A new stem nematode, *Ditylenchus weischeri* sp. n. (Nematoda: Tylenchida), a parasite of *Cirsium arvense* (L.) Scop. in the Central Region of the Non-Chernozem Zone of Russia

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Summary. A new species of the stem nematode, *Ditylenchus weischeri* sp. n. parasitising creeping thistle, *Cirsium arvense* (Asteraceae), is described from material collected in the Moscow region, Russia. *Ditylenchus weischeri* sp. n. differs from *D. dipsaci* by shorter tails in adults, larger c index, shorter spicules, longer vulva-anus distance, larger vulva-anus distance to tail length ratio and longer posterior sac. *Ditylenchus weischeri* sp. n. differs from other *Ditylenchus* in ITS-rRNA and hsp90 gene sequences and chromosome numbers. *Ditylenchus weischeri* sp. n. did not develop and complete its life cycle in onion and garden strawberry seedlings, which are typical hosts for *D. dipsaci*. Diagnostic PCR-ITS-RFLP profiles are given for *D. weischeri* sp. n. and *D. dipsaci.*

Key words: *hsp90* gene, *Ditylenchus dipsaci*, onion, PCR, RFLP, strawberry.

Creeping thistle, *Cirsium arvense* (Asteraceae), has been considered as a host-plant for the stem nematode *Ditylenchus dipsaci* and several experimental studies showed that different host races of *D. dipsaci* can infect and develop on this plant (Salentiny, 1957; Nolte, 1959; Anon, 2008). *Cirsium arvense* is a weed, which is widely distributed in the territory of the European region of Russia. The creeping thistle plants infected by the stem nematode, which causes stem swellings, were sometimes found at the edges of agricultural fields, abandoned agricultural fields, along roadside ditches and banks of small rivers and streams, where regular grass cutting does not occur and the plant forms continuous brushwood for many years. However, several studies showed that populations of stem nematode from *Cirsium* plants found in natural conditions are different from the races of *D. dipsaci* commonly infecting agricultural crops, or *D. dipsaci sensu stricto*, in morphology, karyology, biology (Ladygina, 1978; Barbashova, 1979) and the sequences of the ITS rRNA gene (Subbotin e*t al.*, 2005). The results of several studies showed that the stem nematode parasitising *C. arvense* should not be considered as a race of *D. dipsaci* but independent species. In this paper we provide the description of this stem nematode as a new species.

MATERIAL AND METHODS

Nematodes were extracted from the stem swellings of *Cirsium arvense,* garden strawberry and infected bulbs of onion collected in the Moscow region, Russia. Crushed plant tissue was placed in Petri dishes with water for 2-4 h and then extracted nematodes were fixed in hot 4% TAF. Nematodes were processed in glycerol and embedded on permanent slides using standard methods (Seinhorst, 1959). All measurements of nematodes were made from permanent slides using Axio Image A1 objective of a Carl Zeiss microscope.

To evaluate the ability to infect onion (*Allium cepa* L.) and garden strawberry (*Fragaria* x *ananassa*), seedlings of these two crops were sprayed in field condition with a suspension of nematodes from *C. arvense*. The inoculum density was 300-350 nematodes of different life stages per

