

Morphology of some cyst-forming nematodes from Iran

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Summary. A survey conducted to identify cyst-forming nematodes in Iran revealed the presence of species belonging to the genera *Heterodera* and *Cactodera*. *Heterodera elachista*, *H. fici*, *H. glycines*, *H. goettingiana*, *H. humuli*, *H. turcomanica* and *Cactodera cacti* obtained from different plants and regions are described for the first time from Iran. Morphological features and morphometric characters with comparative details for these species as well as for *H. mothi* are presented and the relationship of some of the species with similar species is discussed.

Key words: *Cactodera cacti*, cyst-forming nematodes, *H. elachista*, *H. fici*, *H. glycines*, *H. goettingiana*, *H. humuli*, *H. mothi*, *H. turcomanica*.

The first evidence of the presence of cyst-forming nematodes in Iran goes back to Esmailpour & Schäfer (1970) who reported *Heterodera schachtii* Schmidt, 1871 from sugar beet fields in Khorasan province (eastern part of Iran). In a survey of sugar beet fields, Talatchian *et al.* (1976) detected the '*Heterodera avenae* group', *H. iri* Mathews, 1971, *H. mothi* Khan and Husain, 1965, *H. latipons* Franklin, 1962, *H. trifolii* Goffart, 1932, *H. galeopsidis* Goffart, 1936, and *H. schachtii*. Noori *et al.* (1980) reported *H. trifolii*, *H. galeopsidis*, *H. latipons*, *H. iri*, *H. rosii* Duggan and Brennan, 1966, and the '*H. avenae* group' from sugar beet fields in the west of Iran. *Heterodera avenae* Wollenweber, 1924 was reported for the first time by Barooti & Loof (1990) from wheat fields in the Marvast area; later, it was also reported from cereal fields in the Bakhtaran province (Hojat Jalali, 1991). *Heterodera mani* Mathews, 1971, another member of the '*H. avenae* group', was recorded from sugar beet fields in the Khorasan Province along with *H. cruciferae* Franklin, 1945 and *H. carotae* Jones, 1950, both members of the '*H. goettingiana*' group

(Mehdikhani & Kheiri, 1995; Mehdikhani, 1998). Sturhan (1996), after surveying the '*H. avenae* group' from 21 localities in Iran, reported the presence of *H. filipjevi* (Madzhidov, 1981) Stelter, 1984 and considered *H. filipjevi* and *H. latipons* as the only well documented species of this group in Iran. Recently, Pedramfar *et al.* (2001) reported *H. oryzae* Luc and Berdon-Brizuela, 1961 from a rice field in the Gilan province in the north of Iran. Finally, Tanha Maafi *et al.* (1999) reported *H. glycines* Ichinohe, 1952 from soybean fields from the north of Iran.

A survey of different plant species in various regions of Iran made in the period 1998-2000 yielded several populations of cyst-forming nematodes. A total of 21 *Heterodera* and *Cactodera* species were identified, among which were seven described species, seven new species for Iranian fauna and seven species obviously new to science (Tanha Maafi, 2002). Their molecular characterisation and phylogeny have been published recently (Tanha Maafi *et al.*, 2003). The results of morphological observations on eight of the species are presented here; the description of

the new species will be published separately.

MATERIALS AND METHODS

The cysts were extracted from the soil by a combination of a modified Cobb sieving method and the sugar flotation technique (Caveness & Jensen, 1955; Dunn, 1969). The vulval cone region was excised and embedded in a heated drop of glycerine jelly on a cover slip and sealed between cover slips held in aluminium holders. The juveniles and eggs obtained from the same cysts were fixed in heated TAF (2 ml triethanol amine, 7 ml formaldehyde, 91 ml distilled water) and transferred to dehydrated glycerine according to the method of De Grisse (1969). The juveniles were mounted in a small drop of dehydrated glycerine, a paraffin ring sealed the cover glasses held in an aluminium slide. The morphological and morphometric characters were studied by using LM. All measurements are expressed in micrometers and presented as a range, with the mean and the standard error of the mean. The light microscopic photographs of cyst vulval cones and second stage juveniles were taken with an automatic Olympus camera attached to a compound microscope Olympus BX50 having an interference contrast system; the photographs of females and cysts were taken with the same camera attached to a dissecting microscope. For scanning electron microscopic (SEM) observations, the juveniles preserved in glycerine were first dehydrated by ethanol and dried by CO₂ before they were placed on the stubs and coated with gold. The specimens were observed with a Scanning Electron Microscope model JMS 840 working at 15KV.

