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# *HETERODERA CICERI* SP. N. (NEMATODA: HETERODERIDAE) ON *CICER ARIETINUM* L. FROM NORTHERN SYRIA

by

N. VOVLAS, N. GRECO and M. DI VITO

During a survey on plant-parasitic nematodes of pulse crops undertaken in Syria in 1983 (Greco *et al.*, 1984), several fields of chickpea (*Cicer arietinum* L.) showed marked decline of the plants. Soil and root samples collected in these fields revealed the occurrence of second stage juveniles, males, females and cysts of a species of a cyst forming nematode. The first report of this nematode is probably that of Bellar and Kebabeh (1983) who reported that in Syria 19% of the lentil fields surveyed in 1979-1980 were infested with a cyst nematode, *Heterodera* sp.. Unfortunately the authors gave no measurements nor microphotographs of the nematode for comparison with our population. Mamluk *et al.* (1983) also reported a *Heterodera* sp. close to *H. rosii* Duggan and Brennan in the same area. However, detailed examination on our population indicated that it is a previously undescribed species.

## Materials and Methods

Nematodes were collected from the roots of chickpea in naturally infested fields at the type locality and reared on chickpea in a greenhouse at the Istituto di Nematologia Agraria, C.N.R., Bari. Newly developed males, females and cysts and newly emerging 2nd-stage juveniles, were used in this study. Males and juveniles were killed

and fixed in hot aqueous 4% formaldehyde 1% propionic acid, dehydrated in ethanol vapour and mounted in dehydrated glycerin (Hooper, 1970). Cysts and eggs were fixed and mounted in 2.5% formalin. Second stage juveniles and males for SEM observations were killed and fixed in formalin-propionic acid 4:1, transferred to 1% osmium tetroxide solution for 12h, then infiltrated with Spurr's resin and mounted on SEM specimen stubs (De Grisse, 1973; Clark and Stone, 1973).

Cysts preserved in glycerin were used for SEM observations of the perineum, the head and neck portions. The specimens were coated with gold and observed in the SEM at 5 kv accelerating voltage.

### Description

#### *Heterodera ciceri* sp. n.

*Holotype female*: Body length (excluding neck) = 880  $\mu$ m; body width = 450  $\mu$ m; L/W ration = 1.9; neck length = 135  $\mu$ m; stylet length = 29  $\mu$ m; excretory pore = 160  $\mu$ m from anterior end; vulva anus distance = 84  $\mu$ m; median bulb rounded, 41  $\mu$ m in diameter.

*Embryonated eggs*: (n = 26) length = 134 (123-143)  $\mu$ m; width = 50 (48-53)  $\mu$ m; L/W ration = 2.5 (2.4-2.8); egg shell unsculptured and hyaline. Second stage juvenile folded four times within the egg shell.

*Second-stage juveniles*: (n = 30) L = 525 (440-585)  $\mu$ m; a = 27 (25-29); b = 2.5 (2.3-2.7); c = 8.7 (8.2-8.9); stylet length = 28.6 (27-30)  $\mu$ m; maximum width at mid-body = 21 (19-22)  $\mu$ m; tail width at anus = 14-15  $\mu$ m; tail length = 60 (53-72)  $\mu$ m; length of hyaline tail portion = 36 (31-42)  $\mu$ m; head tip to median bulb distance = 82 (80-86)  $\mu$ m; head tip to excretory pore distance = 118 (105-128)  $\mu$ m; lateral field width = 4-5  $\mu$ m, 1/4 of body width; DGO = 5-6  $\mu$ m behind stylet knobs. Head hemispherical slightly off set, 4-5  $\mu$ m in length and 8-9  $\mu$ m in width, with three post-labial annules; an oral disc plate dorso-ventrally elongated and two rounded lateral sectors bearing large semilunar amphidial apertures (Figs. 1 B, C; 2 A, B). Stylet robust, knobs 2-2.5  $\mu$ m long, 4-5  $\mu$ m wide with concave anterior