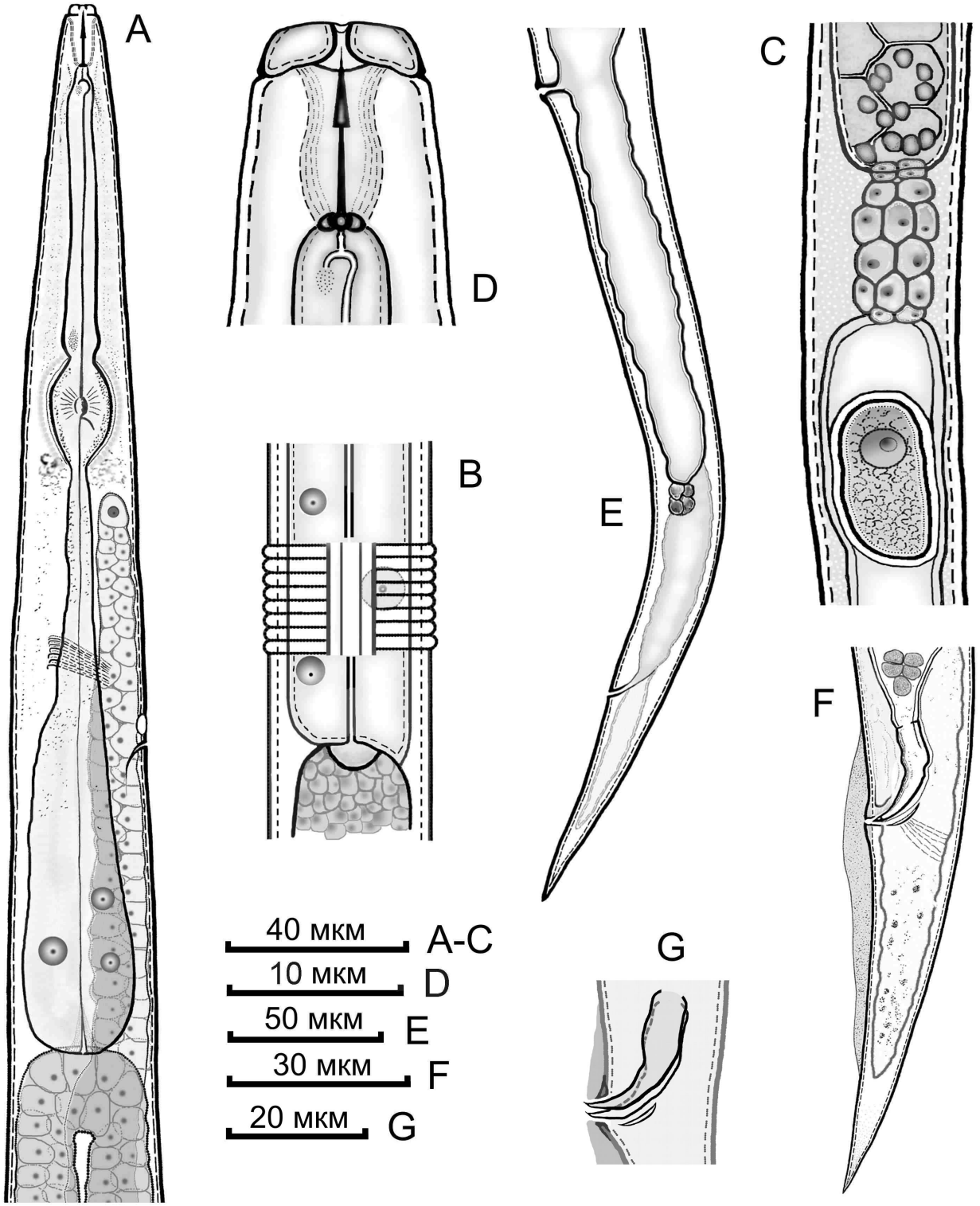

Fig. 1. *Ditylenchus weischeri* sp. n. A: Anterior region of male; B: Lateral field; C: Region of cristaformeria; D: Head region of male; E: Posterior end of female; F: Posterior region of male; G: Spicules.

Table 1. Morphometrics of *Ditylenchus weischeri* sp. n. and two populations of *D. dipsaci sensu stricto* (all measurements in μm).