DESCRIPTIONS

Heterodera humuli Filipjev, 1934 (Figs. 1, 2, 5; Tables 1, 3)

Lemon shaped cyst, occasionally nearly spherical; small size, with low but distinct vulval cone, with small egg-sac, yellow to pale brown, subcrystalline layer present, cuticle with irregular zigzag pattern, and fine punctuation. Vulval cone bifenestrate, abullate, rarely with bullae-like structures; underbridge slender, weak with furcated ends. Second stage juvenile vermiform; body short, slender, curved ventrally after fixation. Lip region rounded, with three distinct annules and a labial disc, slightly set off from the rest of the body. Stylet rather strong, stylet knobs large, rounded slightly concave anteriorly. Lateral field with four incisures, phasmids small, seldom visible. Ten to

13 annules posterior to anus level, tail narrowly tapering to a fine, rounded terminus. SEM photographs of the lip region of second stage juveniles in en face view show distinct fusion of submedian lips with labial disc.

The morphological features and morphometric characters of Iranian specimens of *H. humuli* agree well with *H. humuli* Filipjev, 1934 (Stone & Rowe, 1977). Bifenestrate cysts without bullae, weakly developed underbridge and presence of a long vulval slit places this species within the 'humuli group' (Mathews, 1971; Subbotin *et al.*, 1997). *H. humuli* is morphologically similar to *H. riparia* and there are no significant differences in morphology and morphometrics between cysts of Iranian populations of *H. humuli* and the original description of *H. riparia*. The second stage juveniles of *H. humuli*, however, differ from *H. riparia* by their longer tail length (average 49 vs 40-47 µm) and the longer hyaline part of the tail (average 26-26.5 vs 18-23 µm).

Heterodera humuli was found on common nettle (*Urtica dioica*) in two different regions in the north of Iran, Amol (Mazandaran province) and Siahkal (Gilan province), where common nettle is widely distributed. The presence of numerous individuals at different stages on the roots of *U. dioica* and in the surrounding soil indicates that common nettle is a suitable host for *H. humuli*.

Heterodera fici Kirjanova, 1954 (Figs. 1, 2, 5; Tables 1, 3)

Lemon to nearly spherical shaped cyst with low but distinct vulval cone, light to dark brown. Subcrystalline layer absent, cuticle with irregular zigzag pattern. Vulval cone bifenestrate in young cysts, ambifenestrate in old cysts; bullae small, scattered around underbridge plane, underbridge weak with furcated ends. Second stage juvenile vermiform, body curved ventrally after fixation; lip region high, hemispherical to rounded, with two annules and a labial disc, slightly set off from the rest of the body. Stylet well developed, stylet knobs large, rounded slightly forwarded anteriorly. Lateral field with four incisures starting five to six annules below the lip region and ending at the second half of the tail. Phasmids small, seven to nine annules posterior to the anus level, tail narrowly tapering to a fine, rounded terminus. SEM photographs of the lip region of second stage juveniles in en face view show fusion of submedian lips with labial disc and to some extent with the first head annule.

The morphological features and morphometrics of *H. fici* found in Iran agree well with the original

description of the species, except for the length of the underbridge of the cyst vulval cone which is longer than previously reported data (92.5 vs 75; 70; and 53-75 μm in Maryland, Pakistan population and original data (Kirjanova), respectively) (Golden *et al.*, 1988). *H. fici* has frequently been placed in different groups (Mathews, 1971; Golden & Birchfield, 1972; Mulvey, 1972; Stone, 1975, 1979; Mulvey & Golden, 1983; Golden *et al.*, 1988; Baldwin and Mundo-Ocampo, 1991; Subbotin *et al.*, 1997, 2001). The bifenestrate cysts with small and scattered bullae in the underbridge plane, as well as the weakly developed underbridge and the long vulval slit, place this species in the 'humuli group' (Mathews, 1971; Subbotin *et al.*, 1997). This grouping was recently confirmed by molecular data (Subbotin *et al.*, 2001; Tanha Maafi *et al.*, 2003). *H. fici* was recovered from *Ficus elastica* in a greenhouse in Kordestan province in the west of Iran.

