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Heterodera cruciferae Franklin, 1945, a parasite of *Brassica oleraceae* L. from floodland fields in the Moscow region, Russia

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Summary. During a nematological survey the cabbage cyst nematode, *Heterodera cruciferae,* was found from cabbage growing areas along the Oka River, Ozery and Serpukhov districts of the Moscow region, Russia. It is the first report of this nematode in the Moscow region. Rape, rutabaga and radish were identified as additional host-plants for this nematode. Morphological, morphometrical and molecular characterisation as well as description of symptoms induced by *H. cruciferae* are given.

Key words: cabbage, cabbage cyst nematode, *Heterodera cruciferae,* morphology PCR-RFLP, rRNA.

In 2005-2006 during a nematological survey the cabbage cyst nematode, *Heterodera cruciferae,* was found in several cabbage fields along the Oka River in the south part of the Moscow region, Russia. The population density of the cabbage cyst nematode in infested areas reached 180 cysts per 100 g of rhizosphere soil. Kirjanova & Krall (1971) reported *H. cruciferae* in several regions of Russia and republics of the former USSR; this is the first report of this nematode in the Moscow region. Cysts were collected from the infested fields and reared on *Brassica oleracea* L. *var. capitata* for further studies. Morphometrical, molecular characterisation, and life cycle details are provided.

MATERIAL AND METHODS

Nematode population. The descriptions and molecular studies were based on the population of *H. cruciferae* collected from Ozery and Serpukhov districts in the Moscow region. Cysts were isolated by the sieving-decanting method. In addition, some cysts and males were isolated from roots of *Brassica oleracea* L. *var. capitata* maintained in the glasshouse. Juveniles were usually released from crushed cysts or extracted from soil using the Baermann funnel method.

Light microscopy. Second-stage juveniles and males were killed and fixed in hot 4%TAF and processed to glycerin. The specimens were mounted in dehydrated glycerin on permanent slides, examined and measured. The cyst vulval cone was mounted in glycerin jelly. The photographs were taken with an Axio Imager A1 Carl Zeiss light microscope equipped with Nomarskii differential contrast optics.

Molecular study. The methods used for DNA extraction, PCR amplification, cloning and sequencing were similar to those described by Tanha Maafi *et al.* (2003). Primers TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') were used in the PCR reaction. Several microlitres of the PCR product were digested by one of the following restriction enzymes: *Alu*I, *Ava*I, *Bsh*1236I, *Bsu*RI, *Hin*6I, *Mva*I and *Rsa*I in the buffer stipulated by the manufacturer. The digested DNA was run on a 1.5% TAE buffered agarose gel, stained with ethidium bromide and photographed. Two clones of PCR products were directly sequenced in both directions using TW81 and AB28. Sequences were submitted to the GenBank under accession number: GU126667 and GU126668. Original and known sequences of *H. cruciferae* were aligned using ClustalX 1.64 with default options with *H. carotae* (Subbotin *et al.*, 2001; Madani *et al.*, 2004).

