Heterodera vallicola sp. n. (Tylenchida: Heteroderidae) from elm trees, *Ulmus japonica* (Rehd.) Sarg. in the Primorsky territory, the Russian Far East, with rDNA identification of closely related species

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Summary. *Heterodera vallicola* sp. n. is described from the rhizosphere and roots of elm plants *Ulmus japonica* (Rehd.) Sarg. (Ulmaceae Urticales), growing in a forest along the Ilistaija river in the Mikhailovsky district, Primorsky region, the Russian Far East. This species belongs to the *Humuli* group. It differs from *H. riparia* by its more rounded cysts, narrower semifenestral width, longer length stylet and longer hyaline part of tail for the second stage juveniles. It can be distinguished from *H. humuli* by having a shorter fenestral length, and smaller body length females and males. Restriction enzyme analysis of the ITS regions of ribosomal DNA obtained with *Alu*I, *Alw*26I, *Cfo*I, *Dde*I, *Pvu*II, and *Rsa*I clearly distinguished *H. vallicola* sp. n. from *H. humuli* and *H. riparia*. Phylogenetic relationships of *H. vallicola* sp. n. with other *Humuli* group species are given based on analyses of the ITS sequences.
Key words: cyst nematodes, elm, *Heterodera fici, H. humuli, H. riparia,* ITS of rDNA, phylogeny, RFLP,.

In July 1997, during a nematological survey of cedar broad-leaved forests of the Primorsky territory, females, males, and cysts of the genus *Heterodera* were found on roots and in the rhizosphere of elm plants. Detailed morphological and morphometrical studies revealed that this nematode belongs to the *Humuli* group, and is very similar to *H. humuli* and *H. riparia*. However, subsequent molecular comparison study of this population with *H. riparia* and *H. humuli* showed differences in the ITS-rDNA-RFLP profiles between these species. Evidently, this cyst nematode population may be considered as representing a sibling species of *H. riparia*. A morphological and morphometrical description, and molecular differentiation of this new species from related species of the *Humuli* group are provided.

MATERIALS AND METHODS

Nematode populations. A single population of the new species was collected from elm trees, *Ulmus japonica* (Rehd.) Sarg. in a broad-leaved forest growing in a valley of the river Ilistaij Otradnoe, the Primorsky territory, the Russian Far East. Populations of *H. riparia* from *Urtica laetevirens* Maxim., the Ussurijskii natural reserve, the Russian Far East, and *H. humuli* from *Humulus lupulus* L., Poperinge, Belgium, were used for comparative rDNA-RFLP analysis. Cysts and females were isolated from soil and root samples by a flotation and sieving method. Second stage juveniles were isolated directly from cysts, and males from soil samples by a centrifugal-flotation method (Jenkins, 1964).

Light microscopy study. Females, males and juveniles were fixed in 4% formalin and mounted in glycerol on permanent slides following Seinhorsts's (1959) method. Cyst vulval cones were mounted in glycerine-gelatine. Nematodes were examined and measured with a MBI-11 light microscope. All

measurements are presented as the mean and a standard deviation of the mean, followed by the range in parenthesis.

DNA extraction, amplification and sequencing. The DNA extraction method from a single cyst described by Subbotin *et al.* (1997) was used for this study. After centrifugation, 1.5 µl of the DNA suspension were added to the PCR reaction mixture containing 2.5 µl of 10X *Taq* incubation buffer, 5 µl of Q-solution, 200 µM of each dNTP (*Taq* PCR Core Kit, Qiagen, Germany), 1 µM of each primer (synthesised by Life Technologies, Merelbeke, Belgium), 0.5U *Taq* Polymerase (*Taq* PCR Core Kit, Qiagen, Germany) and double distilled water to a final volume of 25 µl. The forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') were used in the PCR reaction for amplification of the ITS1-5.8S gene-ITS2 region with flanking areas of the 18S and 28S genes of the rDNA. The amplification profile was carried out in a GeneE (New Brunswick Scientific, Wezembeek-Oppem, Belgium) DNA thermal cycler consisted of 4 min at 94 °C; 35 cycles of 1 min at 94 °C, 1.5 min at 55 °C, and 2 min at 72 °C; followed by a final elongation step of 10 min at 72 °C. After DNA amplification, 2 µ of product was run on a 1% agarose gel. The remainder was stored at −20 °C. Three to 7 µl of each PCR product was digested with one of the following restriction enzymes: *Alu* I, *Alw*21I, *Alw*26I, *BsiZ*I, *Cfo*I, *Dde*I, *Ita*I, *Msp*I, *Mva*I, *Pvu*II, *Rsa*I, and *Tru*9I in the buffer stipulated by the manufacturer. The digested DNA was loaded on a 1.5% agarose gel, separated by electrophoresis (100V, 1.5 h), stained with ethidium bromide, visualised on a 2011 Macrovue UV transilluminator, and photographed with a Polaroid MP4+ Instant Camera System. Procedures for obtaining PCR amplified products and endonuclease digestion of these products were repeated several times to verify the results. The exact length of restriction fragments of rDNA-ITS regions for the *Humili* group species were revealed by a virtual digestion of sequences using WEBCUTTER (2.0, http://www. firstmarket.com /cutter/cut2.html).