Species	*D. wischeri* sp. n.			*D. dipsaci*			
Host	*Circium arvence*			*Allium cepa*		*Fragaria x ananassa*	
Characters	Holotype female	Paratype females	Paratype males	Females	Males	Females	Males
n	1	23	25	23	22	21	22
L	1575	1371–1619 (1545±72.8)	1281–1578 (1433±78.8)	1250–1708 (1392±98.3)	1201–1473 (1362±69.6)	1203–1480 (1340±62.1)	1182–1390 (1264±55.6)
a	38.9	35.5–44.8 (40.6±2.7)	50.1–60.4 (54.3±2.9)	31.3–45.5 (40.0±3.6)	39.7–53.5 (44.4±3.6)	30.2–47.1 (38.0±4.1)	35.4–52.7 (46.1±4.3)
b	8.4	7.0–9.0 (8.2±0.5)	6.3–8.2 (7.4±0.6)	6.4–8.2 (7.1±0.5)	6.3–7.8 (6.9±0.4)	6.0–8.8 (6.9±0.6)	5.5–6.4 (6.0±0.3)
c	22.6	18.3–28.1 (23.2±2.3)	18.4–26.4 (23.4±2.4)	13.5–19.5 (15.1±1.5)	13.9–16.3 (14.9 ±0.6)	14.1–18.7 (16.3±1.4)	14.0–17.3 (16.1±1.1)
c'	4.0	2.9–4.8 (3.7±0.4)	3.5–5.0 (4.2±0.5)	4.2–5.5 (4.8±0.4)	4.7–6.2 (5.3±0.4)	3.7–6.0 (4.9±0.6)	4.0–6.4 (5.1±0.7)
e*	2.8	2.4–3.8 (3.0 ±0.3)	–	1.4–2.1 (1.7±0.1)	–	1.6–2.4 (2.0±0.2)	
Stylet	10	9–13 (11±0.7)	9–11 (10±0.4)	10–12 (11±0.5)	10–12 (11±0.4)	9–11 (10±0.3)	9–11 (10±0.4)
V%	83	81–85 (83±1.0)	–	80–86 (82±1.5)	–	79–84 (81±1.2)	–
Spicules	–	–	20–24 (21±1.4)	–	22–28 (26 ±1.6)	–	20–27 (24±0.7)
Gubernaculum	–	–	6–9 (7±1.3)	–	8–11 (9±1.2)	–	8–10 (9±0.6)
Body width	40	32–44 (37±3.7)	23–29 (26±1.8)	31–45 (35±3.5)	24–34 (28±2.8)	27–43 (36±4.3)	24–35 (28±3.1)
Oesophagus length	188	170–203 (184±8.5)	176–214 (194±10.6)	175–208 (194±8.3)	159–207 (189±11.9)	153–245 (196±21.6)	193–230 (213±11.4)
Tail length	69	54–84 (65±7.0)	50–73 (61 ±6.2)	85–103 (93±5.5)	80–97 (87±4.3)	75–105 (83±7.6)	70–93 (79±6.7)
Anal body diameter	17	14–19 (17±1.1)	13–16 (14±1.0)	17–23 (20±1.2)	14–18 (16±1.1)	14–22 (17±2.5)	12–18 (16±1.6)
Anterior end to:median bulb	69	65–75 (71±2.7)	67–86 (75±4.5)	67–78 (73±3.0)	68–85 (73±4.3)	67–80 (72±4.2)	70–82 (75±3.1)
Anterior end to nerve ring	107	101–119 (108±6.2)	95–124 (112±7.3)	112–130 (119±5.8)	94–134 (116±9.4)	102–138 (116±10.7)	95–135 (112±11.1)
Anterior end to hemizonid	139	127–145 (137±6.8)	128–145 (139±6.5)	127–158(144±7.8)	131–151 (142±5.7)	130–155 (142±7.5)	125–158 (142±8.6)
Anterior end to excretory pore	144	133–150 (143±4.3)	130–150 (143±4.8)	140–168 (153±6.5)	136–162 (151±5.6)	137–165 (149±8.5)	135–165 (151±8.9)
Anterior end to vulva	1305	1132–1308 (1240±56.3)	–	1020–1475 (1144±97.2)	–	1025–1212 (1097±53.3)	–
Vulva-anus distance	201	172–240 (194±21.2)	–	132–175 (150±13.5)	–	145–188 (166±11.8)	–
Post-vulval uterine sac	114	101–150 (121±14.7)	–	70–100 (83±9.2)	–	63–118 (80±12.3)	–
Bursa length	–	–	48–78 (65±9.8)	–	62–97 (82±11.3)	–	50–93 (73±9.1)
Tail part length without bursa	–	–	12–30(21±5.7)	–	25–34 (29±2.8)	–	18–40 (29±8.6)

*e – vulva anus distance to tail length ratio.