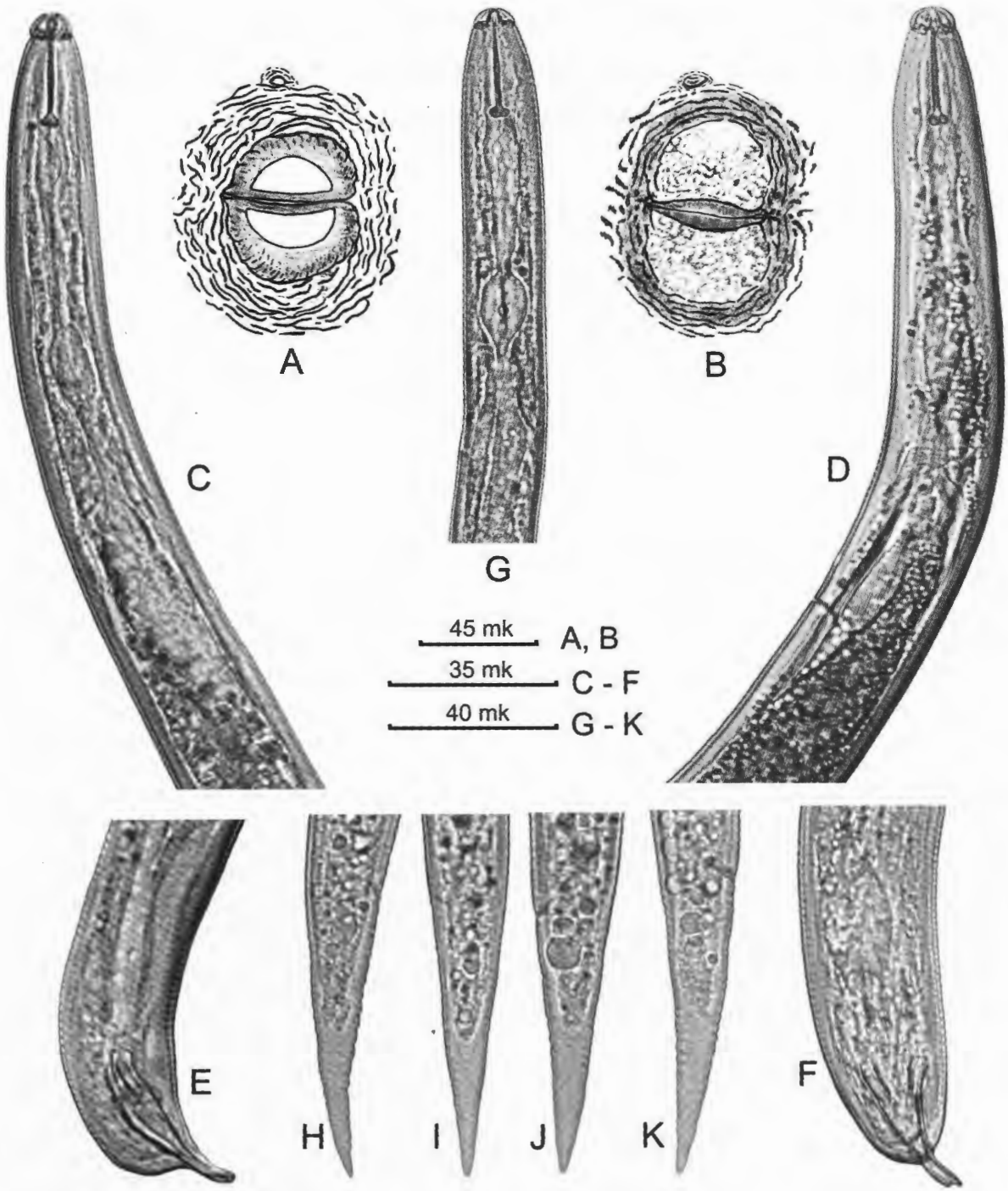

Fig. 1. *Heterodera cruciferae*. A-B; vulval plates showing fenestration and vulva slit; C, D: Anterior region of male; E, F: Posterior region of male; G: Anterior region of second-stage juvenile; H-K: Tail variation of second-stage juvenile.