Amplified products were excised from 1% TBE buffered agarose gels using the QIAquick Gel Extraction Kit (Qiagen), cloned into the pGEM®-T vector and transformed into JM109 High Efficiency Competent Cells (Promega Corporation, USA). PCR products obtained from clones were purified using a QIAquick PCR Purification Kit (Qiagen Ltd.). DNA fragments were sequenced in both directions with TW81, AB28, 5.8SM2 (5'-CTTATCGGTGGATCACTCGG-3') or 5.8SM5 (5'-GGCGCAATGTGCATTCGA-3') primers with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, UK). Only one clone from each sample was sequenced. The resulting products were purified using a Centriflex Gel Filtration Cartridge (Edge BioSystems Inc., Gaithersburg, Maryland, USA). Sequences were run on a 373 DNA sequencer (PE Applied Biosystems, Warrington, UK). The ITS sequences of *H. vallicola* sp. n and *H. riparia* (Russian Far East) are deposited in the GenBank database.

Alignment and phylogenetic analysis. Sequences were edited using Chromas 1.45 (© 1996-1998, Conor McCarthy), aligned using ClustalX 1.64 with default options (Thompson *et al.*, 1997), and then optimized manually using GeneDOC 2.5.0 (Nicholas & Nicholas, 1997). Sequences for *H. fici* (Sukhumi, Georgia), *H. riparia* (*U. dioica*, Germany) and *H. humuli* (*Humulus lupulus*, Tsivilsk, Chuvashija, Russia) for phylogenetic analyses were obtained from the GenBank: AF274409, AF274407, AF274408 (Subbotin *et al.*, unpublished results). *Heterodera fici* was used as an outgroup. Maximum parsimony (MP) analyses were performed using PAUP* 4b4a (Swofford, 1998). Gaps were coded in two ways, either as missing data or as a fifth character. The *g*1 statistic was computed by generating 100000 random trees using the randtrees option in PAUP. Robustness of the clade was assessed by the bootstrap support and decay index. Phylogenetic reconstructions were also obtained by the maximum likelihood method (ML) using PAUP with the HKY85 model of sequence evolution (six substitution types, base frequencies, transition/transvertion parameter and the gamma distribution parameter alpha for four categories were estimated from the data set). Trees were displayed with TreeView 1.6.1 (Page, 1996).

DESCRIPTION

Heterodera vallicola sp. n. (Figs. 1 & 2, Tables 1 & 2)

Holotype cyst: L (excluding neck) = 487 µm; width = 420 µm.

Paratype cysts: see Table 1.

Paratype females (n=14): L (excluding neck) = 248±37 (210-351) µm; width = 196±49 (120-234) µm; a = 1.3±0.19 (1.1-1.7); length of neck =