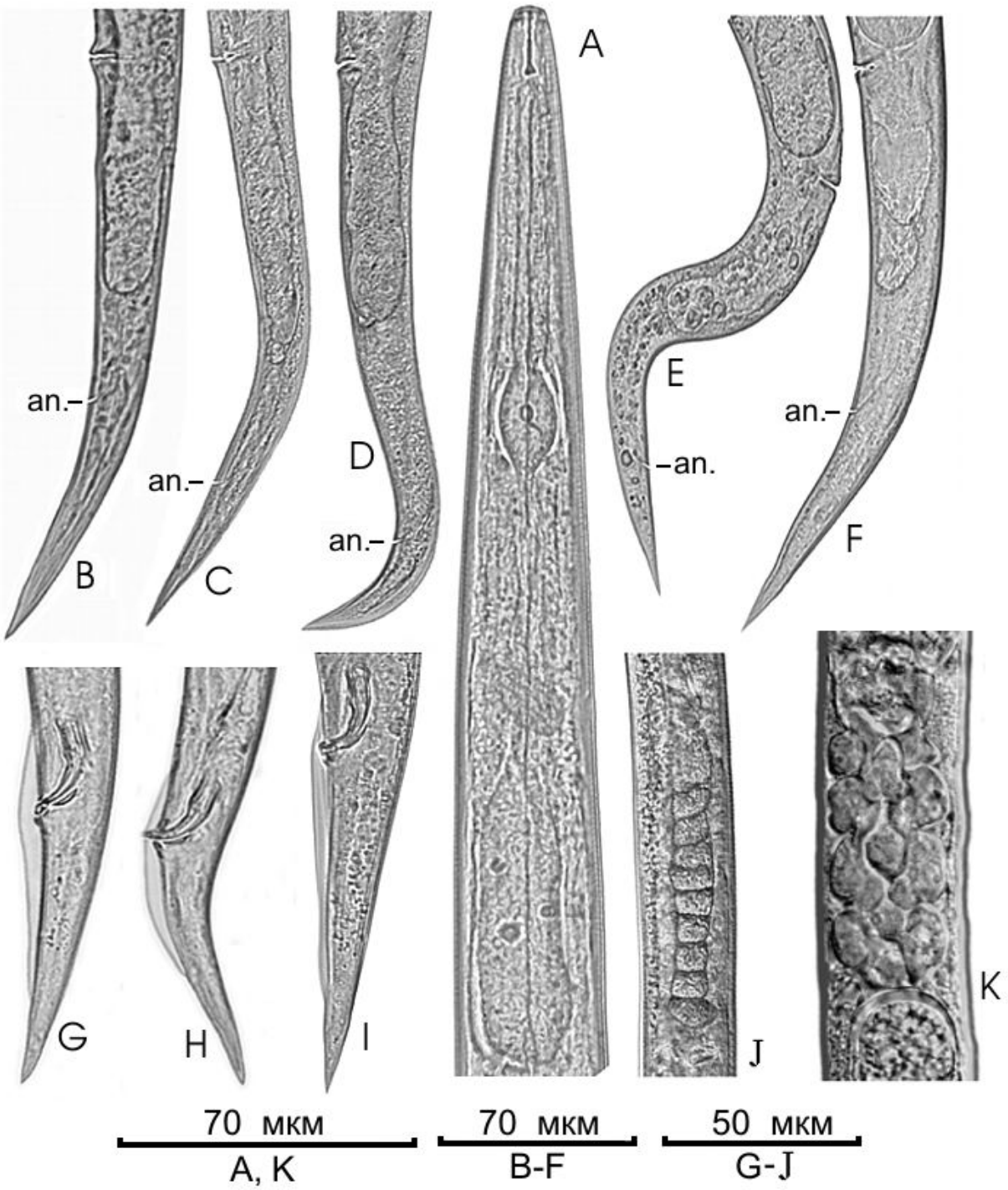

Fig. 2. *Ditylenchus weischeri* sp. n. - A: Anterior region of female; C-E: Posterior region of female; G, H: Tail region of male; J: Posterior region of male with testis; K: Region of uterus. *Ditylenchus dipsaci* - B, F: Posterior region of female; I: Tail region of male; an – anus.

seedling. After this treatment the seedlings were covered by polyethylene caps overnight. At least five plants of each crop were included in the test. The plant tissues were examined 1 month after

inoculation. The life cycle of the new species was studied in a naturally infected area. The samples were taken in 2 weeks intervals.

DNA extraction, PCR, PCR-RFLP cloning and sequencing were made as described by Subbotin *et al*. (2005) and Mundo-Ocampo *et al*. (2008). The ITS-rRNA and *hsp90* gene (Mundo-Ocampo *et al*., 2008) were amplified using TW81 and AB28, U831 and L1110 primers, respectively. Newly obtained sequences were submitted to the Genbank under the accession numbers: HM778133-HM778140. Sequences were aligned with ClustalX 1.83 and then analysed using Bayesian inference using MrBayes 3.1.2 as described by Mundo-Ocampo *et al*. (2008).