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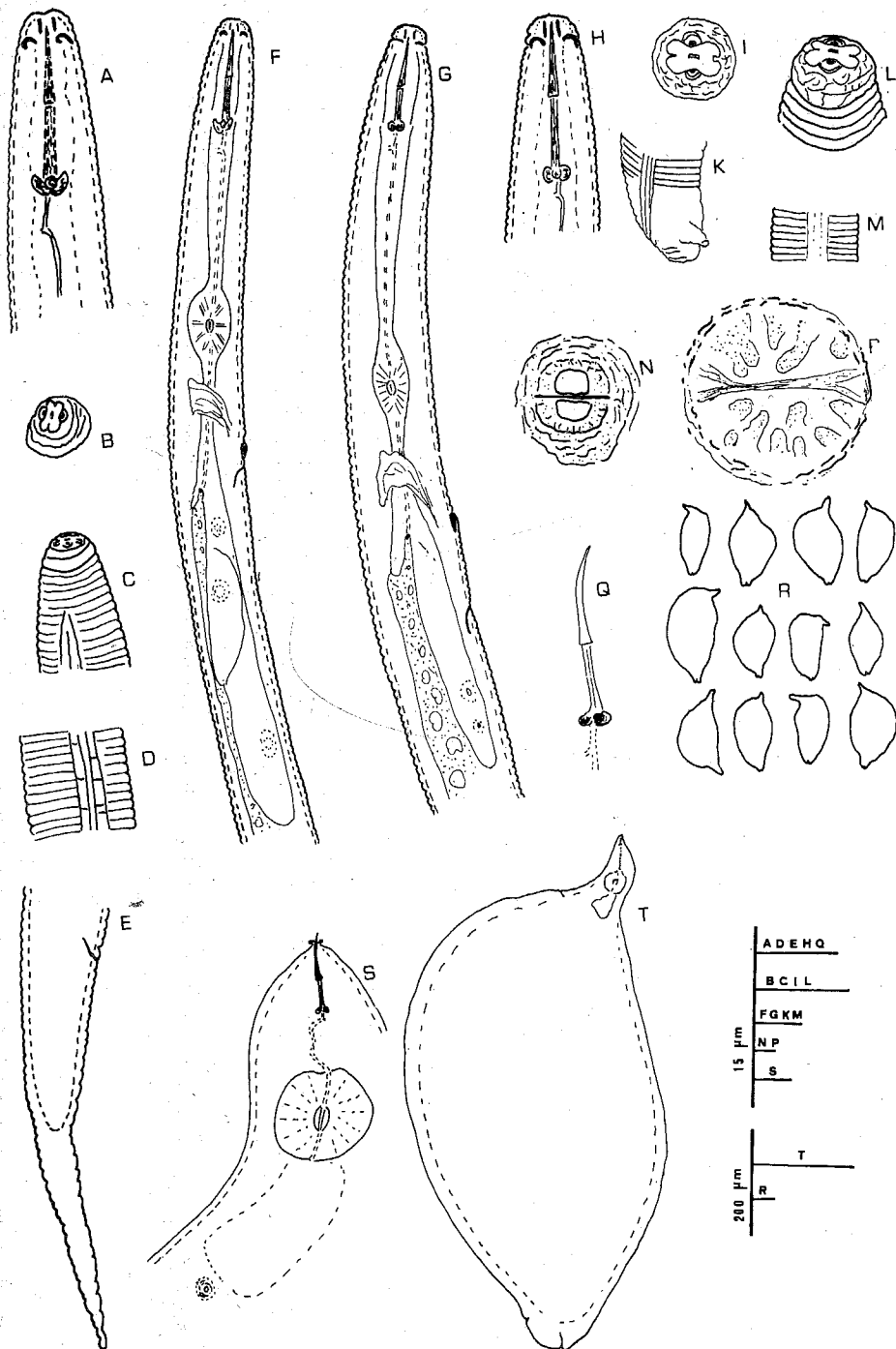


Fig. 1 - *Heterodera ciceri* sp. n.: A) Anterior end of second stage juvenile; B, C) second stage juvenile lip pattern and profile; D) 2nd-stage juvenile mid-body lateral field; E) 2nd-stage juvenile tail; F, G) Oesophageal region of second stage juvenile and male respectively; H) Anterior end of male; I, L) Lip pattern and profile of male; K) Male tail; M) Male mid-body lateral field; N) Fenestrae and vulval slit; P) Bullae and underbridge; Q) Female stylet; R) Outline drawing of cysts; S) Anterior portion of female; T) Outline drawing of a gravid female body.

surfaces. Oesophageal glands well developed, 37% (30-42) body length from head. Hemizonid distinct and two annules long, 1-2 annules anterior to excretory pore. Oval genital primordium, 13-15  $\mu\text{m}$  long, 10-13  $\mu\text{m}$  wide, 218 (210-232)  $\mu\text{m}$  anterior to tail tip. Cuticular annulations distinct. Lateral field with four incisures, 20-25% of body width having outer bands aerolated (Figs. 1 D, 2 C, F). Tail irregularly annulated, tapering uniformly and rather abruptly to a finally rounded terminus, with a terminal hyaline portion 54 (48-58)% of tail length (Fig. 2 E-H).

*Adult females:* (n = 12) L (excluding neck) = 773 (550-950)  $\mu\text{m}$ ; width = 451 (300-520)  $\mu\text{m}$ ; L/W ration = 1.7 (1.5-2.0); stylet length (n = 8) = 30 (29-31)  $\mu\text{m}$ ; DGO (n = 6) = 56  $\mu\text{m}$ ; excretory pore = 158 (154-169)  $\mu\text{m}$  from anterior end. Female body typically lemon-shaped with well defined neck and prominent terminal cone (Figs. 1 T, 3 A, B, D). Adult females opaque-white in colour, turning yellow and then yellowish-brown at the start of tanning. Occasionally a thin sub-crystalline layer may cover the entire female body. Gelatinous matrix (without eggs) present after the female becomes gravid. Vulva a terminally positioned transverse slit. Anus subterminal, located in a depression 7-10  $\mu\text{m}$  in diameter (Figs. 3 D, F, G). The cuticle is ornamented by zig-zag external ridges at mid body, but ornamentation of the neck region and in the cone consists of a series of unbroken lines which in SEM end-on view appears as a number of concentric circles surrounding (Fig. 3 E) the vulva area.

*Cysts:* (n = 20) L (excluding neck) = 796 (570-930)  $\mu\text{m}$ ; W = 452 (350-550)  $\mu\text{m}$ ; L/W ration = 1.77 (1.56-2.37); neck = 115 (90-160)  $\mu\text{m}$ .