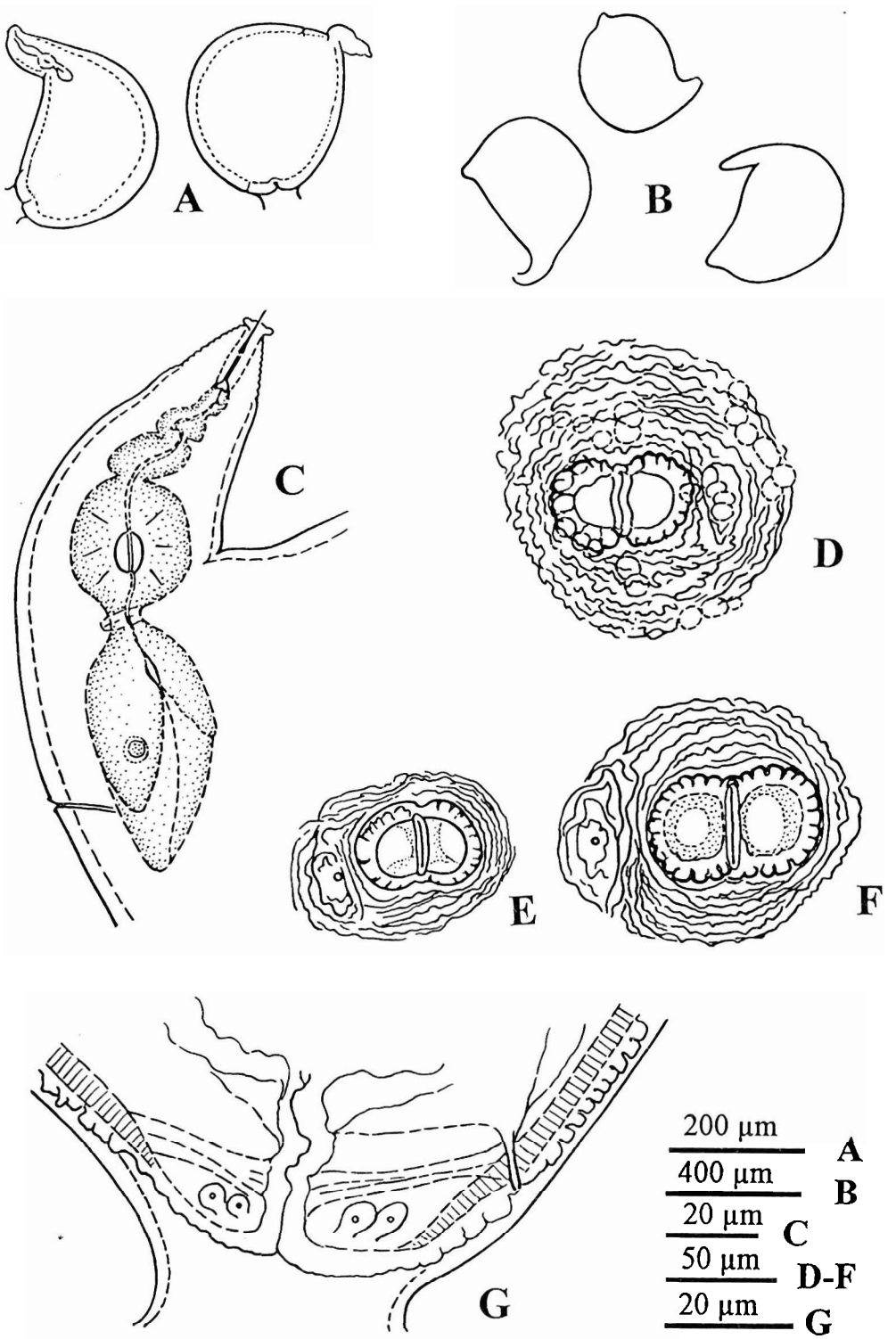

Fig. 1. *Heterodera vallicola* sp. n. A: Females; B: Cysts; C: Anterior end of female; D-F: Vulval plates; G: Female terminal region.