Heterodera turcomanica
Kirjanova & Shagalina, 1965
(Figs. 1, 2, 5; Tables 1, 3)

Rather rounded cysts with very small projection at the end of the body, pale yellow to pale brown, with subcrystalline layer; cuticle transparent with irregular striae. Vulval cone bifenestrate, the nearly circular fenestra separated by a wide vulval bridge directed slightly sublateral, small 'saddle-like' depression around vulva; vulval slit small, bullae large and thick located slightly below fenestral region with deep yellow colour, underbridge absent. Second stage juvenile vermiform slightly curved ventrally after fixation; lip region low and almost rounded, set off from the rest of the body, with two annules and a labial disc. Stylet delicate with small rounded knobs (2-2.5 μm width), anterior surface of knobs slightly directed forward. Lateral field with 4 incisures, starting five annules below the lip region and extending almost at one third of tail length. Phasmids, unlike in other members of the '*H. avenae* group', pore-like and located three body annules posterior to the anus level. Tail short with bluntly rounded terminus, rarely with conoid terminus; hyaline part of tail short, slightly more than half of the tail length. In some specimens en face view SEM photographs of the lip region of second stage juveniles show the fusion of submedial lips with labial disc and partial division in dorsal and ventral boundaries; in other specimens this fusion extends up to the first head annule and to some extent includes this annule, so that it forms almost a united structure elongated dorsally and ventrally. Therefore, according to Stone's (1975) grouping based on the lip region,

H. turcomanica falls between the '*goettingiana* group' and '*avenae* group'.

All morphological features and morphometrics of the Iranian populations of *H. turcomanica* are in agreement with the redescription of the species by Sturhan & Wouts (1995). Among the *Heterodera* species, *H. turcomanica* possesses the smallest second stage juvenile with short body length, short stylet length, and short length of the hyaline part of the tail, but also small eggs. Compared with the other species of the genus, the cyst vulval cone of *H. turcomanica* possesses the shortest vulval slit. The soil sample containing *H. turcomanica* was further composed of *H. hordecalis* and a *Heterodera* sp., member of the '*avenae* group'. Mixed *Heterodera* populations are not uncommon as Sturhan & Wouts (1995) found mixed population in permanent slides of type material and specimens from Turkmenistan. Based on Stone's grouping (1975) and on SEM observation of the lip region of second stage juveniles, *H. turcomanica* falls between the '*goettingiana* group' and the '*avenae* group', while all members of the '*avenae* group' are exactly grouped in Stone's (1975) pattern '4', which corresponds with 'group 3' of Mulvey (1972). Considering both the SEM observations of the lip region of second stage juveniles and the clustering of *H. turcomanica* with the '*humuli* group' with high bootstrap support (Tanha Maafi *et al.*, 2003) as well as results from previous studies (Gäbler *et al.*, 2000), we conclude that *H. turcomanica* does not belong to the '*avenae* group'.

With their short vulval slit and circular semi-fenestrae separated by a wide vulval bridge, cysts of *H. turcomanica* resemble those of *H. latipons* and *H. hordecalis*, but differ from these species in lacking an underbridge (very strong in both species) and the presence of a saddle like-depression around the vulva (absent in both species). Moreover, *H. turcomanica* differs from *H. hordecalis* in having a very short vulval slit (4-6 μm vs 17-23.5 μm) The eggs of *H. turcomanica* are much smaller than those of *H. latipons* and *H. hordecalis* (93.6 \pm 6 x 45.16 \pm 3 μm vs 124 \pm 6.7 x 51.2 \pm 2, and 115 \pm 3 x 52.9 \pm 2.5 μm) in the Iranian population of *H. latipons* and *H. hordecalis*, respectively. Second stage juveniles differ from the two similar species in having a shorter body length, a shorter stylet, a shorter tail length and length of the hyaline part of tail (396 vs 450, 462; 17.8 vs 24, 21.5; 37 vs 53.5, 50; 21 vs 34.3, 28, respectively) (Tanha Maafi, 2002).

Heterodera turcomanica was found in Meshkin Shahr in Ardabil province at the north west of

Table 1. Morphometric characters of second stage juveniles of some *Heterodera* species from Iran (measurements in μm).