Cysts are typically lemon-shaped with a distinct neck and a prominent cone. Neck may be off set and curved posteriorly (Fig. 1 R). Cyst well ornamented with irregular zig-zag ridges, forming a fine network over the body. A radial pattern of 15-20 straight ridges on the apex of the vulval cone, including the semifenestral areas before

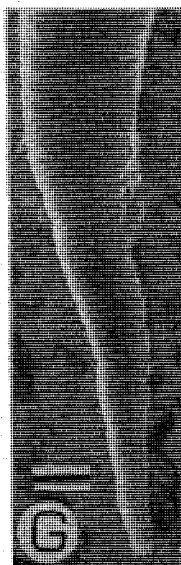
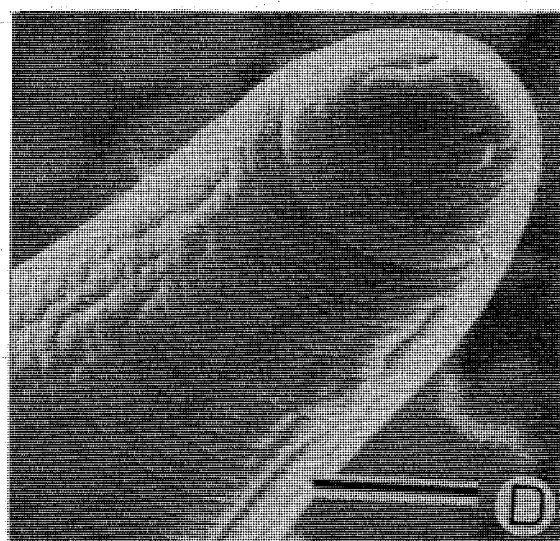
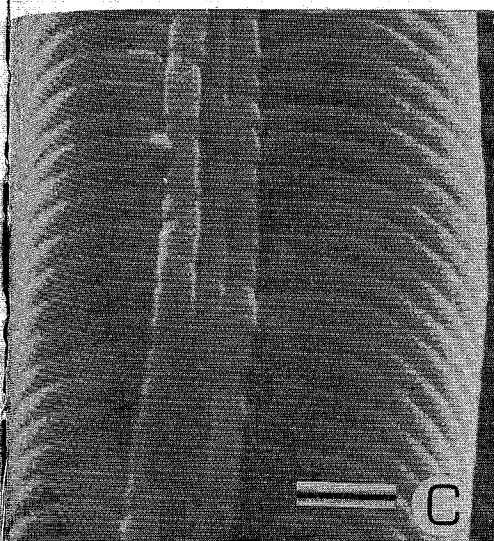
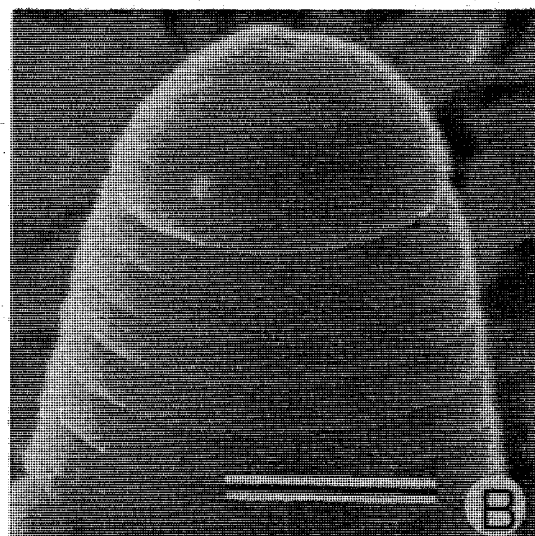
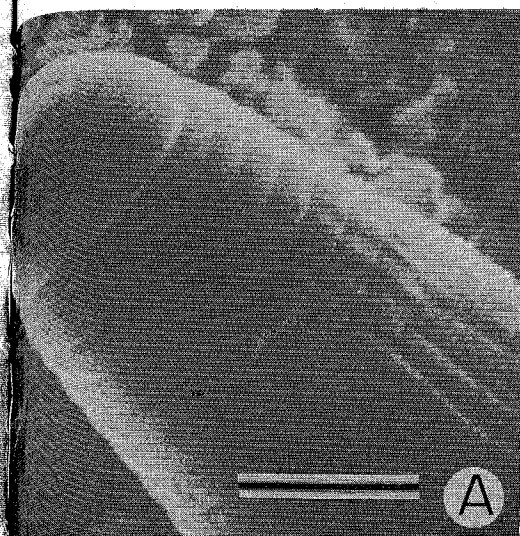
Fig. 2 - *Heterodera ciceri* sp. n. scanning electron photomicrographs: A, B) Lateral view of 2nd-stage juvenile anterior end; C) 2nd-stage mid-body lateral field. Note the outer band areolated; D) Anterior 2nd-stage juveniles body portion showing 3 distinct head annules; E, F, G) 2nd-stage juvenile tails; H) Portion of second stage juveniles tail showing the end of areolated lateral fields. (Scale bar = 5  $\mu\text{m}$ ).

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fenestration (Fig. 3 E, F). Excretory pore 10-14  $\mu\text{m}$  across within a depression and encircled by 3-4 continuous cuticular ridges (Fig. 3 C). Cysts covered partially or entirely with a thin, white subcrystalline layer.

*Vulval cones*: fenestral length = 40 (32-52)  $\mu\text{m}$ ; maximum fenestral width = 27 (20-37)  $\mu\text{m}$ ; semifenestral length = 17 (13-20)  $\mu\text{m}$ ; length of vulval slit = 50 (43-60)  $\mu\text{m}$ . Cysts ambifenestrate; semifenestrae semicircular and sub-equal (Fig. 4 A, B). Numerous prominent, dark-brown bullae irregularly distributed at the periphery of the vulval cone at and below the underbridge level in most cysts (Fig. 4 C-F). Underbridge well developed 125 (115-160)  $\mu\text{m}$  long, lying 78-86  $\mu\text{m}$  below apex of the vulval cone, with furcate ends and central thickening (Fig. 4 C, D).

*Males*: (n = 20) L = 1308 (1235-1488)  $\mu\text{m}$ ; a = 44 (42-51); b = 6.0 (5.8-6.4); maximum body width = 29-30  $\mu\text{m}$ ; stylet length = 29 (28-30)  $\mu\text{m}$ ; T = 62 (61-68); spicule length along axis = 36 (34-38)  $\mu\text{m}$ ; gubernaculum = 9-10  $\mu\text{m}$ ; head tip to excretory pore = 170 (153-180)  $\mu\text{m}$ .

