (31-58)	52 \pm 1.6 (45-58)	49 \pm 1.8 (45-58)	46 \pm 1.5 (38-56)	58 \pm 1.5 (48-70)	56 \pm 2.1 (45-65)	57 \pm 1.4 (48-65)
Mean semifenestral width	26.3 \pm 0.7 (16.6-38.2)	25.0 \pm 0.7 (22.5-27.5)	26.4 \pm 0.7 (25.0-30.0)	24.8 \pm 0.9 (18.0-31.7)	25.9 \pm 0.5 (22.5-30.0)	26.3 \pm 0.8 (22.5-30.0)	26.9 \pm 0.9 (22.5-30.0)
Vulval slit length	33.8 \pm 0.6 (28.2-41.5)	33.9 \pm 1.2 (27.5-37.5)	36.3 \pm 0.8 (33.8-40.0)	33 \pm 0.9 (26-41)	36.2 \pm 0.5 (32.5-40.0)	39.1 \pm 0.5 (37.5-40.0)	35.8 \pm 0.7 (30.0-37.5)
Vulval bridge width	10.7 \pm 0.9 (6.6-18.2)	10.5 \pm 0.7 (7.5-12.5)	10.5 \pm 0.9 (8.8-15.0)	9.0 \pm 0.5 (6-14)	12.2 \pm 0.5 (8.8-17.5)	12.2 \pm 0.7 (10.0-15.0)	10.5 \pm 0.3 (8.8-12.5)
Underbridge length	78 \pm 2.3 (70-88)	76 \pm 1.3 (75-77)	85 \pm 2.5 (83-88)	69 \pm 2.2 (50-90)	88 \pm 2.1 (75-100)	83 \pm 2.7 (75-90)	88 \pm 1.6 (83-93)
Vulva-anus distance	47 \pm 1.4 (36-63)	49 \pm 1.9 (42-58)	53 \pm 2.3 (45-60)	43 \pm 3.9 (24-75)	46 \pm 1.1 (40-58)	53 \pm 1.6 (48-60)	50 \pm 1.5 (43-60)

Table 2. Morphometrics of second stage juveniles of *Heterodera riparia* sp. n. and *H. humuli* populations (measurements in $\mu\text{m} \pm$ standard error).

Population	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Jauza river Moscow region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Valdai Novgorod region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Borok Jaroslavl region Russia	<i>H. riparia</i> sp. n. <i>Urtica dioica</i> Münster Germany	<i>H. humuli</i> <i>Humulus lupulus</i> Cheboksary Chuvashija Russia	<i>H. humuli</i> <i>Humulus lupulus</i> Tsivilsk Chuvashija Russia	<i>H. humuli</i> <i>Humulus lupulus</i> Münster Germany
Juveniles	paratypes						
n	52	12	11	25	20	20	20
Body length (L)	373±2.1 (342-407)	350±2.8 (335-368)	368±6.7 (339-406)	359±2.3 (336-381)	375±4.7 (339-408)	406±3.4 (374-436)	422±7.2 (368-468)
a	20.7±0.1 (18.7-22.8)	19.1±0.1 (18.3-20.1)	20.2±0.4 (18.4-22.4)	21.6±0.1 (19.7-22.6)	20.9±0.2 (19.3-22.8)	21.8±0.2 (20.2-24.5)	20.8±0.3 (16.4-22.5)
b	3.3±0.03 (2.9-4.0)	3.2±0.1 (3.0-3.4)	3.2±0.1 (2.9-3.5)	3.7±0.04 (3.5-4.0)	3.5±0.1 (2.9-4.1)	3.5±0.1 (3.2-3.9)	3.7±0.1 (3.3-4.1)
c	8.0±0.1 (7.4-10.0)	8.7±0.1 (8.3-9.5)	8.2±0.1 (7.9-8.7)	8.1±0.1 (7.1-8.9)	7.5±0.1 (6.5-8.2)	7.7±0.1 (7.2-8.4)	7.4±0.1 (6.6-7.9)
Stylet length	21.7±0.1 (20.3-23.5)	21.8±0.1 (21.4-22.4)	23.4±0.1 (22.4-24.5)	22.1±0.1 (20.8-23.8)	23.1±0.1 (22.4-24.0)	23.2±0.2 (21.4-24.5)	24.5±0.2 (23.5-25.5)
Lip region height	4.1±0.03 (3.8-4.3)	4.2±0.1 (4.1-4.6)	4.0±0.04 (3.8-4.3)	4.1±0.04 (3.8-4.5)	4.0±0.02 (3.8-4.2)	4.2±0.1 (4.1-4.6)	4.2±0.04 (4.1-4.8)
Lip region width	9.2±0.03 (8.7-9.4)	9.1±0.1 (8.5-9.3)	9.0±0.1 (8.7-9.2)	8.5±0.1 (8.0-8.9)	9.1±0.1 (8.5-9.3)	9.1±0.1 (8.7-9.7)	9.4±0.1 (9.2-10.2)
DGO	4.8±0.1 (4.1-5.7)	4.0±0.1 (3.8-4.2)	4.9±0.4 (3.9-4.1)	4.1±0.1 (4.0-5.6)	4.1±0.1 (3.6-4.6)	4.1±0.1 (3.9-4.6)	4.9±0.1 (4.1-6.1)
Anterior end to excretory pore	95±0.6 (84-102)	94±0.7 (91-98)	88±2.1 (80-95)	92±0.6 (85-98)	91±0.9 (84-97)	98±1.1 (92-106)	96±1.2 (86-104)
Anterior end to valve of median bulb (MB)	67±0.4 (61-77)	60±0.4 (57-62)	66±1.3 (59-72)	65±0.6 (60-70)	65±0.9 (57-70)	72±0.9 (65-79)	67±0.8 (60-77)
Oesophagus length	114±0.9 (97-124)	110±1.7 (100-117)	115±2.2 (102-122)	96±0.9 (90-104)	107±1.5 (97-120)	116±1.8 (102-128)	113±1.6 (97-122)
Body width at:							
mid-body	18.0±0.1 (16.3-20.1)	18.3±0.1 (17.3-18.9)	18.3±0.2 (17.3-19.4)	16.7±0.1 (15.2-18.4)	18.0±0.2 (16.3-19.4)	18.7±0.1 (17.3-19.4)	20.3±0.3 (18.4-22.4)
anus (BWA)	11.2±0.1 (9.9-12.8)	11.6±0.1 (11.2-12.2)	12.6±0.2 (11.7-13.3)	10.7±0.1 (10.0-11.6)	11.5±0.1 (11.2-12.2)	11.9±0.1 (11.2-12.8)	12.1±0.1 (11.2-13.3)
Hyaline part of tail length (H)	22.9±0.3 (18.1-27.5)	18.8±0.5 (16.3-20.4)	19.9±0.8 (16.3-23.5)	23.0±0.3 (18.4-25.6)	28.8±0.4 (25.5-31.6)	28.5±0.7 (23.5-35.7)	33.5±1.0 (26.5-43.9)
Tail length	47±0.4 (36-50)	40±0.5 (38-43)	44±1.0 (40-50)	45±0.6 (41-52)	50±0.6 (43-54)	53±0.8 (47-58)	57±1.0 (51-65)
Tail length/BWA	4.1±0.04 (3.4-4.5)	3.5±0.1 (3.2-3.8)	3.5±0.1 (3.3-3.8)	4.2±0.1 (3.8-5.0)	4.4±0.1 (3.8-4.7)	4.4±0.1 (3.8-5.2)	4.7±0.1 (4.4-5.4)
H/stylet length	1.1±0.02 (0.8-1.3)	0.9±0.02 (0.7-1.0)	0.9±0.04 (0.7-1.0)	1.0±0.01 (0.8-1.2)	1.2±0.01 (1.1-1.4)	1.2±0.03 (1.0-1.6)	1.4±0.04 (1.1-1.7)
L/MB	5.5±0.03 (5.0-6.1)	5.8±0.1 (5.5-6.1)	5.6±0.1 (4.9-6.4)	5.6±0.04 (5.3-6.1)	5.7±0.04 (5.5-6.3)	5.7±0.1 (5.2-6.0)	6.3±0.1 (5.4-7.0)