Species	<i>H. humuli</i>		<i>H. fici</i>	<i>H. turcomanica</i>	<i>H. glycines</i>		<i>H. elachista</i>
Locality	Sihkal opulation	Amol population	Sanandaj	Ardabil	Gorgan, Baghoo Kenarehz	Sari, Dasht-e Na	Tonekabon
n	20	12	20	20	20	14	10
L	385±8.9 (376-409)	364 ± 12.3 (336-380)	377 ± 20.4 (328-407)	396 ± 14 (364-436)	436 ±25.44 (392-484)	454±22.22 (425-490)	391 ± 11 (372-410)
a	21.24±0.8 (19.6-23.8)	21.26 ± 1.26 (19.5-23.56)	23.80 ± 1.4 (20.5-26.3)	25 ± 1.02 (22.75-22.25)	23.92±1.25 (21.52-5.88)	24.90±1.05 (23.6-27.22)	22.45 ± 1 (20.77-24)
b	3.89±0.21 (3.53-4.23)		3.86	4.4 ± 0.18 (4.1- 4.7)	4.12±0.17 (3.8-4.45)	4±0.20 (3.7-4.5)	4.31 ± 0.27 (3.9-4.9)
c	7.88±0.43 (7.35-9.4)	7.42 ± 0.50 (6.83-8.60)	7.90 ± 0.45 (6.9-8.5)	10.80 ± 0.86 (9.63-13.1)	8.53±0.28 (8-9)	8.96±0.55 (8.33-10.65)	6.68 ± 0.31 (5.9-7.2)
c'	4.3 ± 0.41 (3.8-5.40)	4.42 ± 0.40 (3.82-5.30)	4.58 ± 0.32 (4.09-1.45)	8.32 ± 0.54 (7.54-9.5)	4.26±0.19 (4-4.5)	4.25±0.21 (3.83-4.5)	5.73 ± 0.41 (5-6.5)
Stylet length	22.4±0.4 (21.9-23.3)	22.7 ± 0.75 (22-24)	21.4 ± 0.6 (20-22)	17.8 ± 0.48 (16-18)	22.6±0.63 (22-24)	22.7± 0.6 (22-24)	20 ± 0.69 (18-21)
Lip region height	3.4±0.3 (2.85-3.8)	4	3.7 ± 0.44 (3-4)	3.1 ± 0.31 (3-4)	3.3±0.48 (3-4)	3.3±0.47 (3-4)	3
Lip region width	8±0.43 (7.6-9)	8	8	7.9 ± 0.31 (7-8)	8	8	7.8 ± 0.36 (7-8)
D.G.O.	5±0.4 (4.3-5.7)	5.4 ± 0.5 (5-6)	5.2 ± 0.52 (4-6)	4.4 ± 0.82 (3-7)	4.3±0.7 (3-6)	4±0.7 (3-5)	5.2 ± 0.77 (4-7)
Anterior to exc. pore	93±2.5 (88-97)	89 ± 3.4 (83-93)	93 ± 2.95 (89-101)	85 ± 4.14 (78-94)	97±0.74 (89-106)	99±4.7 (90-106)	84 ± 2.75 (79-88)
Median bulb length	64.3±2.8 (57-70)	57 ± 6 (48-65)	65 ± 2.39 (60-70)	55 ± 2.18 (51-60)	66±3.6 (59-73)	67±3.4 (59-73)	59 ± 3.57 (52-67)
Body width at mid body	18.1±0.39 (17.1-19.)	17.2 ± 0.94 (16-19)	15.8 ± 0.6 (15-17)	15.8 ± 0.36 (15-16)	18.2±0.41 (18-19)	18.3±0.4 (18-19)	17.4± 0.75 (16-19)
Body width at anus	11.5±0.42 (10.5-12.5)	11.2 ± 0.58 (10-12)	10.5 ± 0.5 (10-11)	10.5 ± 0.47 (10-11)	12±0.4 (11-13)	11.7±0.42 (11-13)	10.2 ± 0.55 (9-11)
Hyaline part	26±2.71 (20.5-31)	26.25 ± 3.22 (22-30)	25.6 ± 2.8 (21-32)	21 ± 2.08 (17-25)	29.8±2.68 (24-36)	29.7±2.46 (25-33)	32 ± 3.03 (25-39)
Tail length	49.1±2.6 (40.5-52.5)	49.2 ± 3.14 (42-53)	47.8 ± 3.2 (44-56)	37 ± 2.98 (30-41)	51±2.24 (48-54)	50±2.40 (46-54)	59 ± 2.87 (54-63)
Hyaline / Stylet	1.15±0.11 (0.89-1.34)	1.15 ± 0.15 (0.96-1.36)	1.19 ± 0.13 (0.9-45)	1.18 ± 0.1 (1-1.38)	1.31±0.11 (1.09-1.63)	1.31±0.11 (1.13-1.5)	1.63 ± 0.13 (1.3-1.85)
L / Median bulb length	5.98±0.23 (5.58-6.63)	6.54 ± 0.62 (5.82-7.40)	5.81 ± 0.35 (4.96-6.31)	7.28 ± 0.23 (6.84-7.7)	6.6±0.27 (5.9-7.15)	6.81±0.27 (6.32-7.31)	6.66 ± 0.38 (5.6-7.4)