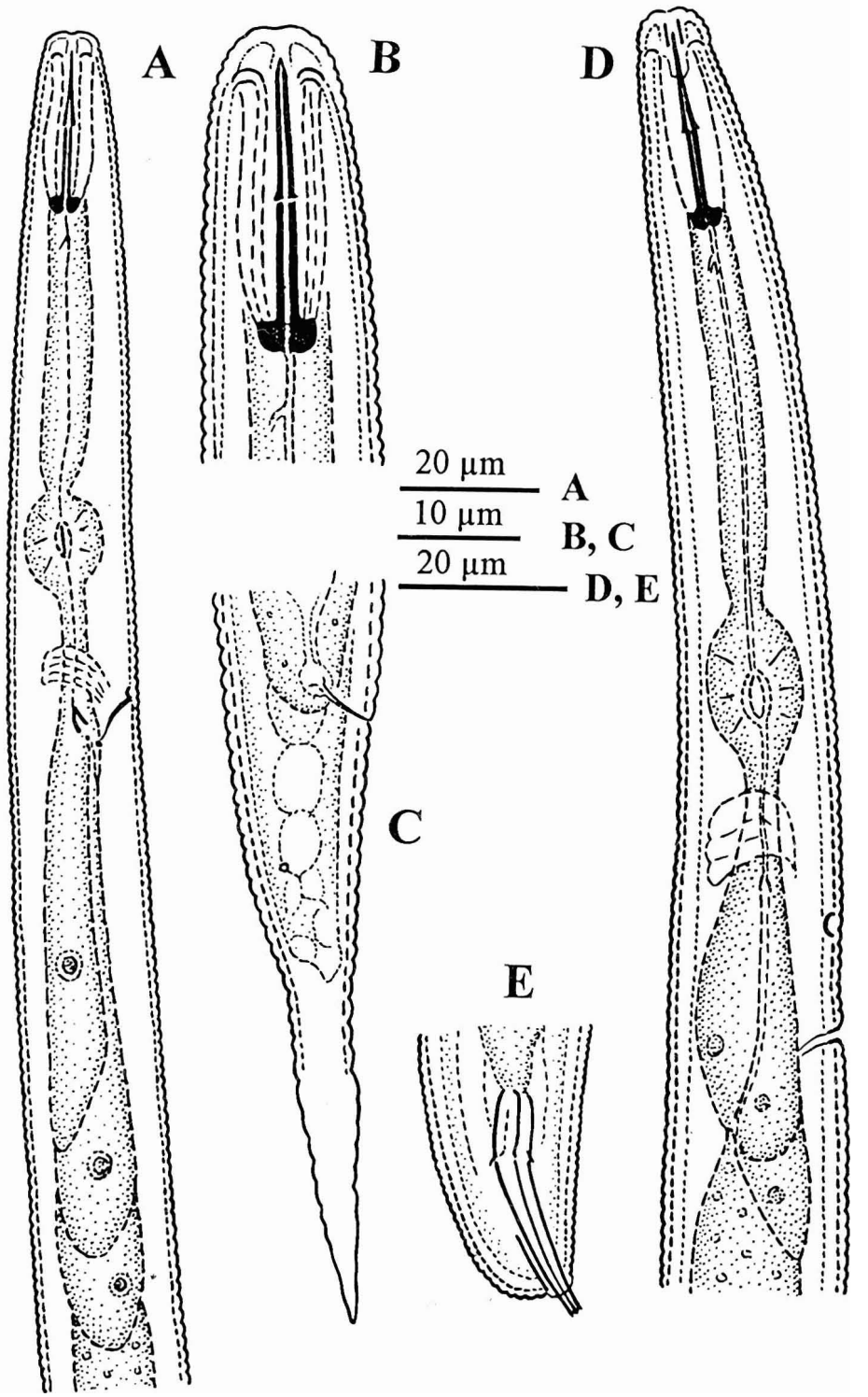

Fig. 2. *Heterodera vallicola* sp.n. A: Anterior end of second stage juvenile; B: Head of juvenile; C: Tail of juvenile; D: Anterior end of male; E: Posterior end of male.

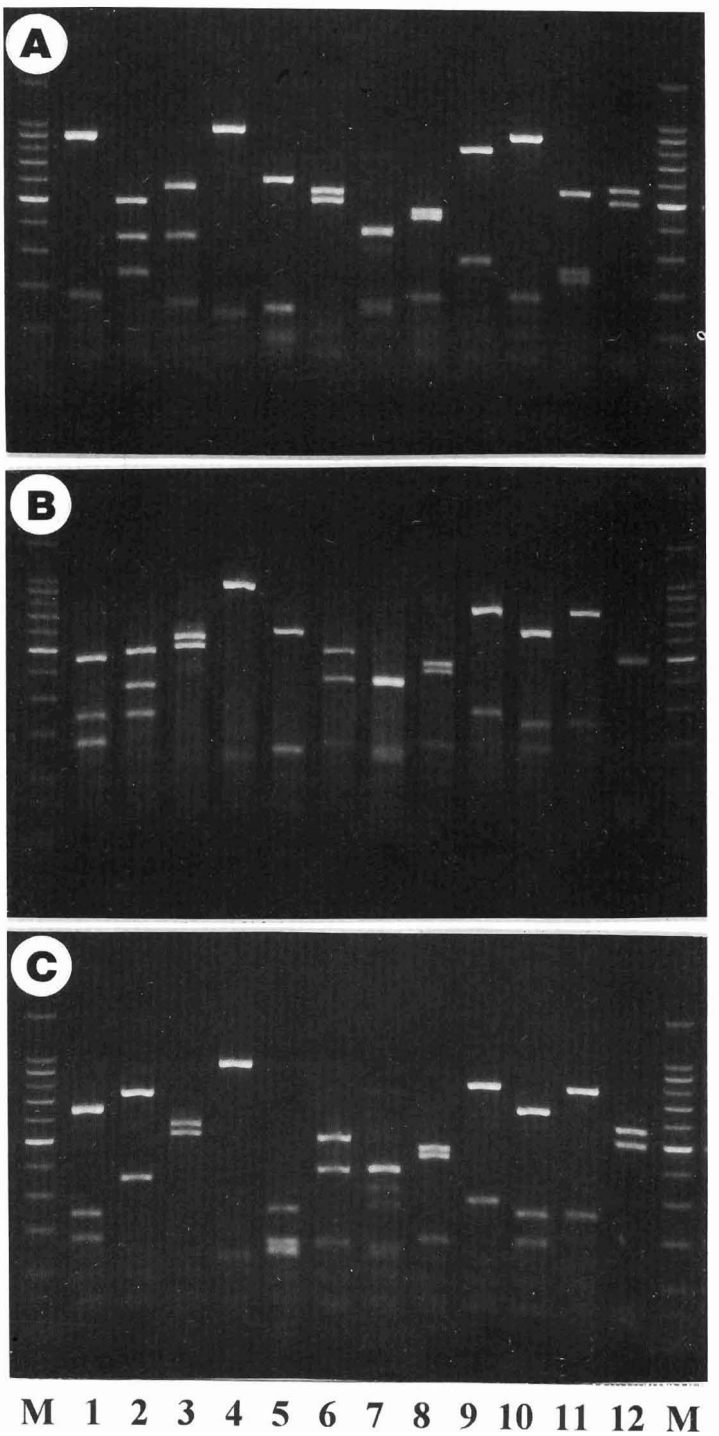

Fig. 3. Restriction fragments of amplified ITS regions of species of the *H. humuli* group. A: *H. vallicola* sp. n.; B: *H. humuli*; C: *H. riparia*. (Codes: Lanes M – 100bp DNA ladder, 1 – *Alu*I, 2 – *Alw*21I, 3 - *Alw*26I, 4 – *Bsi*ZI; 5 – *Cfo*I; 6 – *DdeI*; 7 – *Ita*I, 8 – *Msp*I; 9 – *Mva*I; 10 – *Pvu*II; 11 – *Rsa*I; 12 – *Tru*9I).