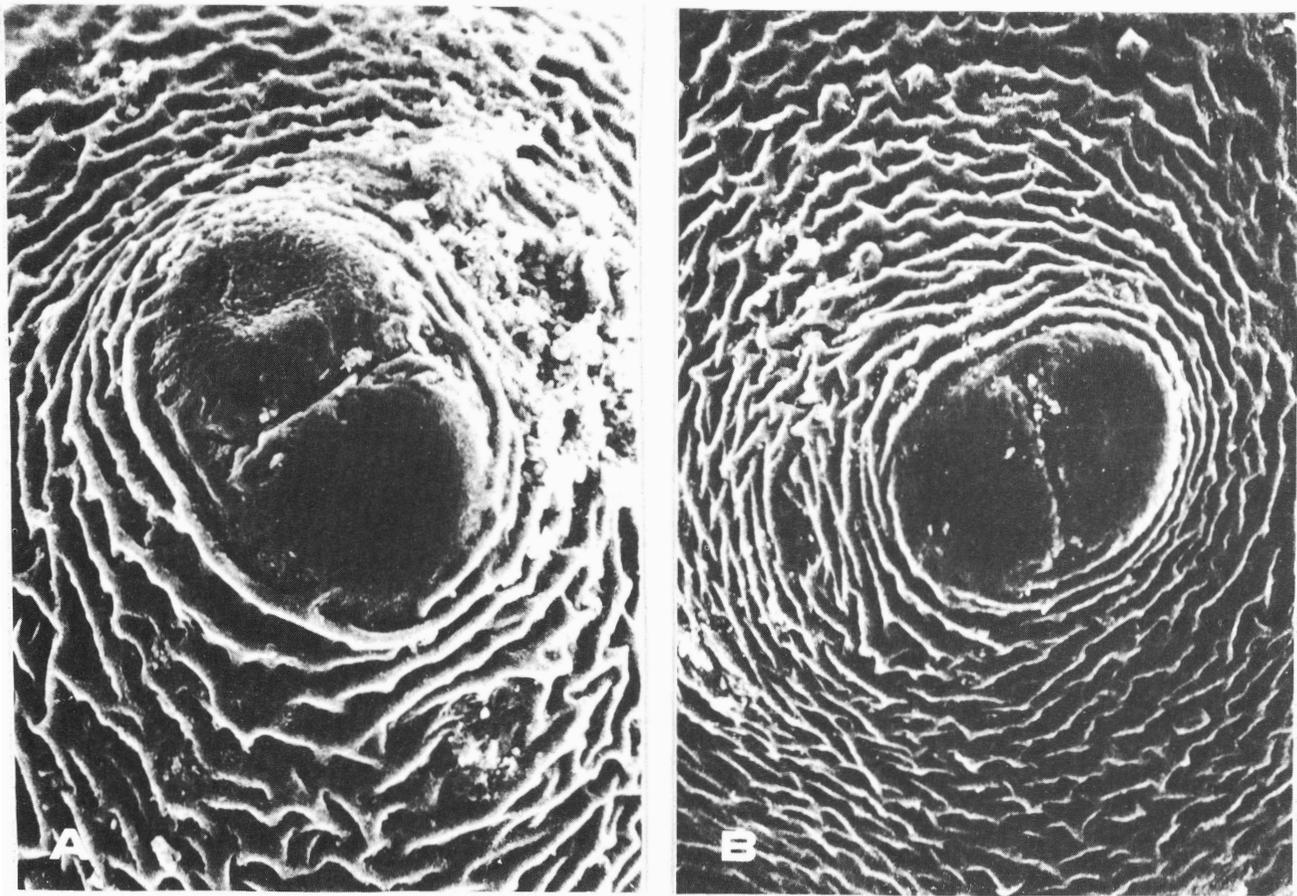


Fig. 4. *Heterodera riparia* sp. n. (paratypes). A, B: SEM of vulval cone.

posterior to hemizonid, which is almost two body annules long. Lateral field with 4 lines, arranged at even distances; outer bands only occasionally areolated. Annules at mid-body 1.5-1.7 μm wide. Genital primordium mostly oval and with 4 nuclei. Phasmids small but distinct, situated 7-10 body annules posterior to anus, frequently located on ventral side of the lateral field. Tail conical, with finely rounded terminus, hyaline terminal portion usually occupying less than half of tail length, limit of pseudocoelom in tail rounded and centrally situated (Figs. 1C, D & 2B, E, F).