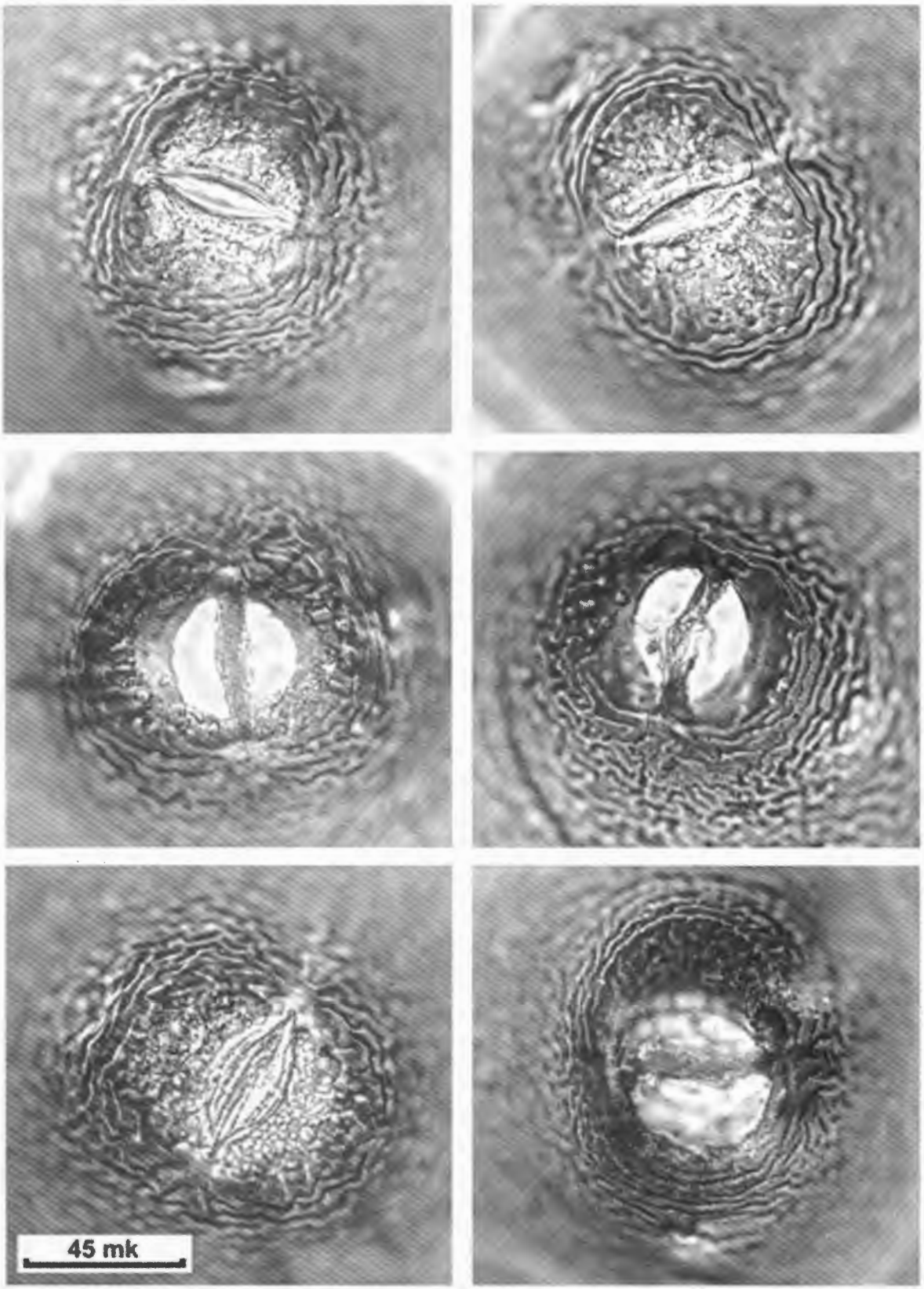

Fig. 2. *Heterodera cruciferae*. Variations of vulval plates.

Table 1. Morphometrics of *Heterodera cruciferae* from different reported populations (measurements in µm, range with mean ± standard deviation).