Body vermiform, typically an open-C-shape after heat relaxation, with a short, bluntly rounded tail, about 1/3 body width long, and with a long (10-14  $\mu\text{m}$ ) cloacal tube ending in a circular openings (Fig. 5 E, F). Head region, hemispherical, 5-6  $\mu\text{m}$  long and 10  $\mu\text{m}$  wide, offset from the body (Fig. 5 A, B). Prestoma rectangular shaped, situated in the centre of the slightly raised oral disc plate. Labial disc plate partially fused with the rounded subdorsal and subventral pairs of lip sectors. Large amphidial apertures lie between the labial disc and the ellipsoidal lateral sectors (Figs. 1 I, L, 5 A, B). Posterior to the lip sectors the head annules are irregular and incomplete, except the basal annule which is larger and less subdivided by longitudinal striae (Fig. 5 A, B). Cephalic framework robust; stylet also robust with rounded basal knobs 5-6  $\mu\text{m}$  wide. DGO opens 5-6  $\mu\text{m}$  behind

Fig. 3 - SEM photomicrographs of *Heterodera ciceri* sp. n.: A) Gravid female attached to chickpea root; B) Female neck and excretory pore (arrowed); C) Excretory pore; D) Terminal cone, a=anus; E, F) Unfenestrated vulval cones with interlacing cuticular pattern extending over larger distance into vulval areas; G) Anal area of cyst cone with rounded anal basin, a=anus. (Scale bar: in Figs. A, B, D = 100  $\mu\text{m}$ ; in Figs. C, E, F, G = 10  $\mu\text{m}$ ).

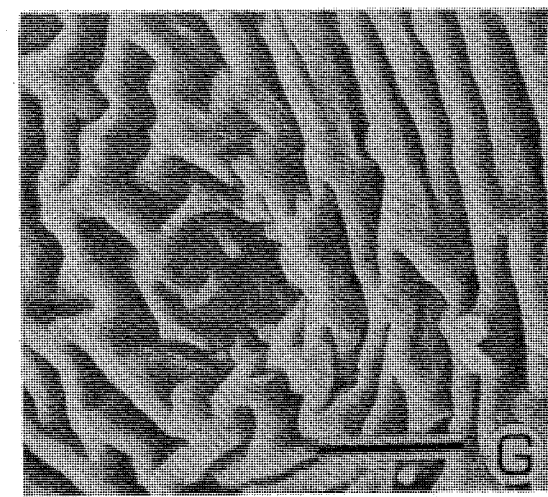
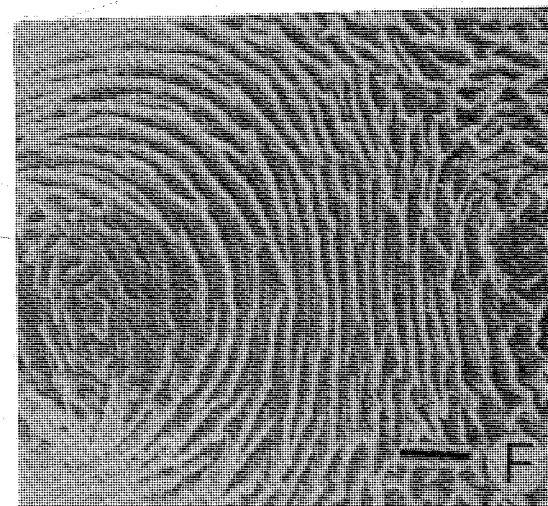
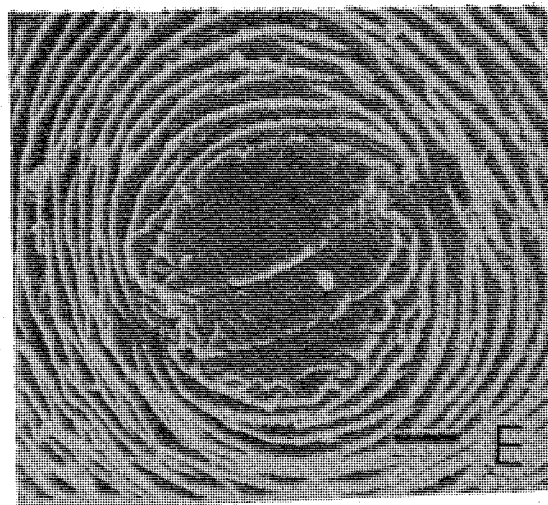
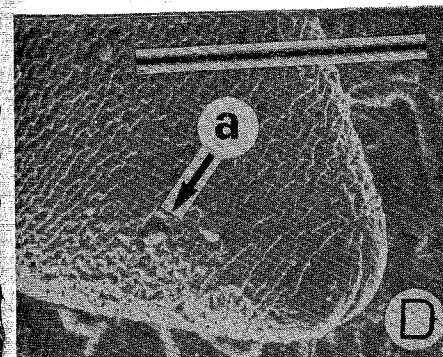
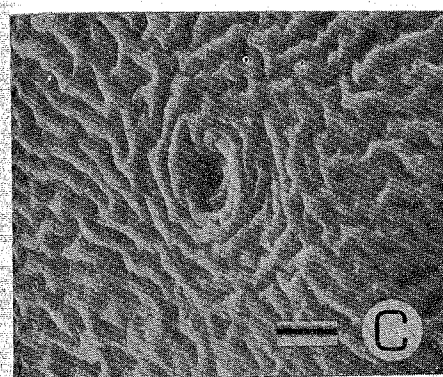
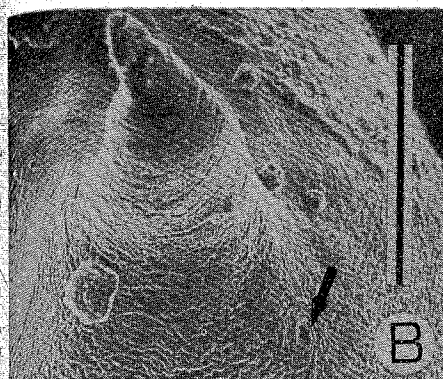
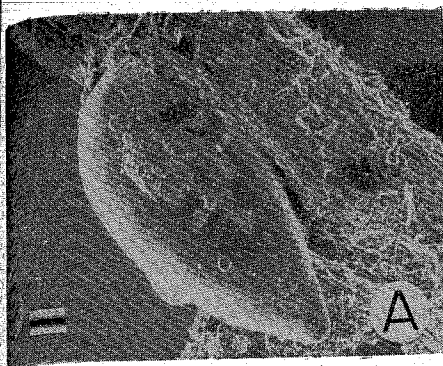