Type host and locality. Soil samples and roots of *Urtica dioica* L. growing on the bank of the Jauza river in the Mytishchi district, Moscow region, Russia. In a glasshouse test done in Moscow with two different populations of *H. riparia* sp. n. from Kabardino-Balkarija and the Moscow region, white females and cysts did not develop on the roots of hemp (*Cannabis sativa* L.) and hop (*Humulus lupulus* L.) (Subbotin, 1986). Danilova (1996) also confirmed these results with a Tsvilsk population of this species.

Other localities. *Heterodera riparia* sp. n. is widely distributed along banks of rivers, streams and lakes in the Moscow region. It was also found in other regions of Russia (Novgorod, Kalinin, Sverdlovsk, Kostroma, Penza, Jaroslavl, Leningrad, Vologda regions, Kabardino-Balkarija, Chuvashija), in Estonia, Latvia, Armenia, Moldova, Ukraine, Bulgaria (Poghossian, 1962; Kirjanova & Krall, 1971; Kirjanova & Lobanova, 1972; Nikitin, 1972, 1981; Subbotin & Chizhov, 1985; Subbotin, 1986) and the Slovak Republic (Lišková, unpublished data). In Germany the new species has been recorded from 38 localities throughout the country, mainly from banks of rivers, brooks or ponds or from moist woodland. Only three records are from meadows and other grassland biotopes with *Urtica dioica* stands not in vicinity of rivers or lakes. In Belgium *H. riparia* sp. n. was found in six soil samples collected from banks of rivers or streams and moist woodland from different provinces (Fig. 9).

Type material. Holotype cyst collected by S.A. Subbotin, September 1996. Holotype slide deposited in the Nematode Collection of the Institute of

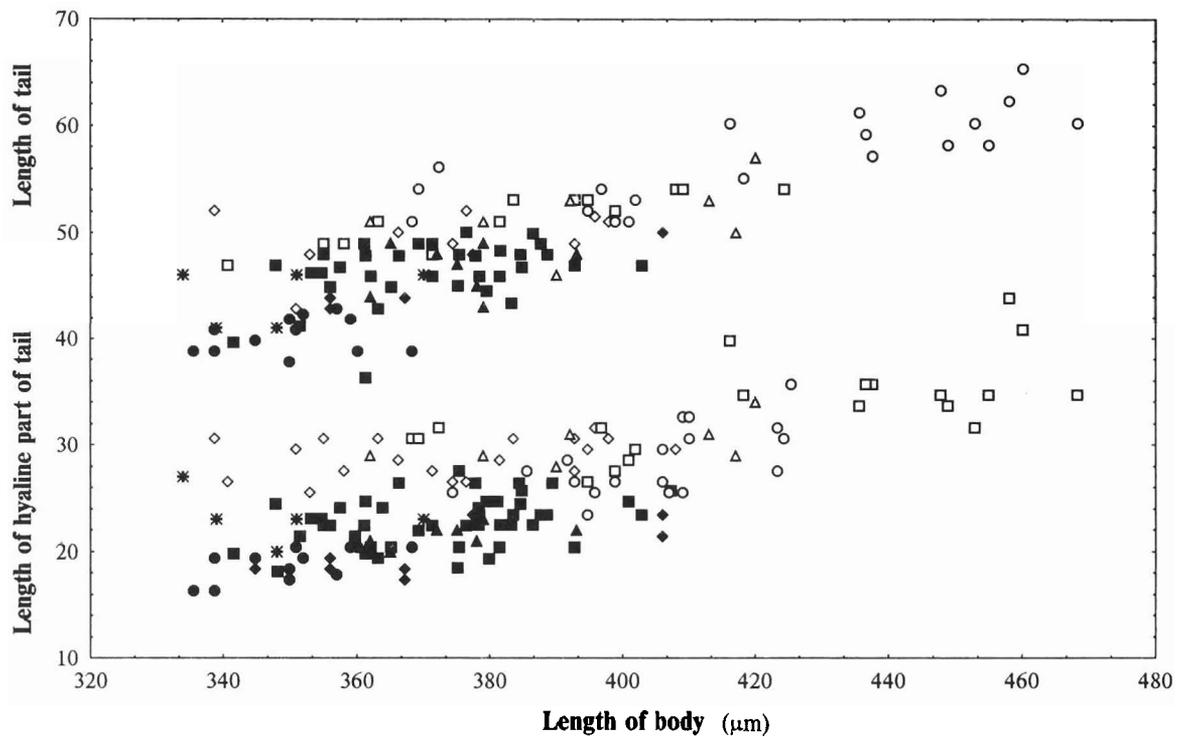


Fig. 5. Scatter diagram of body length and tail length or length of hyaline part of tail of different populations of *H. riparia* sp. n. and *H. humuli*. *Heterodera riparia* sp. n.: ◆ - Borok, Russia; ● - Valdai, Russia; ■ - Mytishchi, Russia; * - Münster, Germany; ▲ - Han-sur-Lesse, Belgium; *H. humuli*: ◇ - Cheboksary, Russia; ○ - Tsvilsk, Russia; □ - Münster, Germany; △ - Popering, Belgium.

Parasitology of the Russian Academy of Sciences, Moscow, Russia. Paratypes of cysts, males and juveniles distributed as follows: German Nematode Collection, Münster, Germany; Nematode collection of the Zoological Institute of the RAS, St. Petersburg, Russia; and Rothamsted Experimental Station, England.