Characters	Russia, Moscow region (original)	Germany (Wouts & Weischer, 1977)	UK (Stone & Rowe, 1976)	Iran (Jabbari & Niknam, 2008)
Second-stage juveniles (n)	28	20	25	
L	377–504 (426±31.8)	426±3.8	431±19	351.2±15
a	14.5–31.8 (20.5±2.7)			
b	4.1–5.1 (4.5±0.2)			
b'	2.1–3.1 (2.4±0.2)			
c	8.2–10.0 (9.1±0.5)			
c'	3.2–4.4 (3.7±0.3)			
Stylet length	21–25 (23±0.9)	24.5±0.13	24.1±1.6	21±0.8
Anterior end to median bulb	62–81 (70±4.1)	72.3±0.81	68.3±3.1	-
Anterior end to excretory pore	91–117 (103±6.2)		101.6±4.8	95-100
Oesophageal length	85–106 (95±5.9)			
Anterior end to oesophageal gland base	141–208 (178±14.5)			
Body width	12–27 (21±2.2)	20.0±0.19	20.8±1.5	
Body width at anus	11–14 (13±0.8)			
Tail length	38–54 (47±4.0)	47.8±0.6	50.0±2.7	41±5
Hyaline part of tail	17– 30 (24±3.3)	24.5±0.5	25.2±2.7	21.3±1.9
Males (n)	24		25	-
L	718–1343 (1156±132.1)		1170±79	961±83
a	18.9–47.2 (35.3±5.8)		40.8±5.8	38.2±2.5
b	5.2–9.5 (8.2±0.9)			
b'	5.2–6.8 (6.1±0.4)			
c	2.0–7.0 (3.8±1.6)			
c'				
Stylet length	22–28 (25±1.7)		24.9±1.1	23±1.6
Body width	23–43 (33±5.4)		29.1±1.4	
Anterior end to median bulb	68–107 (96±8.7)		97.8±4.9	
Anterior end to excretory pore	140–182 (159±10.8)		154.4±12.3	
Oesophageal length	119–168 (144±14.3)			
Anterior end to oesophageal gland base	163–208 (192±14.8)			
Spicule length	29–38 (34±2.4)		34.5±2.4	21±4.4
Gubernaculum length	7–11 (9±1.6)		10.0±1.0	7±1.1

RESULTS AND DISCUSSION

Heterodera cruciferae Franklin, 1945 (Figs. 1 & 2)

Cysts (n=25): Mature cysts lemon shape with relative small vulval cone. Subcrystalline layer usually not observed; however, in some young females the layer is rather thin. Cyst length = 355–690 (557 ± 71.6) µm; cyst width = 300–460 (398 ± 72.3) µm; L/W ratio = 0.6 – 1.8 (1.4 ± 0.3) µm; neck length = 40–90 (66 ± 17.4) µm. Vulval plates have the following size = 47–75 (59 ±6.1) µm x 55–95 (72±9.9) µm, ambifenestrate, semifenstral width = 12–25 (17±3.5) µm; semifenstral length = 27–53 (41 ± 5.5) µm, vulval slit length = 32–55 (46 ± 6.3) µm; vulva-anus distance = 37–65 (51 ± 6.5) µm.

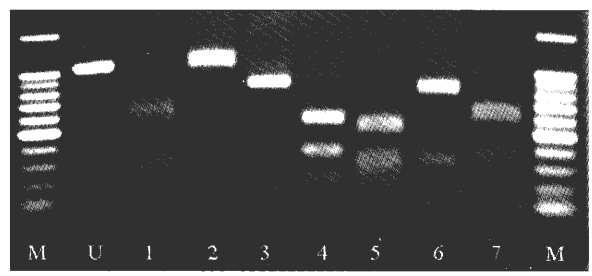

Fig. 3. PCR-ITS-RFLP profile for *Heterodera cruciferae*. Code: M – 100bp DNA ladder (Promega), U – unrestricted PCR product, 1- *Alu*I, 2- *Ava*I, 3 - *Bsh*1236I, 4 - *Bsu*RI, 5 - *Hin*6I, 6 – *Mva*I, 7 – *Rsa*I.