Table 1. Morphometrics of cysts of *Heterodera vallicola* sp. n., *H. riparia* and *H. humuli* populations (All measurements in μm).

Population Host Locality	*H. vallicola* sp. n. *Ulmus japonica* Primorsky territory Paratypes	*H. riparia* *Urtica laetevirens* Primorsky territory	*H. riparia* *Urtica dioica* Moscow region (Subbotin *et al.*, 1997)	*H. humuli* *Humulus lupulus* Cheboksary, Chuvashija (Subbotin *et al.*, 1997)
Cysts				
n	25	25	50	29
Length (excluding neck)	468±38 (418-554)	505±44 (416-585)	462±7.9* (448-580)	515±15.3* (336-664)
Width	419±49 (308-541)	375±52 (299-520)	327±5.0 (212-454)	367±11.4 (248-504)
Length/width	1.1±0.08 (1.0-1.3)	1.3±0.02 (1.1-1.6)	1.4±0.03 (1.1-1.8)	1.4±0.02 (1.2-1.7)
Vulval areas				
n	25	25	35	20
Fenestral length	46±6.9 (34-58)	48±5.7 (40-55)	46±0.8 (31-58)	58±1.5 (48-70)
Mean semifenestral width	21±3.9 (18-28)	27±4.3 (22-34)	26±0.7 (17-38)	25.9±0.5 (23-30)
Vulval slit length	31±3.6 (23-36)	37±4.0 (31-43)	34±0.6 (28-42)	36.2±0.5 (33-40)
Vulval bridge width	11±1.1 (9-12)	12±2.2 (9-16)	11±0.9 (6.6-18)	12±0.5 (8.8-18)
Underbridge length	–	(71, 78)	78±2.3 (70-88)	88±2.1 (75-100)
Vulva-anus distance	53±7.8 (40-65)	45±5.3 (37-53)	47±1.4 (36-63)	46±1.1 (40-58)

Table 2. Morphometrics of second stage juveniles of *Heterodera vallicola* sp. n., *H. riparia* and *H. humuli* populations (All measurements in μm).

Population Host Locality	*H. vallicola* sp. n. *Ulmus japonica* Primorsky territory Paratypes	*H. riparia* *Urtica laetevirens* Primorsky territory	*H. riparia* *Urtica dioica* Moscow region (Subbotin *et al.*, 1997)	*H. humuli* *Humulus lupulus* Cheboksary, Chuvashija (Subbotin *et al.*, 1997)
n	25	25	52	20
Body length (L)	383±20 (356-420)	370±26 (330-432)	373±2.1* (342-407)	375±4.7* (339-408)
a	20±1.4 (16-22)	21±1.7 (18-24)	21±0.1 (19-23)	21±0.2 (19-23)
b	3.5±0.1 (3.2-3.7)	3.4±0.2 (3.2-3.7)	3.3±0.03 (2.9-4.0)	3.5±0.1 (2.9-4.1)
c	7.5±1.7 (6.7-9.5)	7.7±1.7 (7.1-10.1)	8.0±0.1 (7.4-10.0)	7.5±0.1 (6.5-8.2)
Stylet length	25±0.5 (24-26)	24±0.7 (22-25)	22±0.1 (20-24)	23±0.1 (22-24)
Lip region height	3.8±0.3 (3.2-4.0)	3.4±0.14 (2.8-3.5)	4.1±0.03 (3.8-4.3)	4.0±0.02 (3.8-4.2)
Lip region width	8.4±0.5 (7.2-8.8)	8.7±0.5 (8.4-9.8)	9.2±0.03 (8.7-9.4)	9.1±0.1 (8.5-9.3)
DGO	3.5±0.5 (3.0-4.8)	3.8±0.2 (3.6-4.9)	4.8±0.1 (4.1-5.7)	4.1±0.1 (3.6-4.6)
Anterior end to excretory pore	93±6.0 (78-98)	90±4.5 (80-97)	95±0.6 (84-102)	91±0.9 (84-97)
Anterior end to valve of median bulb (MB)	68±3.9 (62-74)	62.2±4.9 (52-75)	67±0.4 (61-77)	65±0.9 (57-70)
Oesophagus length	106±3.8 (101-114)	100±5.8 (86-108)	114±0.9 (97-124)	107±1.5 (97-120)
Body width at:				
mid-body	20±1.5 (16-24)	19±1.0 (18-21)	18±0.1 (16-20)	18±0.2 (16-19)
anus (BWA)	12±0.6 (11-13)	11±1.2 (9-12)	11±0.1 (9.9-13)	12±0.1 (11-12)
Hyaline part of tail length (H)	29±2.7 (26-36)	23±2.5 (20-32)	23±0.3 (18-28)	29±0.4 (26-32)
Tail length	49±4.7 (40-57)	46±4.7 (38-54)	47±0.4 (36-50)	50±0.6 (43-54)
Tail length /BWA	4.2±0.4 (3.6-5.1)	4.3±0.5 (3.4-5.2)	4.1±0.04 (3.4-4.5)	4.4±0.1 (3.8-4.7)
H/ stylet length	1.1±0.04 (1.0-1.4)	1.0±0.09 (0.8-1.2)	1.1±0.02 (0.8-1.4)	1.2±0.01 (1.1-1.4)
L/MB	5.7±0.3 (4.8-6.6)	6.0±0.5 (5.1-6.5)	5.5±0.03 (5.0-6.1)	5.7±0.04 (5.5-6.3)