Differential diagnosis. *Heterodera riparia* sp. n. is similar to the hop cyst nematode *H. humuli* and can be considered as a sibling species. It differs from *H. humuli* by its smaller average cyst size (415-468 µm vs 452-524 µm in *H. humuli*) and shorter average fenestra length (46-52 µm vs 56-61 µm). The second stage juveniles of *H. riparia* sp. n. have a smaller average body length (350-373 µm vs >375 µm), a shorter tail (40-47 µm vs 50-57 µm) and a shorter hyaline part of tail (19-23 µm vs >27 µm). The hyaline tail end is less than 50 % of the tail length in *H. riparia* sp. n. and more than 50 % in *H. humuli* (Figs. 2D, E & 5). While *H. riparia* sp. n. second stage juveniles have 3 lip annules (+ labial plate), usually only 2 are present in *H. humuli* juveniles (Wouts & Weischer, 1977; own observations). The new species is also distinguished by reproducing on *U. dioica*, which is not a good host for *H. humuli*.

Heterodera riparia sp. n. is also similar to the fig cyst nematode *H. fici*, but cysts of this species are considered ambifenestrated, rather than bifenestrated (Mulvey, 1972; Golden *et al.*, 1988) and bullae are mostly present. Also, the fenestrae are generally slightly longer and wider in *H. fici* than in *H. riparia* sp. n. (45-68 µm and 35-37 µm: Mulvey, 1972) and the vulval slit longer (35-60 µm: Mulvey, 1972). In second stage juveniles, the tail and hyaline tail end are only slightly longer (Fig. 2C & G) than in *H. riparia* sp. n. (average values: 380-419 µm, 45-54 µm and 22.0-29.3 µm, respectively: Wouts & Weischer, 1977; Golden *et al.*, 1988; Subbotin *et al.*, 1989; Shahina & Maqbool, 1995). From personal observations and published data, the body diameter of *H. fici* second stage juveniles is not smaller than in, e.g., *H. humuli*, as stated by Wouts and Weischer (1977). While these authors give 1 (-2) lip annules for *H. fici* second stage juveniles and Golden *et al.* (1988) 3 or 4 lip annules, we observed 2 (+ labial plate) in specimens of different origin (as compared with 3 lip annules + labial plate in *H. riparia* sp. n.). In *H. riparia* sp. n. the male tail in lateral position shows projecting ridges of the lateral field, similar to those described as a distinguishing feature for *H. fici* (Golden *et al.*, 1988). *Heterodera fici* is not known

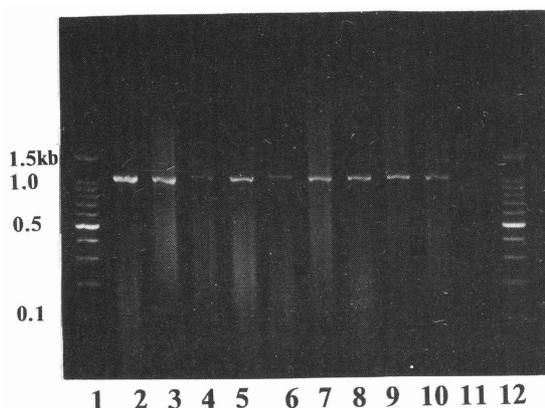


Fig. 6. Amplified Internal Transcribed Spacers of *Heterodera riparia* sp. n., *H. humuli* and *H. fici*. Lanes 1 and 12: 100 bp DNA ladder; Lanes 2-6, *H. riparia* sp. n. 2: Mytishchi, Russia, 3: Lesnoi, Russia, 4: Münster, Germany, 5: Knokke, Belgium, 6: Han-sur-Lesse, Belgium; Lanes 7-9, *H. humuli*, 7: Poperinge, Belgium, 8: Tsvil'sk, Russia, 9: Münster, Germany; Lane 10: *H. fici*, Abkhazija; Lane 11: Control without DNA.

to reproduce on *H. lupulus* or *Urtica* species.

The new species can be readily distinguished from *H. urticae*, which also infects nettles, as the cyst of *H. urticae* has fenestration of the ambifenestrated type and the second stage juvenile has a longer body, stylet, tail and hyaline tail end (average values: 541 μm , 27 μm , 58 μm and 29 μm , respectively: Matthews, 1971).

Biology. In the Moscow region infective second stage juveniles of *H. riparia* sp. n. invaded nettle roots from early May up to the beginning of June. Third stage juveniles were observed in the roots from the middle of May with the fourth stage juveniles and young females developing about one week later. Males developed in the same time, and were found in the soil up to end of July. The first mature females with eggs were observed on nettle roots in early June. Only cysts with juveniles were found at the end of October. Only one generation of the nematode developed during the vegetative season in the Moscow region (Subbotin & Chizhov, 1985).

PCR amplification and RFLP analysis. The amplification of the ITS regions of each population of the three species gave one fragment of approximately 1.1 kb. PCR products were not obtained in the negative control lacking DNA template (Fig. 6). The RFLP pattern obtained with *AluI* clearly distinguished the species *H. riparia* sp. n., *H. humuli* and *H. fici* from each other (Fig. 7A). The enzymes *CfoI* (Fig. 7B) and *PstI* (Fig. 7C) distinguished the new

species from the other two. *BsuRI*, *MspI*, *MvaI* (Fig. 7D-F) and *RsaI* (Fig. 8A) distinguished *H. riparia* sp. n. and *H. humuli* from *H. fici*. Three enzymes: *Bsh1236I*, *Bsp143I* (Fig. 8B & C) and *TaqI* (data not shown) restricted the ITS regions without generating any polymorphism between the species. Two enzymes: *HinI* (Fig. 8D) and *HindIII* (data not shown) did not restrict the amplified regions. Intraspecific variation was not observed with any of the enzymes.

DISCUSSION

The first report of this nematode species appears to be that by Poghossian (1960) who found many cysts infecting nettle, hemp and two *Rumex* species at several localities in Armenia and identified them as *H. humuli* (= *H. urticae*?). Two years later this author published a short description and morphometrics and referred to the nematode as *H. urticae* (Poghossian, 1962). This nematode species was found in ten districts of Armenia, and hemp considered the probable host. Subsequently, when reporting investigations on root-knot and cyst nematodes in Armenia, Poghossian (1978) indicated that only nettle and hemp were host plants of this *Heterodera* species. This species was also recorded in the Ukraine (Nikitin, 1972) and in different regions of Russia (Kirjanova & Lobanova, 1972). Comparative studies of morphometrics and drawings of cysts, males and juveniles from these publications and examination of slides from the Nematode Collection of the Zoological Institute of the Russian Academy of Sciences confirmed these populations as representing *H. riparia* sp. n.