DESCRIPTION

Ditylenchus weischeri sp. n. (Table 1; Figs. 1 & 2)

Holotype female. See Table 1.

Females. Body shape is typical for parasitic nematodes of the genus *Ditylenchus* and became straight, sometimes slightly curved ventrally in posterior region, after killing by heating. Lip region offset body, 6-8 (7) μm wide and 2-3 (2.5) μm height. Stylet small, short with stylet knobs 2 μm in width. The orifice of dorsal pharyngeal gland in 1-2 μm from stylet basis. Cuticle with fine annulation, which is uniform throughout the entire length of body. Annules 0.7 to 0.9 μm wide. Lateral field indistinct, with four incisures, occupies 15-30% of body width, or 4-6 μm, two internal incisures poorly visible. Distance between incisures almost equal, but sometimes it is larger between internal than between external incisures. Procorpus cylindrical, narrowing slightly toward the median bulb. Median bulb oval with developed valve, which is situated in a bulb center or slightly displaced anteriorly. Median bulb 17-23 (30) μm long and 10-16 (13) μm wide. Isthmus narrow and relatively long. Basal bulb well developed, occupies nearly 75% of body width. Basal bulb with a slight overlap over intestine. Nerve ring at the level of posterior half of isthmus. Hemizonid and excretory pore at the level of anterior half of basal bulb. Posterior edge of hemizonid within few annules, or 1-10 μm anterior to excretory pore. Ovary outstretched, 80-188 (130) μm long, sometimes reaching basal or median bulb, with one flexure. Spermatheca sac-shaped. Crustaformeria in form of quadricolumella consists of 16-20 cells arranged in 4 rows, per 4-5 cells in a row. Oocytes and ooginia arranged in 2-3 rows. Post-vulval uterine sac well developed, more than 50% of vulva to anus length. Anus well observed. Tail relatively short, elongate conical with pointed terminus.

Males. Body elongated and slender. Structure of anterior region is similar to that for females. Testis outstretched, reaching basal bulb basis, with one flexure. Spermatozoa oval shape, 7-10 (9) μm. Bursa does not enclose a tail tip. Spicules tylenchoid type. Tail elongate-conical, tapers to pointed tip.

Eggs (n=15): L = 69-91 (78) μm, W = 26-40 (32) μm.

Biology. In Moscow region, three-four generations occur during a vegetation season. Symptoms on infected plants can be observed from the second half of summer and become clearly visible at the beginning of flowering. Stem swellings or galls are formed at a distance of nearly 1 m and higher above soil level (Fig. 3). The galls were characterised by extensive hypertrophy and hyperplasia, differentiation of nutritive tissue, nuclear modification, and a central cavity containing nematodes (Watson & Shorthouse, 1979). At the end of flowering during the second half of August, when stem swellings become black and start cracking, the majority of nematodes start leaving the plant tissues and move into soil, where the fourth-stage juveniles overwinter. The fourth-stage juveniles infect seedlings of creeping thistle plants during early spring. Nematodes can survive in plant tissues in an anhydrobiotic condition for at least up to three years.

The results of the host tests revealed that *D. weischeri* sp. n. did not develop and complete its life cycle in onion or garden strawberry seedlings.

Type host-plant. Creeping thistle, *Cirsium arvense* (L.) Scop.

Type locality. Village Maikovo, Pushkin district, Moscow region, Russia.

Other localities. *Ditylenchus weischeri* sp. n. was found in other north and north-west districts of the Moscow region and in several regions of Russia: Jaroslavl, Tver, Ivanov and Vladimir regions. Watson and Shorthouse (1979) reported the stem nematodes from *C. arvense* collected in a pasture near Regina, Saskatchewan, Canada, which, perhaps, belongs to *D. weischeri* sp. n. Janezic (1994) also found the stem nematode from *Cirsium oleraceum* leaves in Croatia.