* - standard error of mean.

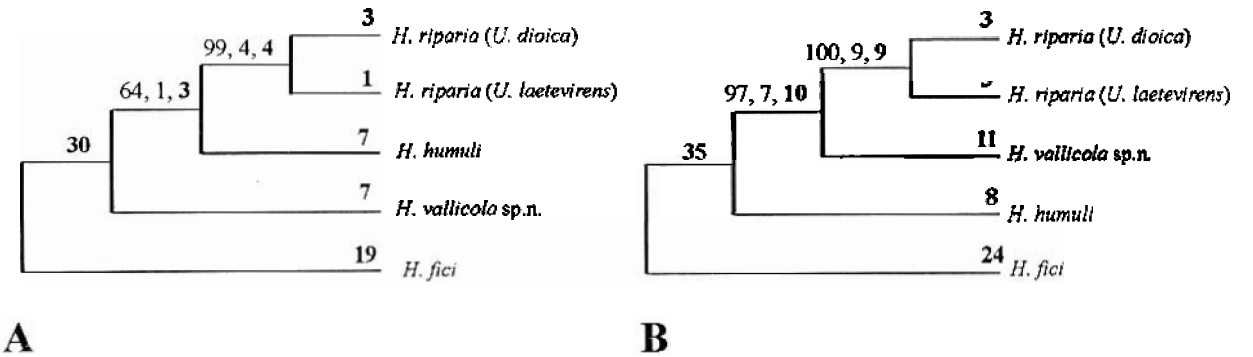

Fig. 4. Most parsimonious trees from phylogenetic analyses of the ITS sequence data for the *Humuli* group species. **A**: Tree from parsimony analysis with gaps treated as missing data [informative characters = 9, tree length = 74, Consistency index (CI) excluding uninformative characters = 0.8182, Homoplasy index (HI) excluding uninformative characters = 0.1818, Retention index (RI) = 0.7778, Rescaled consistency index (RC) = 0.7568, g1= - 1.0163], **B**: Tree from parsimony analysis with gaps coded as a fifth character [informative characters = 22, tree length = 103, CI excluding uninformative characters = 0.8800, HI excluding uninformative characters = 0.1200, RI = 0.8636, RC = 0.8385, g1= - 1.106821]. Number above the branches indicate bootstrap percentage, decay index, and number of character state changes in bold font.

61±0.24 (33-117) µm; length of stylet = 25±1.4 (23-27) µm; distance from stylet base to opening of dorsal oesophageal gland = 4.0-5.6 µm; width of median bulb = 24 µm; distance vulva-anus = 48±6.7 (40-56) µm; distance from anterior end to excretory pore = 104±10.4 (100-130) µm.

Paratype males (n=16): L = 756±30.6 (715-820) µm; a = 28±1.2 (26-30); b = 7.3±0.5 (6.9-8.4); b' = 5.2±0.5 (4.4-6.0); c = 356±73 (253-438); maximum width of the body = 25±2.9 (18-28) µm; height of lip regoin = 4.1±0.5 (3.6-4.8) µm; width of lip region = 9±0.4 (8.4-9.6) µm; length of stylet = 24±0.8 (22-25) µm; height of stylet knobs = 4.1±0.5 (3.6-4.8) µm; width of stylet base = 3.9±0.4 (3.0-4.8) µm; distance from stylet base to opening of dorsal oesophageal gland = 4.2 µm; distance from anterior end to valve of median bulb = 68±8.1 (60-75) µm, distance from anterior end to excretory pore = 113±13.1 (90-133) iµm; length of oesophagus from anterior end to oesophago-intestinal junction =102±6.9 (86-111) µm; length of spicules = 28±1.2 (26-30) µm; length of gubernaculum = 8.2±0.6 (7.2-9.0) µm; tail = 2.3±0.5 (1.8-3.0) µm.