H. urticae Cooper, 1955 does not appear to be present in the territory of the European part of the former Soviet Union and all earlier records of *H. urticae* should be considered as representing *H. riparia* sp. n. In Belgium (own unpublished data), Germany and possibly other parts of Western Europe, *H. urticae* is widely distributed (Sturhan, 1976). *H. riparia* sp. n. and *H. urticae* an occasionally present together in soil samples.

Heterodera riparia sp. n., *H. humuli* and *H. fici* represent a species complex of specialised parasites of the plant order Urticales with *H. lupulus* and *Cannabis sativa* of the Cannabaceae family, *Ficus* spp. of the Moraceae family, and *U. dioica* and *U. urens* of the Urticaceae family being the only hosts. Small lemon-shaped cysts, which are at early stages bifenestrated and often become progressively ambifenestrated with age, and a long vulval slit situated in a cleft between the thickened vulval lips (cp. also Green, 1975) are main characteristics separating these species from most other *Heterodera* species. *H. litoralis* with similar vulval configuration differs, e.g.,

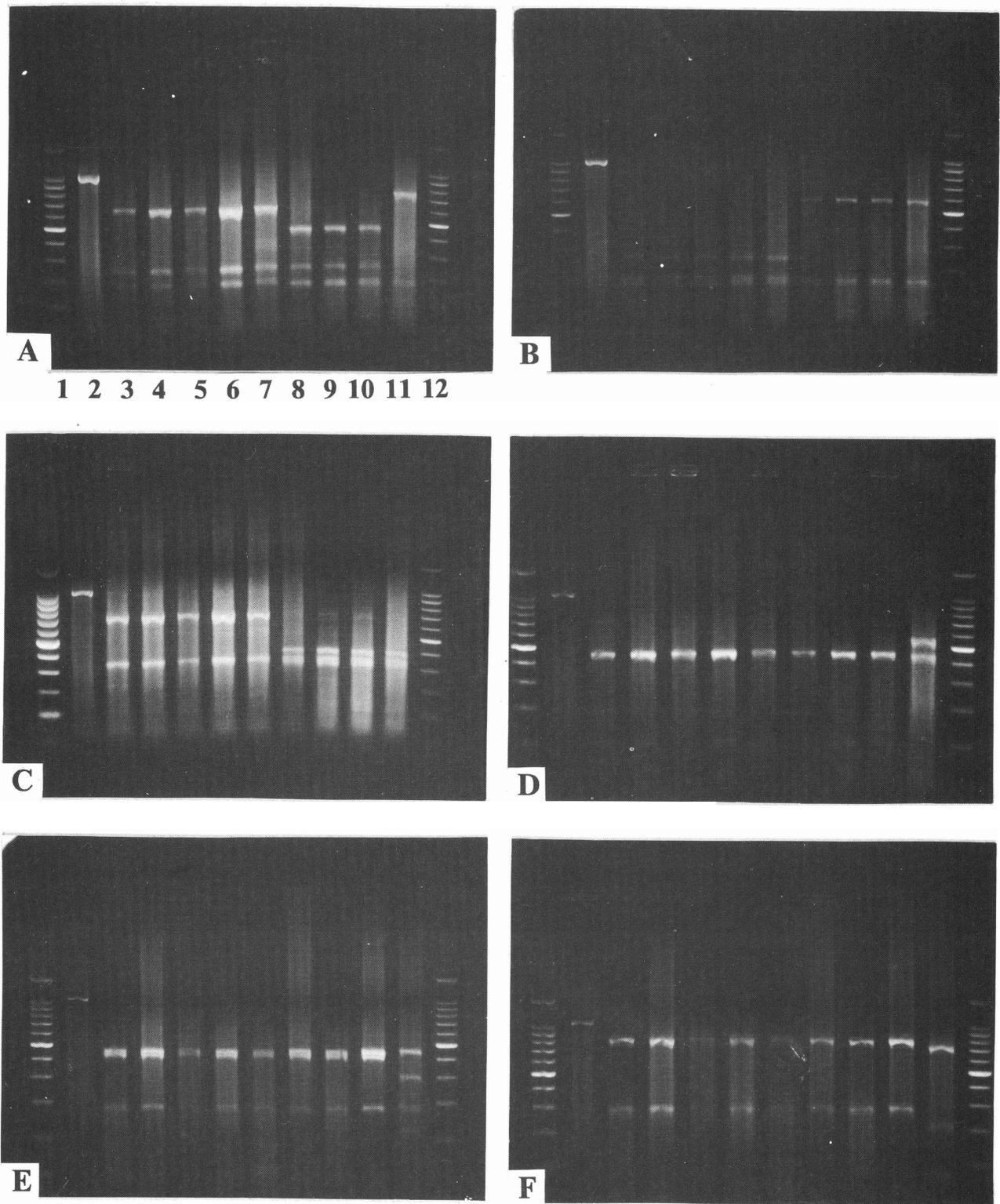


Fig. 7. Restriction fragments of amplified Internal Transcribed Spacers of *Heterodera riparia* sp. n., *H. humuli* and *H. fici*. A: *AluI*; B: *CfoI*; C: *PstI*; D: *BsuRI*; E: *MspI*; F: *MvaI*; Lanes 1 and 12: 100 bp DNA ladder; Lane 2: unrestricted *H. riparia* sp. n.; Lanes 3-7, *H. riparia* sp. n. 3: Mytishchi, Russia, 4: Lesnoi, Russia, 5: Münster, Germany, 6: Knokke, Belgium, 7: Han-sur-Lesse, Belgium; Lanes 8-10, *H. humuli*. 8: Poperinge, Belgium, 9: Tsivilsk, Russia, 10: Münster, Germany; Lane 11: *H. fici*, Abkhazija.