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stylet knobs. Median oesophageal bulb oval,  $25 \times 10 \mu\text{m}$ , with moderately developed « valve »  $98\text{--}117 \mu\text{m}$  from head tip. Hemizonid conspicuous, 2 annules long, located 9-12 annules anterior ( $13\text{--}21 \mu\text{m}$ ) to the excretory pore. The excretory pore is located  $170 (153\text{--}180) \mu\text{m}$  from the anterior end and is visible by SEM as a small pore about half an annule width in diameter (Fig. 5 C). Testis single not reflexed and occupying 61-68% of the body length. Spicules arcuate, tapering distally. Gubernaculum with slight ventral curve, not ornamented. The lateral fields have four equidistant incisures and are about  $1/4$  of the body width at mid-body (Fig. 5 D). Anteriorly the field begins at the 6-8th body annules as three lines forming two regularly areolated bands (Fig. 5 A).

*Holotype*: Female slide V.13/2/1 deposited at the Istituto di Nematologia Agraria C.N.R., Bari, Italy.

*Paratype*: Males, female and cyst cones, 2nd-stage juveniles slides (V.13/2/2-25), Istituto Nematologia Agraria C.N.R., Bari, Italy. Second-stage juveniles, males and cyst cones deposited in Nematology Department, Rothamsted Experimental Station, Harpenden, England; Plantenziektenkundige, Dienst, Wageningen, The Netherlands; USDA Department of Agriculture, Nematode Collection, Beltsville, Maryland, USA; Division of Nematology, University of California, Davis, California, USA; German Nematology Collection Institut für Nematologie, Münster, West Germany.

*Type host*: chickpea (*Cicer arietinum* L.).

*Type locality*: Idleb, Syria.

*Distribution and host plants*. *Heterodera ciceri* sp. n. is abundant and widespread in Northern Syria in the provinces of Hama, Idleb and Aleppo, where it causes severe yield losses of chickpea and lentil (*Lens culinaris* Medic.), which are among the major crops in the area. Greenhouse investigations (Greco *et al.*, unpublished) showed

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Fig. 4 - *H. ciceri* sp. n. cyst vulval cones; A, B) Fenestra and vulval bridge; C, D, E) Underbridge, and bullae; F) Underbridge, vagina and bullae. (Scale bar =  $20 \mu\text{m}$ ).



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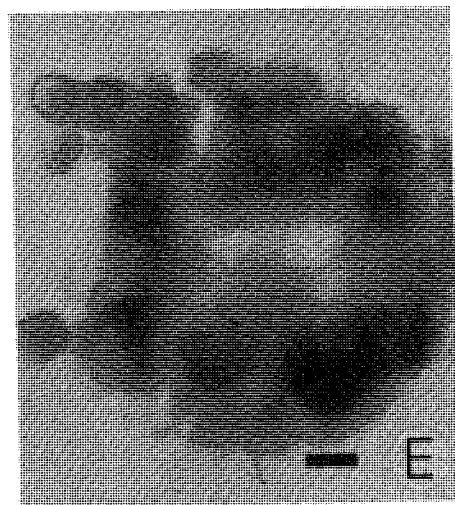
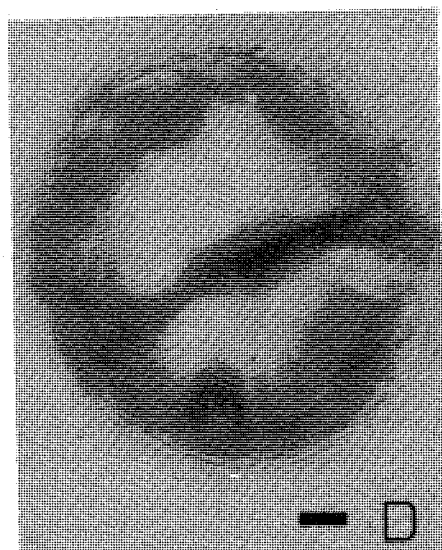
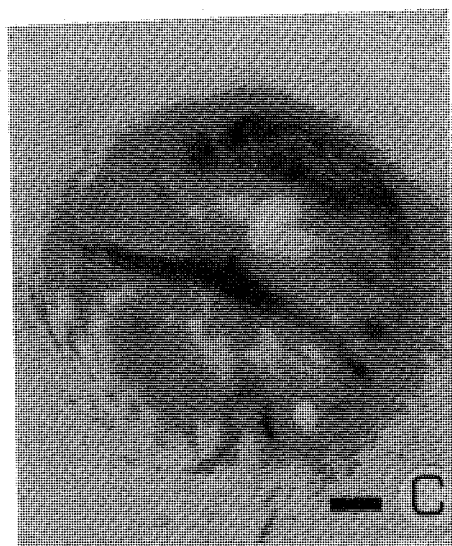
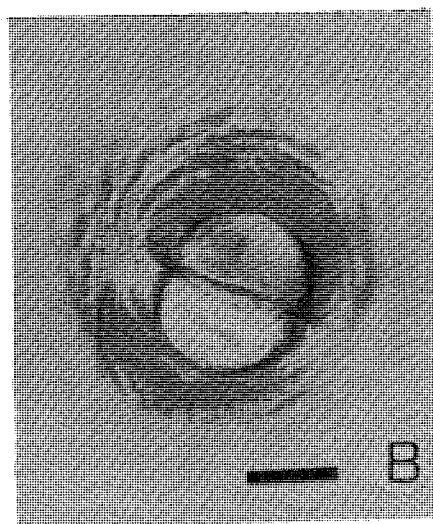
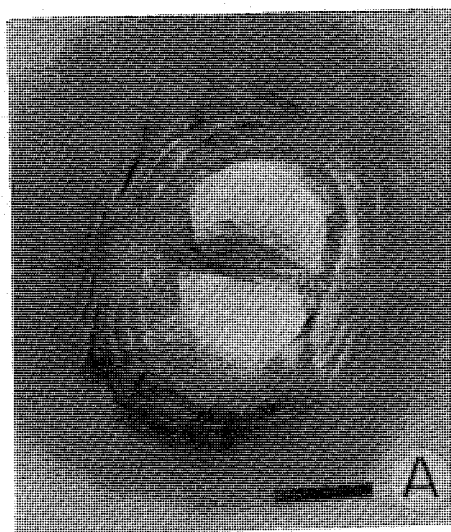
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that pea (*Pisum sativum* L.) is also a good host for the nematode. Other moderately good hosts include the legumes cowpea (*Vigna unguiculata* Walp.) and gross pea (*Lathyrus sativus* L.). A few cysts have been observed on bean (*Phaseolus vulgaris* L.), lupin (*Lupinus albus* L.), broad bean (*Vicia faba* L.), annual medic (*Medicago rigida* Desr.), alfalfa (*Medicago sativa* L.), vetch (*Vicia sativa* L.), and occasionally on carnation (*Dianthus cariophyllus* L.). No reproduction occurred on sugarbeet (*Beta vulgaris* L.), curled dock (*Rumex crispus* L.), and *Trifolium* spp., among the tested species.