Paratype juveniles: see Table 2.

Paratype eggs (n=25): L = 88±3.8 (78-93) µm; width = 32±2.7 (27-36) µm; length/width ratio =2.7±0.28 (2.3-3.4).

Cysts. Lemon shaped, with distinct vulval cone. Colour varying from yellow to pale brown. Cyst wall with ridges forming an irregular zig-zag pattern, thicker in vulval cone. Vulval cone bifenestrate, vulval bridge wide. Underbridge absent or weak. Bullae absent, small bullae-like structures only occasionally present. Conspicuous perianal pattern present.

Females. Body swollen, lemon shaped without distinct vulval cone. Usually neck displaced laterally. Body of females covered by subcrystalline layer, interrupted in vulval field. Thickness of cuticle in mid-body 8.8±1.0 (8.0-10) iµm. Cephalic region with indistinct labial disc, and wide anterior lip annule. Stylet slender, with small oval knobs. Length of metenchium about half the stylet length.

Males. Lip region dome-shaped, distinctly set-off, with 4 lip annules and a labial disc. Stylet strong, with small knobs slightly sloping anteriorly. Length of metenchium about half of the stylet length. Median bulb slender oval, valve posterior to centre. Hemizonid situated 7-9 annules anterior to the excretory pore. Lateral field with 4 lines. Spicules ventrally curved. Gubernaculum simple. Lateral field with 4 lines.

Second stage juveniles. Lip region rounded, about twice as wide as high, with 3-4 lip annules and a labial disc. Annules at mid-body 1.4 µm wide. Lateral field with 4 lines, areolated. Stylet strong, knobs flattened anteriorly or weakly con-

Table 3. Length (bp) of restriction fragments of rDNA-ITS regions for cyst nematodes from the *Humuli* group based on RFLPs and sequence data.

Enzyme	*Heterodera vallicola* sp. n.	*Heterodera humuli*	*Heterodera riparia*
Alu I	178, 867	171, 175, 241,450	178, 243, 630
*Alw*21I	231, 339, 475	233, 331, 473	338, 713
*Alw*26I	165, 341, 539	500, 537	510, 541
*Bst*ZI	130, 915	127, 910	130, 921
*Cfo*I	74, 81, 148, 152, 590	148, 152, 152, 585	148, 154, 155, 169, 175, 250
*Dde*I	33, 487, 525	33, 164, 355, 485	33, 167, 362, 489
*Ita*I	20, 144, 156, 358, 367	20, 136, 156, 358, 367	20, 107, 143, 156, 256, (299), 399
*Msp*I	179, 420, 446	179, 412, 446	181, 419, 451
*Mva*I	271, 773	272,765	274, 777
*Pvu*II	178, 867	175, 241, 621	178, 243, 630
*Rsa*I	21, 26, 224, 246, 528	21, 26, 243, 747	21, 26, 246, 758
*Tru*9I	7, 9, 485, 544	7, 9, 72, 466, 483	7, 9, 487, 548

cave; height of stylet knobs = 2.7±0.4 (2.4-3.2) μm, width of stylet knobs = 5.0±0.5 (4.0-5.6) μm. Metenchium = 12±0.6 (11-13) μm or 45-52% of stylet length. Median bulb oval, 12.8 x 8.8 m; oesophageal glands well developed. Oesophago-intestinal junction posterior to excretory pore. Distance from lip region to genital primordium = 212±17.7 (186-240) μm. Tail conical, with finely rounded terminus. Phasmids small but distinct, situated 10±2 (8.0-12) μm posterior to anus.

Type locality and host. *Heterodera vallicola* sp. n. was recovered from the rhizosphere and roots of Japanese elm, *Ulmus japonica* (Rehd.) Sarg. (Ulmaceae, Urticales) growing in forests along the Ilistaija river in the Mikhailovsky district, the Primorsky territory, Russia.

Type material. Holotype cyst and paratype cysts, females, males and juveniles deposited in the collection of the Laboratory of Phytonematology, Institute of Biology and Pedology, Vladivostok. Paratype cysts, females, males and juveniles deposited in the nematode collection of the Institute of Parasitology of the Russian Academy of Sciences, Moscow, Russia.