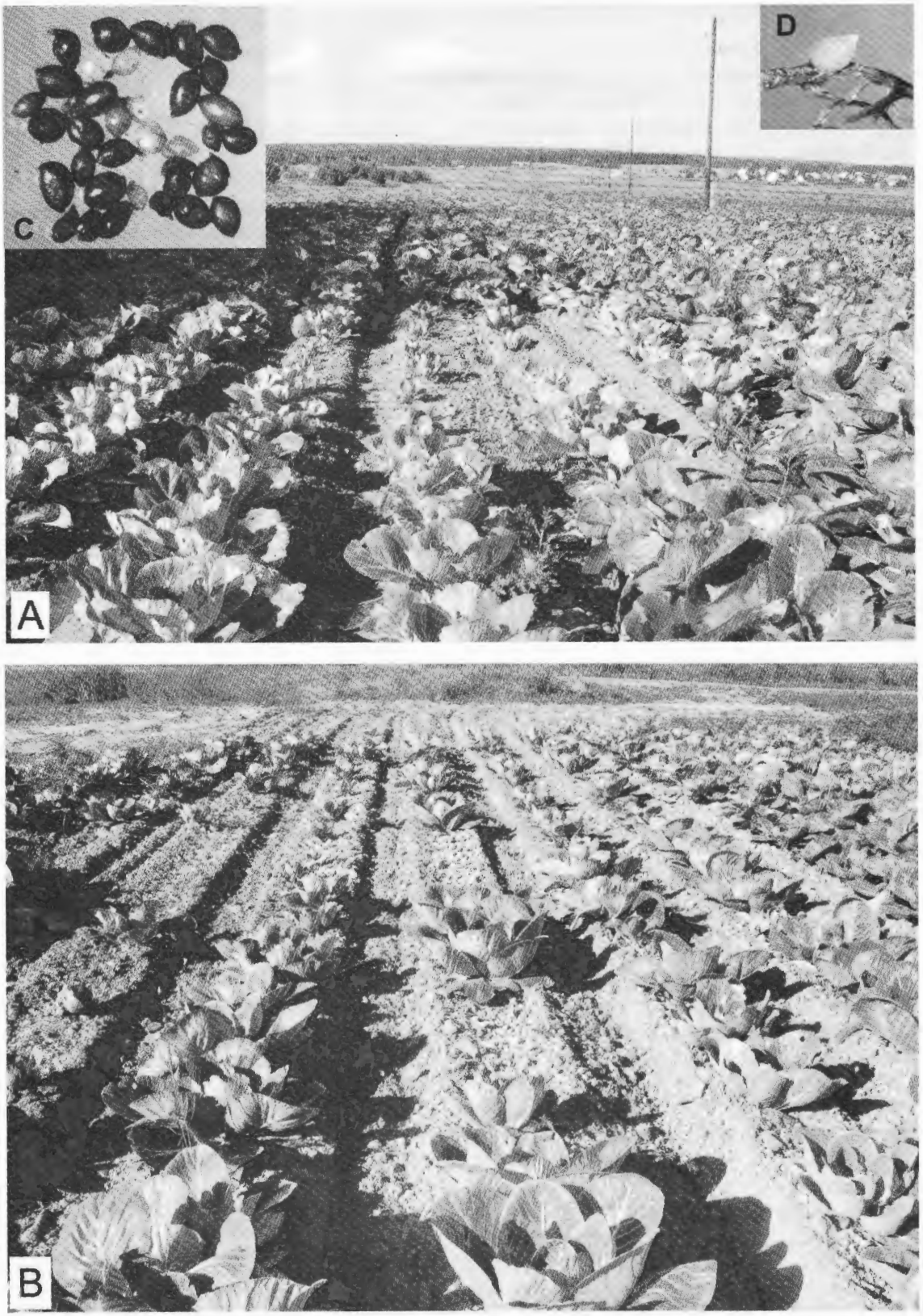

Fig. 4. A, B: Infected area on a field with *Brassica oleracea* L. *var. capitata*. C: Cyst; D: Young cyst on roots.

Bullae absent. Egg sac present. Number of eggs in cysts = 64–271 (121 ± 49).

Males (n=24): See: Table 1.

Vermiform, slightly curved dorsally by the larger bent in posterior region. Cuticle annulated, annule width = 1.5–1.9 (1.7) μm at mid-body. Lateral field with four incisures, 6–8 μm in width, with equal distance between incisures, not areolated. In some specimens the distance between two internal incisures is wider than between internal and external incisures. Lip region width = 10–12 μm; lip region length = 6–8 μm; semispherical, contains 4-5 annuli. Stylet well developed, metenchium is equal of telenchium. Stylet knob backward, width of base knob = 3–4 μm. Distance of the dorsal oesophageal gland opening from the stylet base = 4–6 μm. Procorpus cylindrical; narrow, even more greatly it becomes narrow at junction with median bulb. Median bulb occupies approximately one-third body width. Median bulb valve weakly sclerotised, located somewhat lower than middle of bulb. Isthmus is relatively narrow and short. Nerve ring is located in the lower part of isthmus. Oesophageal glands overlap the base of the intestine from the ventral side to 1.5–2.0 diameters of body at their level. Excretory pore at the level of median bulb, hemizonid 4-6 annuli in front of excretory pore. Distance from anterior end to the beginning of genital tract = 462–728 (546) μm; genital tract length = 239–734 (617) μm. Spermatozoa are round or slightly oval shape, diameter 3 μm. Spicules are nearly terminal. Width of the base of spicules is approximately 3 μm. In some individuals a small protrusion is visible.