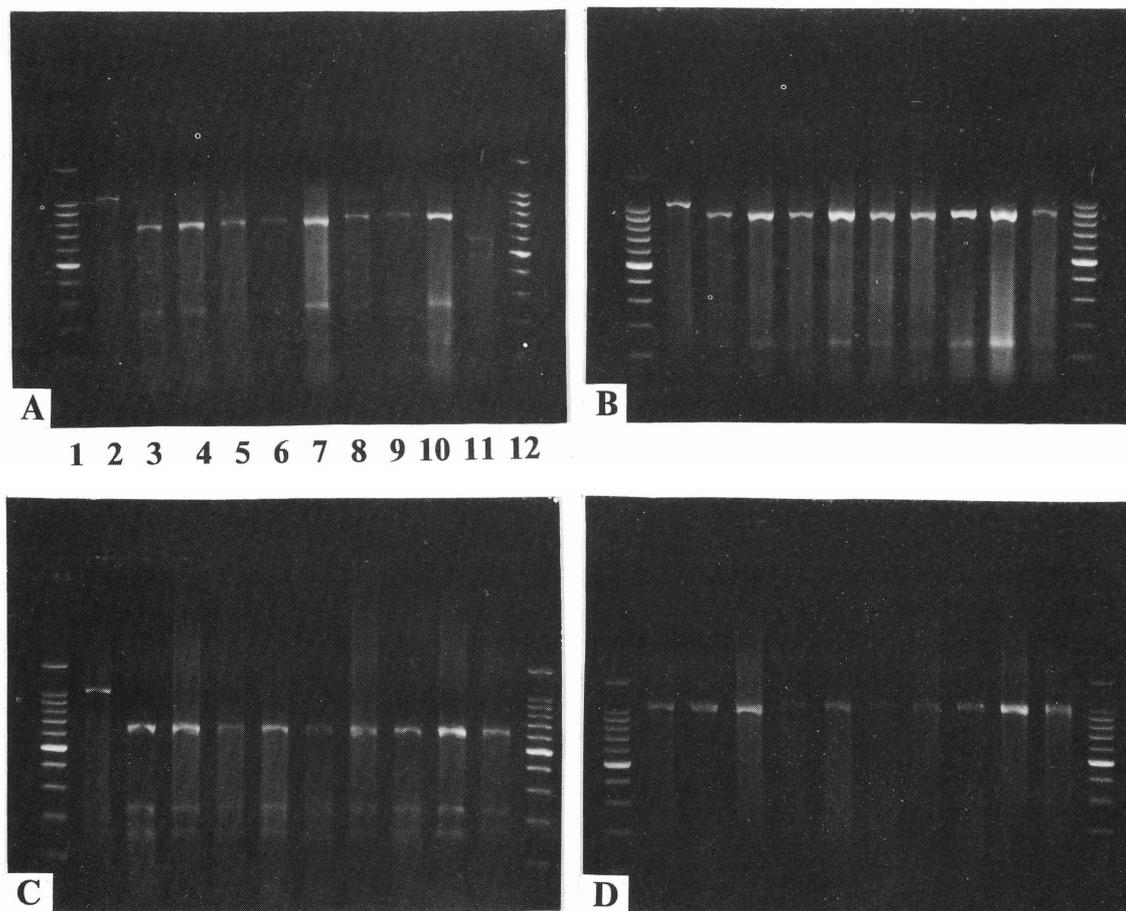


Fig. 8. Restriction fragments of amplified Internal Transcribed Spacers of *Heterodera riparia* sp. n., *H. humuli* and *H. fici*. A: *Rsa*I; B: *Bsh*1236I; C: *Bsp*143I; D: *Hinf*I. See Fig. 7 for lane codes.

in dimensions of cysts and juveniles and in its hosts belonging to Chenopodiaceae (Wouts & Sturhan, 1996). Presence or absence of bullae in cyst cone, which lead Mulvey (1972), Mulvey and Golden (1983), Baldwin and Mundo-Ocampo (1991) and others to place *H. fici* in the *H. schachtii* group and *H. humuli* in the *H. goettingiana* group, are considered of minor taxonomic significance. There is also much variation in this character. Thus Goffart (1954) observed weakly developed bullae in 66 % of all *H. fici* and in 8 % of all *H. humuli* cysts studied. All three species of the *H. humuli* group have short juveniles with four lines in the lateral field and small but distinct phasmids.

If the differences between the species in morphological characteristics are only small, the differences in host range are obvious. *Humulus lupulus* appears to be the main host of *H. humuli*, but *C. sativa*, *U. dioica* and *U. urens* are also hosts. However, other members of the Urticaceae family, including several *Urtica* species, are no-hosts (Winslow, 1954). De Grisse and Gillard (1963) and Mikhailiyukov (1976)

found only a few cysts on *U. dioica* roots but not on *U. urens*, whereas Andersson (1979) reported that this nematode reproduced well on *U. dioica* growing in pots. The *H. humuli* population from Cheboksary, Russia, included in our studies, developed a few cyst on roots of nettle (Subbotin, 1986), but cysts of *H. humuli* were not found during our survey of the common nettle growing near hop plantations heavily infected by the hop cyst nematode in Belgium. Goffart (1954) reported *Ficus elastica* Roxb. as a poor host of *H. humuli*, and the report by Sen and Jensen (1969) of hosts belonging to quite different plant orders requires to be confirmed.

For *H. fici* only several *Ficus* species are hosts and *H. lupulus*, *U. urens* and *U. gracilis* proved to be non-hosts (Sher & Raski, 1956). *H. riparia* sp. n. is known only from *U. dioica*.