#### Differential diagnosis

*Heterodera ciceri* sp. n. belongs to the group of *Heterodera* species (group 4 of Mulvey, 1972), with lemon-shaped ambifenestrated cysts, long vulval slit and well developed bullae and underbridge. *H. ciceri* resembles *H. trifolii* Goffart 1932, *H. rosii* Duggan et Brennan, 1966 and *H. daverti* Wouts et Sturhan, 1978 in its general morphology. It differs from *H. trifolii* by the presence of males, by the different host-range (none of the *Trifolium* spp. tested were hosts of *H. ciceri*), and by the fenestral measurements (shorter fenestral length 40-52  $\mu\text{m}$  vs 45-60  $\mu\text{m}$ , and fenestral width 20-37  $\mu\text{m}$  vs 31-49  $\mu\text{m}$  in the *H. trifolii*, by the deeper (78-86  $\mu\text{m}$  vs 33-38  $\mu\text{m}$ ) and longer (115-160  $\mu\text{m}$  vs 80-100  $\mu\text{m}$ ) underbridge. An additional difference is the lip region of the 2nd-stage juvenile which has 3 annules vs the single distinct annule reported for *H. trifolii*. Hirschmann and Triantaphyllou (1979), in a detailed morphometric comparison of six populations of the *Heterodera trifolii* species complex, define the *H. trifolii* group as those species showing morphology similar to *H. trifolii* that lack males. *Heterodera ciceri* has males and therefore cannot be included in this small group, although it belongs to the large « *H. schachtii* » group (group 4 of Mulvey, 1972), which also includes *H. trifolii* and related species.