Differential diagnosis. *Ditylenchus weischeri* sp. n. differs from *D. dipsaci* by shorter tails in adults (females = 55-84 μm *vs* 70-108 μm; males = 50-73 μm *vs* 80-97 μm), larger c index (18-28 *vs* 11-22), shorter spicules (20-24 μm *vs* 20-30 μm), longer vulva-anus distance (172-240 μm *vs* 132-188 μm), larger vulva-anus distance to tail length ratio (2.4-3.8 *vs* 1.4-2.1), longer post-vulval uterine sac (101-150 *vs* 70-100 μm) (Table 1; Metlitskii, 1968; Brzeski, 1998).

Chromosome numbers. Barbashova (1979) reported 2n = 56 for the stem nematode from *Cirsium* and 2n=24 for *D. dipsaci sensu stricto.*

Etymology: This species is named after Prof. Dr Bernhard Weischer.

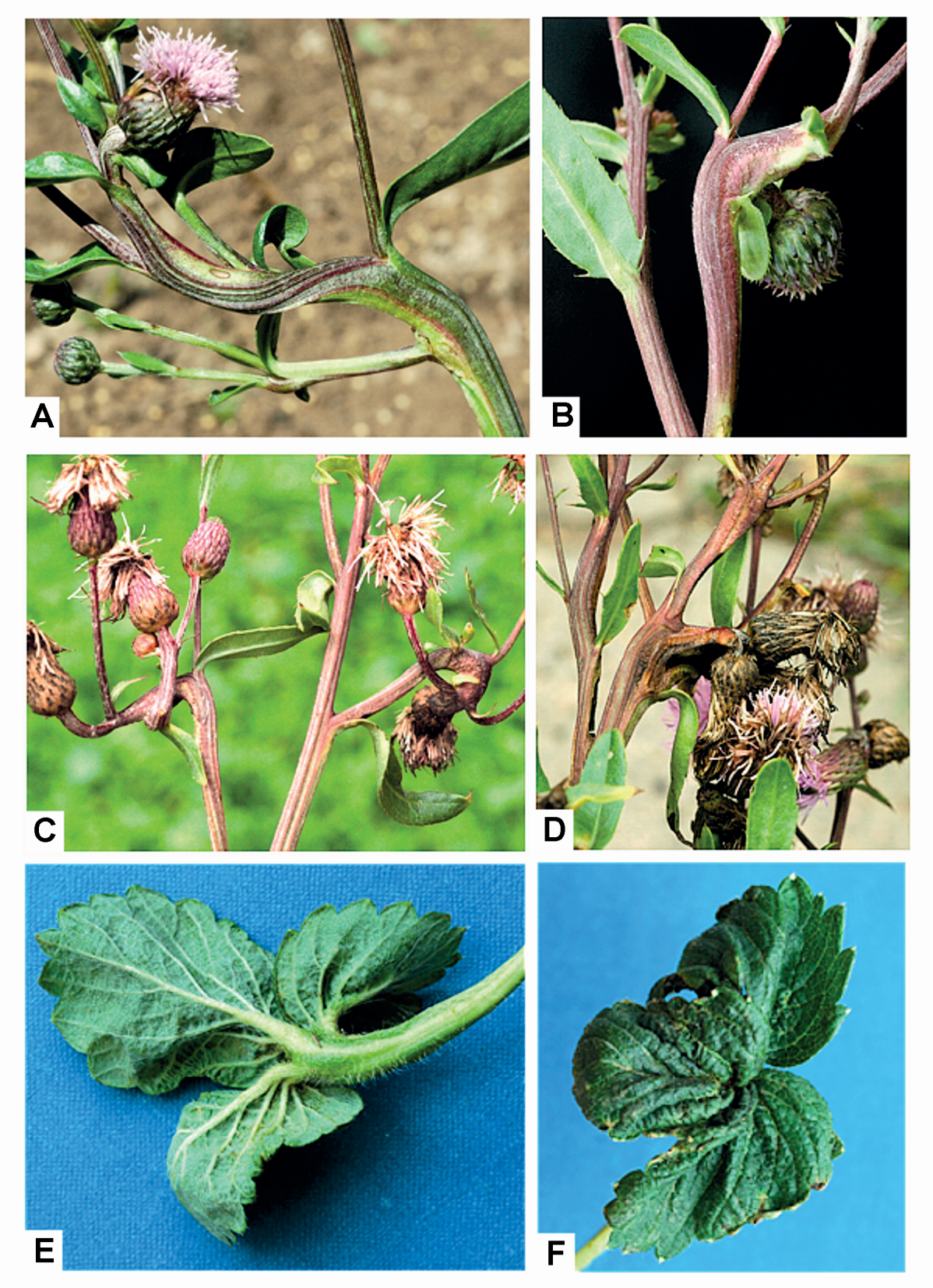

Fig. 3. *Ditylenchus weischeri* sp. n. A-D: Symptoms of infection on *Cirsium arvense*. *Ditylenchus dipsaci.* E, F: Symptoms of infection on strawberry.