Our study of the *H. humuli* group indicates that rDNA-RFLPs differentiate these three species. *H. riparia* sp. n. is distinguished from the two other species of this group by three enzymes (*Alu*I, *Cfo*I, *Pst*I). *H. humuli* can be distinguished only by one

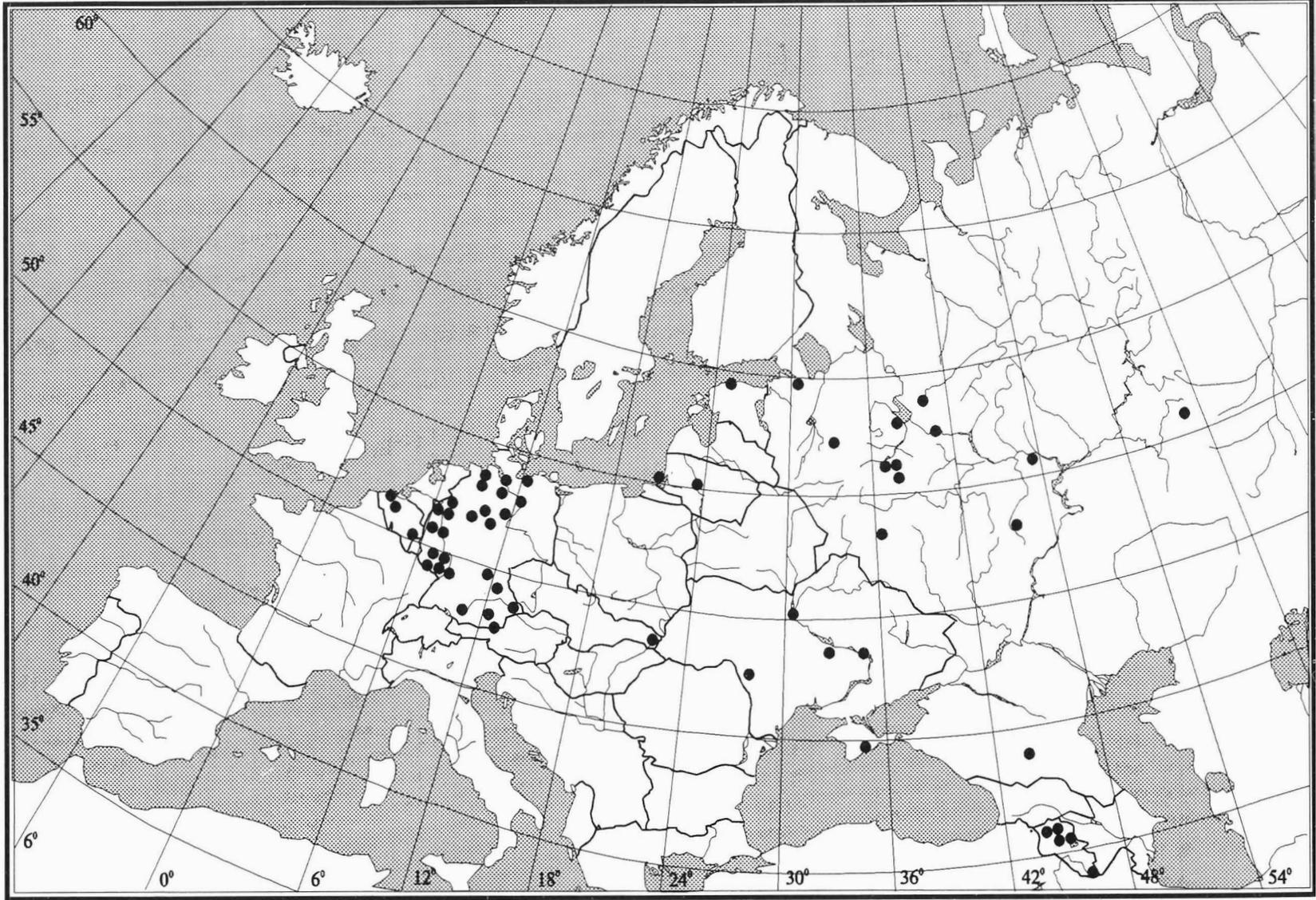


Fig. 9. Distribution of *H. riparia* sp. n. in Europe.

enzyme (*AluI*) and *H. fici* by four enzymes (*AluI*, *BsuRI*, *MspI*, *MvaI* and *RsaI*). In some RFLPs the sum of the DNA fragments lengths did not correspond to the entire length of the PCR product, probably as a result of agarose gels being used for the study. These gels are not suitable for visualising bands with a length less than 100 bp.

Unlike *H. humuli*, *H. riparia* sp. n. is not an agricultural pest able to cause damage in hop plantations. As both species are very similar in morphology and morphometrics, the PCR-RFLP technique is a useful tool for quick and reliable species separation of the *H. humuli* group.