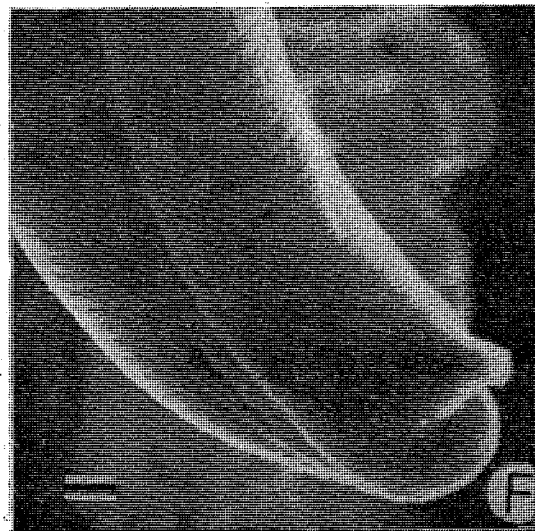
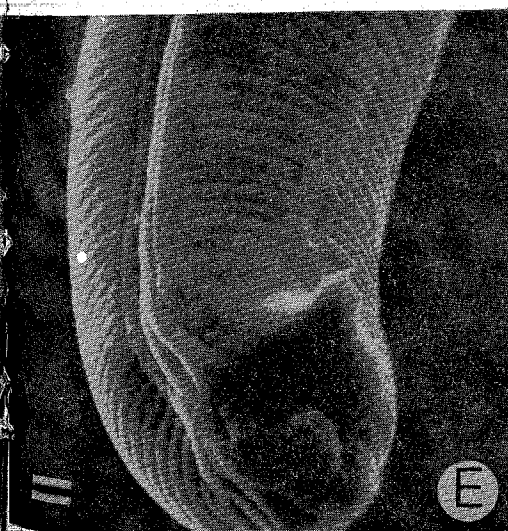
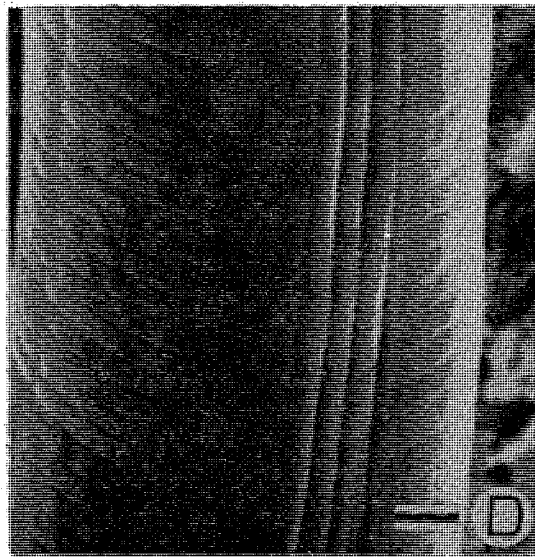
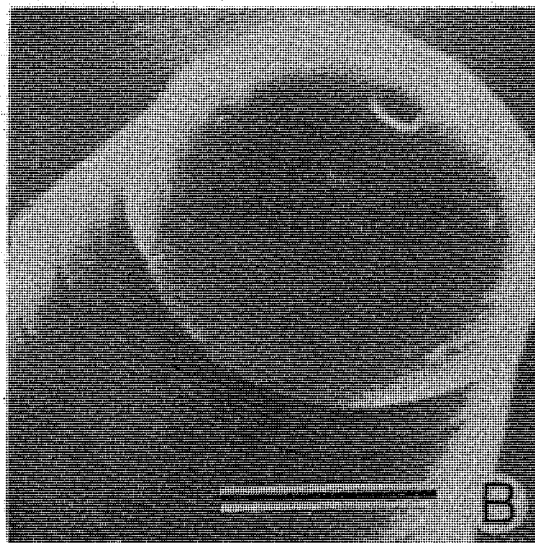
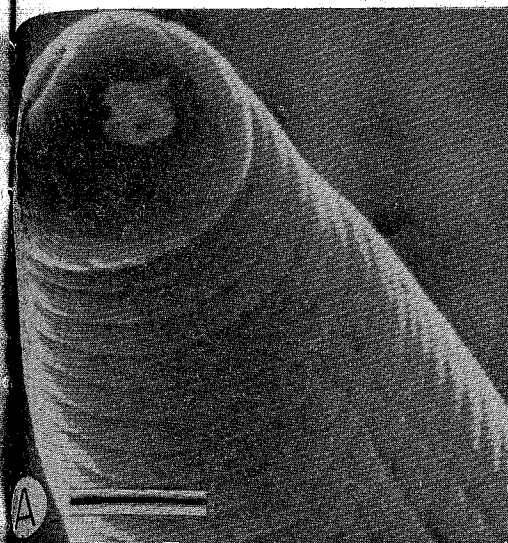
Fig. 5 - SEM photomicrographs of male *H. ciceri* sp. n.: A, B) head region C) Ventral view of excretory pore (arrowed) area; D) Mid-body lateral fields; E, F) Ventral and lateral-ventral view of terminal body portion. Note the long cloacal tube. (Scale bar = 5  $\mu\text{m}$ ).

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e. An additional difference is  
hich has 3 annules vs the single  
irschmann and Triantaphyllou  
parison of six populations of  
, define the *H. trifolii* group  
imilar to *H. trifolii* that lack  
therefore cannot be included  
s to the large « *H. schachtii* »  
n also includes *H. trifolii* and

*H. ciceri* sp. n.: A, B) head region  
area; D) Mid-body lateral fields;  
aninal body portion. Note the long



Bisexual « *H. trifolii* » populations (biological races) have been reported from Southern France (Cuany and Dalmasso, 1975), but *Trifolium* and *Dianthus* are mentioned as hosts. Wouts (1978) in his study on « males of *H. trifolii* reported in literature » concluded that the only *H. trifolii* males appear to be those collected by Norton from a population reared from a single *H. trifolii* cyst. Unfortunately they were male intersexes! In addition, in a French population collected by Cuany and illustrated on page 116 (Wouts, 1978) the J<sub>2</sub> stylet knobs have a slightly concave anterior surface, compared with the distinctly anchor-shaped of *H. trifolii* collected from the type locality and illustrated in the same paper (Wouts, 1978).

*Heterodera ciceri* differs from *H. rosii* mainly in fenestral measurements [fenestral length 40 (32-52)  $\mu\text{m}$  vs 65 (60-67)  $\mu\text{m}$ ]. After Duggan and Brennan O.D. (original description); 48-56  $\mu\text{m}$  (after Mulvey, 1972), fenestral width 27 (20-37)  $\mu\text{m}$  vs 55 (46-57)  $\mu\text{m}$ . O.D., 48-50 in Mulvey measurements, by the deeper underbridge 78-86  $\mu\text{m}$  below the vulval cone vs 70  $\mu\text{m}$  in O.D. and 43-62  $\mu\text{m}$  in Mulvey measurements.

The lip region of *H. ciceri* second stage juveniles is marked by 3 annules in comparison with the two (obscure) reported for *H. rosii* in the original description by Duggan and Brennan.

Moreover, the stylet-length of second stage juveniles of *H. ciceri* is 28.6  $\mu\text{m}$  vs 31.5  $\mu\text{m}$  of *H. rosii*.

Finally *Rumex crispus* L., the type host of *H. rosii*, was found to be a non host for *H. ciceri*, while pea and other leguminosae reported by Duggan and Brennan as non hosts, proved to be very good hosts for *H. ciceri*.