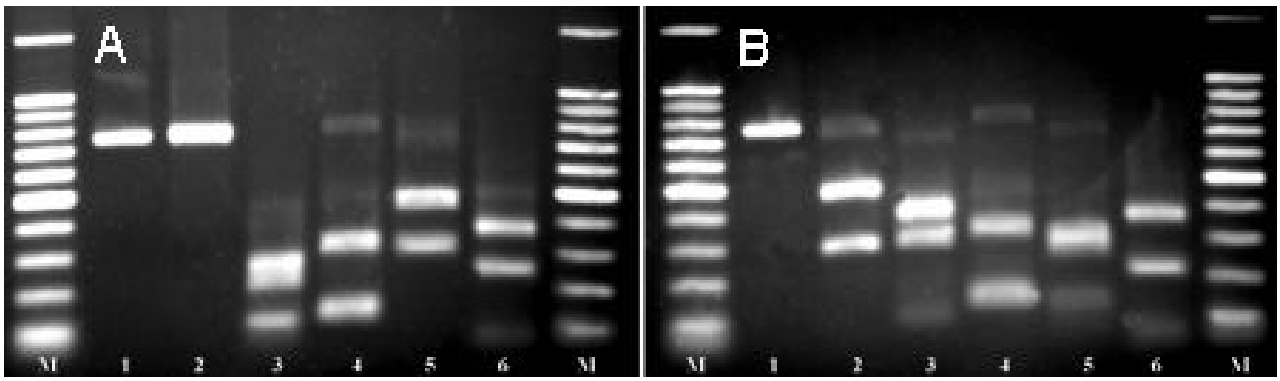

Fig. 4. PCR-ITS-RFLP profiles. A: *Ditylenchus weischeri* sp. n., B: *D. dipsaci*. M – 100 bp DNA marker (Promega); U – unrestricted PCR product; 1 - *Bsh*1236I; 2 – *Hinf*I; 3 – *Msp*I; 4 – *Rsa*I; 5 – *Taq*I.

Table 2. Restriction fragment lengths (bp) for PCR-ITS-RFLP of two plant-parasitic *Ditylenchus* species.

Restriction enzymes	*D. weischeri* sp. n.	*D. dipsaci*
Unrestricted PCR product	750	752
*Bsh*1236I	750	467, 285
*Hin*fI	262, 214, 111, 88, 46, 29	374, 289, 89
*Msp*I	315, 140, 139, 133, 23	316, 140, 139, 134, 23
*Rsa*I	450, 300	300, 264, 127, 61
*Taq*I	358, 242, 65, 51, 34	359, 208, 65, 51, 35, 34