ACKNOWLEDGEMENTS

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REFERENCES

- Andersson, S. 1979.** *Heterodera humuli* Filipjev, humle-cyst-nematoden, funnen i Sverige. *Växtskyddsnotiser* 43: 18-19.
- Baldwin, J.G. & Mundo-Ocampo, M. 1991.** Heteroderinae, cyst- and non-cyst-forming nematodes. In: *Manual of Agricultural Nematology*. (W.R. Nickle, Ed.). pp. 275-362. New York, Basel, Hong Kong. Marcel Dekker Inc.
- Danilova, Yu. S. 1996.** [The hop cyst nematode *Heterodera humuli* Filipjev, 1934: bioecological basis of control]. Candidate thesis. Moscow. 20 pp.
- De Grisse, A. & Gillard, A. 1963.** Morphology and biology of hop cyst eelworm (*Heterodera humuli* Filipjev 1934). *Nematologica* 9: 41-48.
- Goffart, H. 1954.** Über *Heterodera fici* Kirjanova 1954. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 68: 597-599.
- Golden, A.M., Maqbool, M.A. & Shahina, F. 1988.** Re-description of *Heterodera fici* (Nematoda: Heteroderidae) with SEM observations. *Journal of Nematology* 20: 381-391.
- Green, C.D. 1975.** The vulval cone and associated structures of some cyst nematodes (Genus *Heterodera*). *Nematologica* 21: 134-144.
- Joyce, S.A., Reyd, A., Driver, F. & Curran, J. 1994.** Application of polymerase chain reaction (PCR) methods to identification of entomogenic nematodes. In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes*. Proceedings of Symposium & Workshop, St. Patrick's College, Maynooth, Co. Kildare, Ireland. (A.M. Burnell, R.-U. Ehlers & J.P. Masson. Eds.). pp. 178-187. Luxembourg, European Commission, DG XII.
- Kirjanova, E.S. & Krall, E.L. 1971.** [*Parasitic Nematodes of Plants and their Control*]. Vol. 2. Leningrad, Nauka. 522 pp.
- Kirjanova, E.S. & Lobanova, N.A. 1972.** [On the distribution of cyst-forming nematodes. In: *Nematodnye bolezni sel'skokhozjaistvennykh kul'tur i mery bor'by s nimi*. pp. 114-116. Moscow.
- Mathews, H.J.P. 1971.** Morphology of the nettle cyst nematode *Heterodera urticae* Cooper, 1955. *Nematologica* (1970) 16: 503-510.
- Mikhailyukov, V.S. 1976.** [Plant-hosts of the hop cyst nematode]. *Nauchnye Trudy USKHA. Zashchita rastenii ot vreditel'ei i boleznei* 161: 60-61.
- Mulvey, R.H. 1972.** Identification of *Heterodera* cysts by terminal and cone top structures. *Canadian Journal of Zoology* 50: 1277-1292.
- Mulvey, R.H. & Golden, A.M. 1983.** An illustrated key to the cyst-forming genera and species of Heteroderidae in the western hemisphere with species morphometrics and distribution. *Journal of Nematology* 15: 1-59.
- Nikitin, V.S. 1972.** [Nematodes of the genus *Heterodera* from the Ukrainian SSR]. In: *Nematodnye bolezni s/kh kul'tur i mery bor'by s nimi*. pp. 95-96. Moscow.
- Nikitin, V.S. 1981.** [Occurrence of nematodes of the subfamily Heteroderinae in the Ukraine]. In: *Pervaja konferentsija (IX soveschanie) po nematodam rastenii, nasekomykh, pochvy i vod*. pp. 195-197. Tashkent.
- Poghossian, E.E. 1960.** [The incidence of nematodes of the family Heteroderidae (Nematoda) in the Armenian SSR]. In: *Materialy k pjatomy vsesojuznomy soveschaniju hj izytscheniju nematod*. pp. 79-81. Samarkand.
- Poghossian, E.E. 1962.** [The incidence of nematodes of the family Heteroderidae (Nematoda) in the Armenian SSR]. In: *Nematody vrednye v sel'skom khozjaistve i bor'ba s nimi*. pp. 228-250. Samarkand.
- Poghossian, E.E. 1978.** [The results of the study of root-knot and cyst-forming nematodes in the Armenian SSR and the modern systematics of the family Heteroderidae]. In: *Materialy pervoi zakavkazskoi konferentsii po obschei parazitologii*. pp. 133-138. Tbilisi.
- Sen, A.K. & Jensen, H.J. 1969.** Host-parasite relationships of various plants and the hop cyst nematode, *Heterodera humuli*. *Plant Disease Reporter* 53: 37-40.
- Shahina, F. & Maqbool, M.A. 1995.** *Cyst Nematodes of Pakistan (Heteroderidae)*. Karachi. 155 pp.
- Sher, S.A. & Raski, D.J. 1956.** *Heterodera fici* Kirjanova

- 1954 in California. *Plant Disease Reporter* 40: 700.
- Stone, A.R. & Rowe, J.A. 1977.** *Heterodera humuli*. C.I.H. *Description of Plant-parasitic Nematodes*. Set 7, No. 105. 4 pp.
- Sturhan, D. 1976.** Erstnachweise von fünf *Heterodera*-Arten in der Bundesrepublik Deutschland. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Braunschweig)* 28: 167-169.
- Subbotin, S.A. 1986.** [The nettle race of the hop cyst nematode *Heterodera humuli* Filipjev, 1934]. *Byulleten' Vsesoyuznogo Instituta Gel'mintologii* 45: 98-99.
- Subbotin, S.A. & Chizhov, V.N. 1985.** [Cyst-forming nematode *Heterodera* sp., from the nettle *Urtica dioica* L.]. *Byulleten' Vsesoyuznogo Instituta Gel'mintologii* 41: 61-66.
- Subbotin, S.A., Kruchina, S.N. & Chizhov, V.N. 1989.** [*Heterodera fici* Kirjanova, 1954 - parasite of fig in Abkhazija]. *Byulleten' Vsesoyuznogo Instituta Gel'mintologii* 50: 127-128.
- Winslow, R.D. 1954.** Provisional lists of host plants of some root eelworms (*Heterodera* spp.). *Annals of Applied Biology* 41: 591-605.
- Wouts, W.M. & Weischer, B. 1977.** Eine Klassifizierung von fünfzehn in Westeuropa häufigen Arten der Heteroderinae auf Grund von Larvenmerkmalen. *Nematologica* 23: 289-310.
- Wouts, W.M. & Sturhan, D. 1995.** *Heterodera litoralis* sp. n. (Nematoda: Heteroderidae) from Austral glasswort, *Sarcocornia quinqueflora*, in New Zealand. *Nematologica* 42: 62-70.

Субботин С. А., Штурхан Д., Ваенберге Л., Моенс М. Описание *Heterodera riparia* sp. n. (Tylenchida: Heteroderidae) с крапивы двудомной *Urtica dioica* L. и дифференциация на основе rDNA-RFLP видов группы *H. humuli*.

Резюме. По материалам, собранным с корней растений, произрастающих близ берегов рек, прудов и озер России, Германии и Бельгии, описывается *H. riparia* sp. n., наиболее сходный с *H. humuli*, но отличающийся меньшим средним размером цист (415-468 мкм против 452-524 мкм у *H. humuli*) и меньшей длиной фенестр (46-52 мкм против 56-61 мкм). Вторая личиночная стадия *H. riparia* sp. n. имеет меньшую среднюю длину тела (350-373 мкм против > 375 мкм), более короткий хвост (40-47 мкм против 50-57 мкм), а также более короткую гиалиновую часть хвоста (18-23 мкм против обычно > 27 мкм). У нового вида за вегетационный сезон развивается одно поколение. Рестрикционный анализ последовательностей рибосомальной ДНК был использован для дифференциации *H. riparia* sp. n. от близких видов *H. humuli* и *H. fici*. Спектры рестрикционных полос, полученных при использовании *AluI*, четко отличают все эти виды друг от друга, рестриктазы *CfoI* и *PstI* также хорошо дифференцировали новый вид от остальных видов. Приводится карта распространения нового вида в Европе.