*Heterodera ciceri* also differs from *H. daverti* by the longer 2nd-stage total body length 525 (440-585)  $\mu\text{m}$  vs 457 (400-480)  $\mu\text{m}$ ; by its longer J<sub>2</sub> stylet 27-30  $\mu\text{m}$  vs 24-26  $\mu\text{m}$  and by the anteriorly more concave stylet knobs.

In addition *H. ciceri* has longer underbridge 115-160  $\mu\text{m}$  vs 70-120  $\mu\text{m}$  in *H. daverti* and differs by the host range: the white clover (*Trifolium repens* L.), the type host of *H. daverti* resulted non host of *H. ciceri*. The lip region of *H. ciceri* second stage juveniles is marked by 3 annules in comparison with the distinct annule reported for *H. daverti* (Wouts and Sturhan, 1978).

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We are grateful to Dr. M. Luc, Dr. A.R. Stone, and Dr. D. Sturhan for critically reviewing the manuscript and for comments, and to Dr. M.C. Saxena for providing us with facilities in collecting the nematode population.



(biological races) have been (May and Dalmasso, 1975), but as hosts. Wouts (1978) in his « in literature » concluded that those collected by Norton from *trifolii* cyst. Unfortunately they a French population collected (Wouts, 1978) the J<sub>2</sub> stylet knobs, compared with the distinctly from the type locality and il- (1978).

*rosii* mainly in fenestral meas- n vs 65 (60-67) µm]. After Dug- (tion); 48-56 µm (after Mulvey, 55 (46-57) µm. O.D., 48-50 in underbridge 78-86 µm below the µm in Mulvey measurements. stage juveniles is marked by (obscure) reported for *H. rosii* and Brennan.

and stage juveniles of *H. ciceri*

the host of *H. rosii*, was found e pea and other leguminosae non hosts, proved to be very

*H. daverti* by the longer 2nd- m vs. 457 (400-480) µm; by its and by the anteriorly more

underbridge 115-160 µm vs y the host range: the white ost of *H. daverti* resulted non *ciceri* second stage juveniles is h the distinct annule reported (78).

R. Stone, and Dr. D. Sturhan for omments, and to Dr. M. C. Saxena the nematode population.

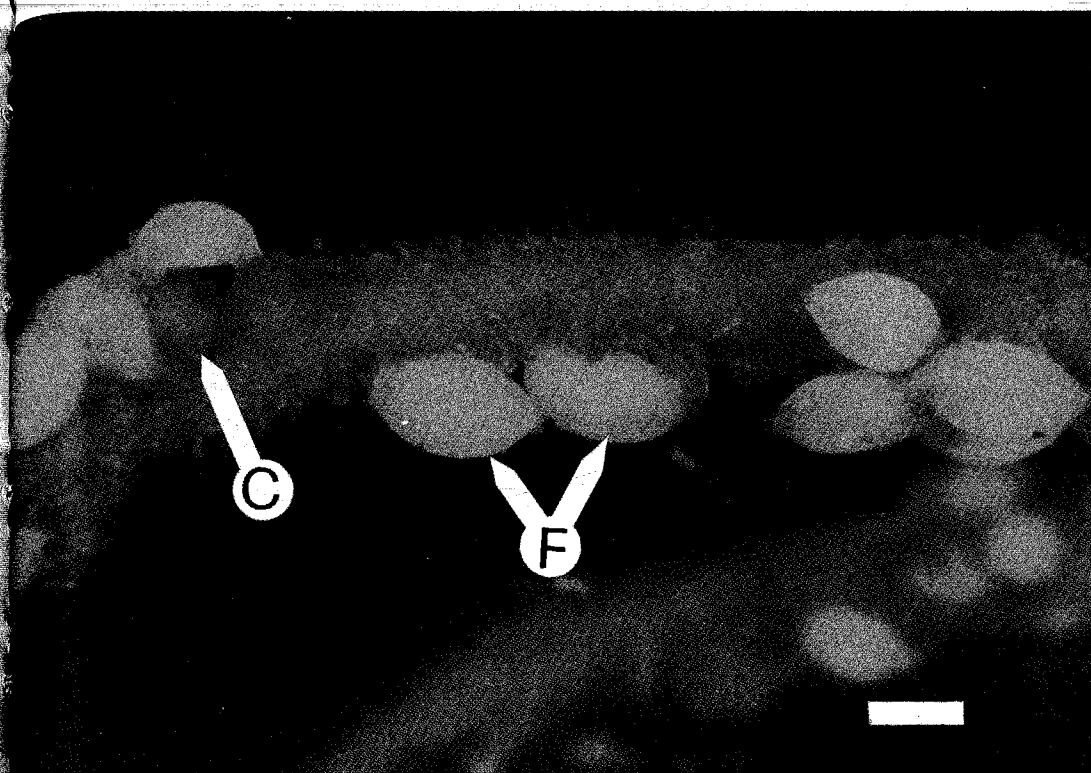


Fig. 6 - Lentil (*Lens culinaris* Medic.) root infected with a colony of *H. ciceri* sp. n.: F = gravid females; C = cyst. (Scale bar = 450 µm).

## S U M M A R Y

A new bisexual *Heterodera* species, belonging to « *schachtii* » group, collected in northern Syria and associated with chickpea, *Cicer arietinum* L. roots, is described and illustrated. *Heterodera ciceri* sp. n., with lemon shaped, ambifenestrated cysts, long vulval slit, well developed bullae and underbridge, anteriorly concave second stage juvenile stylet knobs, and presence of yellow phase, differs from the closely related species *H. trifolii*, *H. rosii*, *H. daverti* by morphological characters, presence of males, and by a different host range.

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Accepted for publication on 15 July 1985.

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