Differential diagnosis. The lemon-shaped, abullate and bifenestrate cysts places *H. vallicola* sp. n. in the *Humili* group. The new species is similar to *H. riparia* and *H. humuli*, both of which infect plants of the order Urticales, consequently the new species represents a sibling species. It differs from *H. riparia* by having more rounded cysts (L/W = 1.1 *vs* 1.3-1.4) and narrower semifenestral width (21 *vs* 25-27 μm). The second stage juveniles of *H. vallicola* sp. n. have a longer length stylet (25 *vs* 22-24 μm) and longer hyaline part of tail (29 *vs* 19-23 μm) (Subbotin *et al.*, 1997). *H. vallicola* sp. n. differs from the hop cyst nematode *H. humuli* by having more rounded cysts (L/W = 1.1 *vs* 1.4-1.5), and shorter fenestral length (46 *vs* 56-58 μm). Also males of *H. vallicola* sp. n. have a shorter body length (756 *vs* 800, 941 μm) (Stone & Rowe, 1977; Subbotin *et al.*, 1997).

rDNA-RELP analysis. The amplification of the ITS regions of *H. vallicola* sp. n., *H. humuli* and *H. riparia* gave fragments of approximately 1.04 kb. PCR products were not obtained in the negative control lacking DNA template. The RFLP patterns obtained with *Alu*I, *Alw*26I, *Cfo*I, *Dde*I, *Pvu*II, and *Rsa*I clearly distinguished *H. villicolla* sp.n. from *H. humuli* and *H. riparia*. The enzymes *Alu*I, *Alw*21I, *Cfo*I, *Ita*I, and *Tru*9I distinguished *H. humuli* and *H. riparia* from each other (Fig. 3). Lengths of restriction fragments of the rDNA-ITS regions produced by identification enzymes for the three species are given in Table 3.

Relationships of *H. vallicola* sp. n. with other *Humuli* group species based on the ITS sequence data. The aligned sequences of the entire ITS region, including the 5.8S gene, were 980 bp long, and sequence length ranged from 958 bp (*H. fici*) to 973 bp (*H. riparia*, the Russian Far East). The pattern of length polymorphisms (indels) among the *Humuli* group involves single nucleotide, 2-nt, 3-nt, 4-nt, or 5-nt, long motifs of two main types. Firstly, length polymorphism occured in deletion or insertion of single similar nucleotide after $(T)_n$, $(A)_n$ or $(G)_n$ repeats. Secondly, for *H. riparia* and *H. vallicola* sp. n. several insertions of identical motifs were obtained in results of the full, and

partly duplications of repeats: $(CTGT)_n$, $(TGGT)_n$ $(TG)_n$, $(AT)_n$. Sequence divergence (adjusted for missing data) between *H. vallicola* sp. n and *H. humuli* was 1.7% (16 pairs), and *H. vallicola* sp. n. and *H. riparia* was 1.6-1.8% (15-17 pairs), whereas between *H. riparia* populations was only 0.4% (4 pairs).

MP analyses of the ITS sequences for the *Humuli* group provided two alternative topologies, with different positions of *H. vallicola* sp. n. within the group. Exhaustive search, when gaps were coded as missing data, revealed a single most parsimonious tree with low bootstrap that supported a *H. riparia* and *H. humuli* clade (Fig. 4A). Topology of the ML tree (*Ln* likelihood = -1767.06) was identical with the previous tree. When gaps were coded as a fifth character, the MP analysis provided a single most parsimonious tree with high bootstrap support for a *H. riparia* and *H. vallicola* sp. n. clade (Fig. 4B).

Consequantly *H. vallicola* sp. n. apparently shows only minor morphometrical differences with *H. riparia* and *H. humuli*, but can be distinguished from them by RFLP and rDNA sequences of the ITS region. Although this species was found only in one locality, it, perhaps, may be widely distributed with its host *Ulmus japonica* growing in the Russian Far East, China, and Japan.

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Ерошенко А.С., Субботин С.А., Казаченко И.П. *Heterodera vallicola* sp. n. (Tylenchida: Heteroderidae) из ризосферы вяза *Ulmus japonica* (Rehd.) Sarg. из Приморья (Дальний Восток России) и идентификация близких видов по рибосомальной ДНК.

Резюме. Из ризосферы и корней вязов *Ulmus japonica* (Rehd.) Sarg. (Ulmaceae, Urticales), произрастающих вдоль реки Илистой в Михайловском районе Приморского края, описан новый вид цистообразующий нематоды *Heterodera vallicola* sp. n. Новый вид принадлежит к гетеродерам группы *Humuli*. От близкого вида *H. riparia* отличается более округлыми цистами, узкими семифенестрами, большей длиной стилета и гиалиновой части хвостового конца личинок 2-й стадии. Новый вид отличается от *H. humuli* более короткими фенестрами и меньшей длиной тела у самок и самцов. Анализ рестрикционных спектров ITS-участка рибосомальной ДНК, полученных с помощью нуклеаз *Alu*I, *Alw*26I, *Cfo*I, *Dde*I, *Pvu*II, и *Rsa*I, позволяет дифференцировать *H. vallicola* sp. n. от *H. humuli* и *H. riparia*. Предлагается анализ филогенетических отношений *H. vallicola* sp. n. с другими видами группы *Humuil* на основании последовательностей нуклеотидов ITS участка.