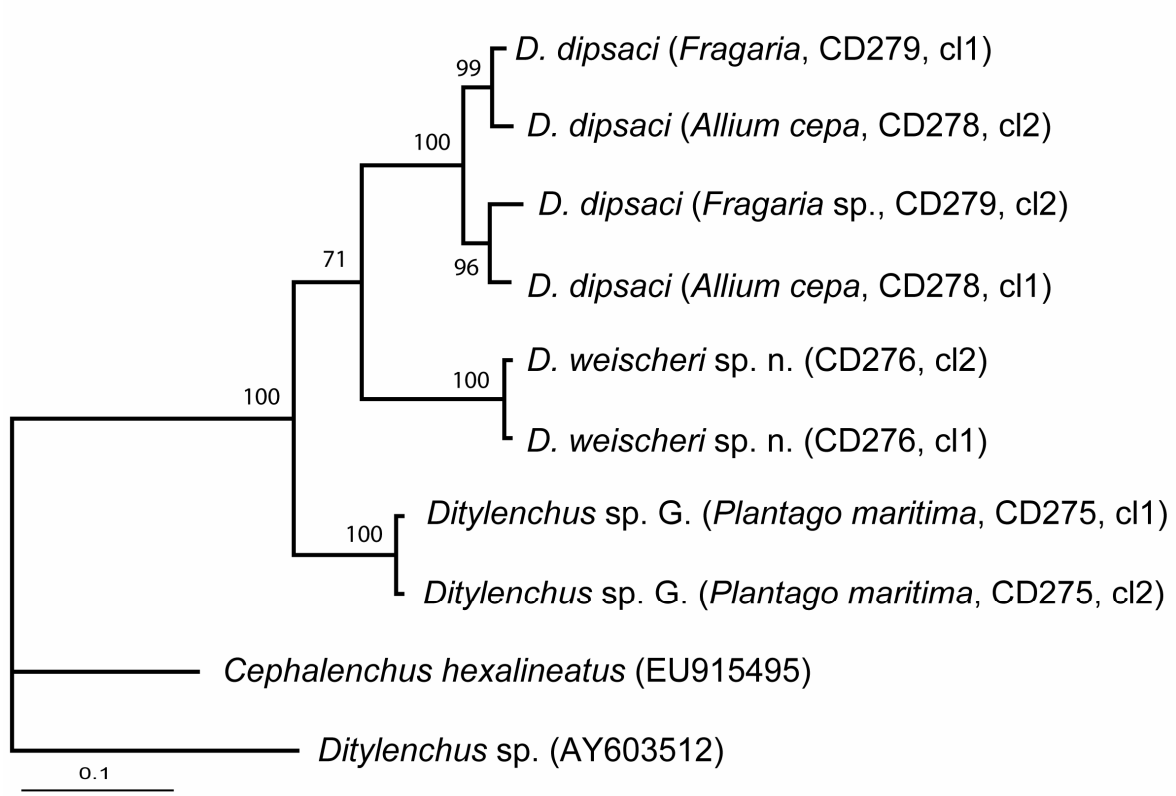

Fig. 5. Phylogenetic relationships of plant-parasitic *Ditylenchus* species as inferred from the analysis of *hsp90* gene sequences using Bayesian analysis.

Slides. Holotype and paratypes of *D. weischeri* sp. n. were deposited under the number 98/33-40 in the Nematological collection of the Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Moscow, Russia.

Molecular characterisation. The analysis of the ITS-rRNA of plant-parasitic *Ditylenchus* species revealed that *D. weischeri* sp. n. (or *Ditylenchus* sp. C) was different from *D. dipsaci sensu stricto* and several other still undescribed *Ditylenchus* species and formed a separate clade (Subbotin *et al.,* 2004, 2005). A similar picture of the phylogenetic relationships of plant-parasitic *Ditylenchus* species was obtained after analysis of *hsp90* gene sequences using Bayesian analysis (Fig. 5). PCR-ITS-RFLP patterns for *D. weischeri* sp. n. and *D. dipsaci sensu stricto* are given in Fig. 4. The restriction of PCR products by enzymes *Bsh1236*I, *Hin*fI, *Rsa*I and *Taq*I enables these two species to be distinguished from each other (Table 2).

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Чижов В. Н., Борисов Б. А., Субботин С.А. Новая стеблевая нематоды *Ditylenchus weischeri* sp. n. (Nematoda: Tylenchida) – паразит *Cirsium arvense* (L.) Scop. в Центральном регионе Нечерноземной зоны России.

Резюме. По материалу, собранному в Московской области, описан новый вид стеблевых нематод *Ditylenchus weischeri* sp. n., паразитирующий на чертополохе *Cirsium arvense* (Asteraceae). *Ditylenchus weischeri* sp. n. отличается от *D. dipsaci* меньшей длиной хвостового конца, более высоким значением индекса «c», короткими спикулами, большим расстоянием от вульвы до анального отверстия, большим отношением этого расстояния к длине хвостового конца и большей длиной рудимента задней матки. *Ditylenchus weischeri* sp. n. отличается от других видов *Ditylenchus* по последовательностям ITS-rRNA и гена *hsp90*, а также по числу хромосом. *Ditylenchus weischeri* sp. n. не способен развиваться и завершать жизненный цикл в растениях лука и саженцах клубники, являющихся типичными хозяевами *D. dipsaci*. Приводятся характерные профили PCR-ITS-RFLP для *D. weischeri* sp. n. и *D. dipsaci.*