Second-stage juveniles: See: Table 1.

Vermiform, lip region semispherical, consists of 4 annuli. Lateral field with 4 incisures. Stylet well developed, metenchium is slightly shorter than telenchium. Stylet knob height = 1.5 μm, stylet knobs width = 3–4 μm. Distance of the dorsal oesophageal gland opening from the stylet base = 3-4 μm. Procorpus becomes narrow before junction to median bulb. Median bulb oval, 70-80% of width of body. Valve below the middle of bulb. Nerve ring in the center of istmus. Excretory pore in the upper part of the cardial bulb. Walls of the excretory pore slightly sclerotised. Hemizonid immediately above the excretory pore, about 2 μm long. Genital primordium lower than the middle of body, at a distance of 213–296 (253±27.6) μm. Rectum prominent. The hyaline part of tail is approximately half of the tail length. Tail terminus rounded.

Eggs (n=35): L = 95–125 (105±7.0); W= 42–67 (56 ± 5.6); L/W ratio = 1.4–2.5 (1.9 ± 0.2) μm.

The morphological and morphometric characters of *H. cruciferae* found in the Moscow region were similar to those of the European population described by Stone and Rowe (1976). However, there were some minor differences between these populations. The Moscow population had larger cyst (557±72 x 398±72 *vs* 429±67 x 333±56 μm) and slightly greater size of vulval plate. The morphometrics of males and second-stage juveniles were also similar (Table 1). The Moscow populations were significantly different from the Iranian population described by Jabbari & Niknam (2008) in some characters: in longer vulval slit length, longer body and spicule lengths in males, and longer body length in second-stage juveniles (Table 1).

Molecular characterization. PCR with universal primers TW81 and AB28 yielded a single product of 1040 bp length. The PCR-ITS-RFLP diagnostic profile with seven restriction enzymes is given in Fig. 3. The length of fragments corresponded to those for *H. cruciferae* from the Netherlands (Subbotin *et al.*, 2000). The ITS-rRNA sequences of two clones were different in 5 nucleotides from each other and they were very similar to *H. carotae* sequences.

Biology. Under the conditions of the middle regions of Russia on the late cultivars of cabbage *H. cruciferae* had three generations per year. In the middle of the host growth period, distinct symptoms of infection were observed on the cabbage fields with mainly cruciferous vegetables (50-60%) in the crop rotation. Infected plants showed delayed formation of heads and the death of many plants was also observed (Fig. 4). Population levels in soil at the edges of the infestation area reached 100 eggs and juveniles g^{-1} soil and in such cases the majority of the infected plants appeared relatively healthy. The increased death of plants in the cropping areas could be explained by the effects of pathogen interactions because after nematode infection, pathogenic fungi and bacteria colonised damaged cabbage roots. In addition to cabbage, a good host for *H. cruciferae* was rape (*Brassica napus* L. var. *napus*). In the territory of the Moscow region the host plants for *H. cruciferae* were also rutabaga, *Brassica napus L. var. napobrassica* (Mill) and radish, *Raphanus sativus* L.

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Резюме. Во время нематологического обследования капустных полей в пойме реки Оки в Озерском и Серпуховском районах Московской области была обнаружена капустная цистообразующая нематода *Heterodera cruciferae*. Это первое сообщение о присутствии этой нематоды в Московской области. Рапс, брюква и редис отмечены как дополнительные растения-хозяева этой нематоды. Дается морфологическая, морфометрическая и молекулярная характеристика *H. cruciferae*, а также описание симптомов поражения этой нематодой.