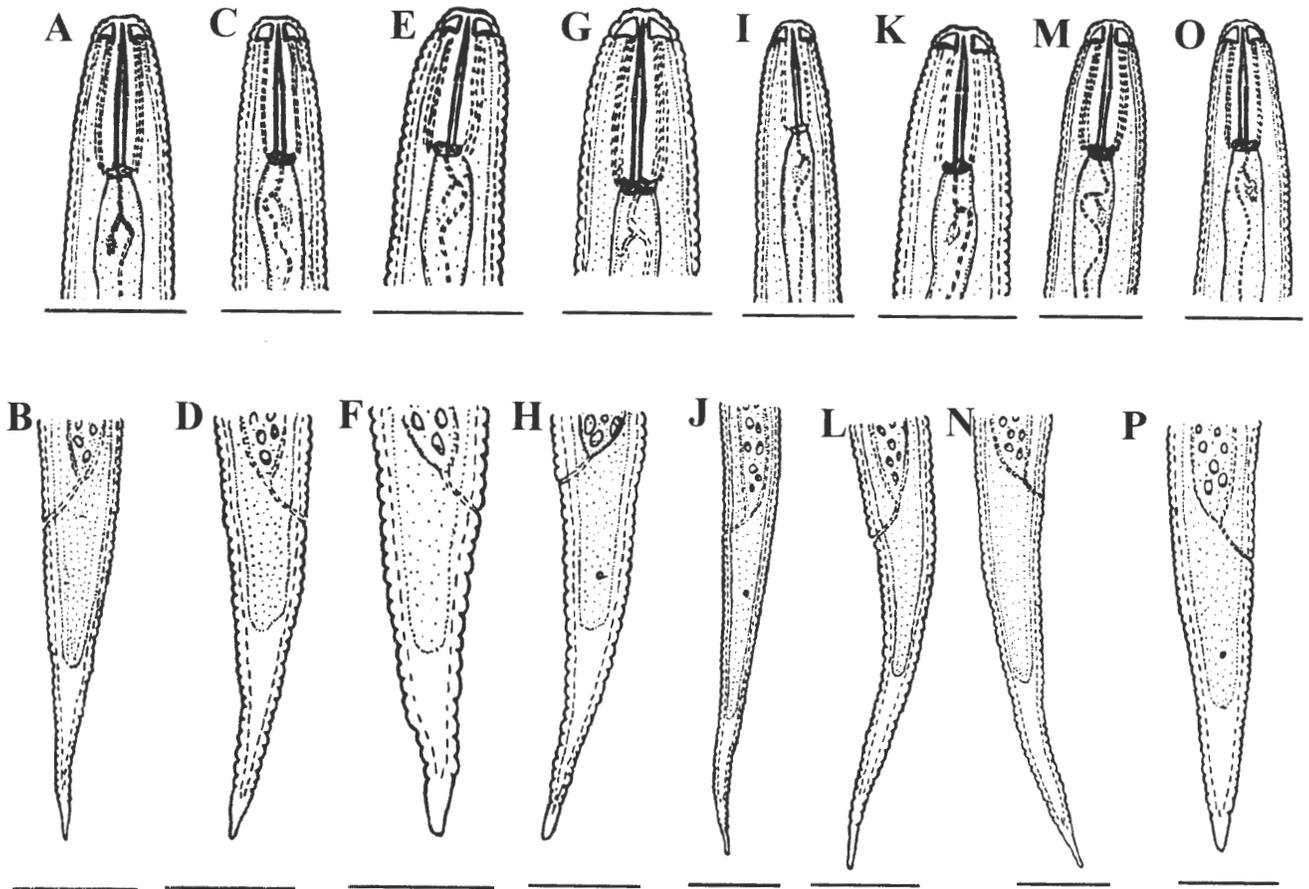


Fig. 1. Lip region and tail of *Heterodera* and *Cactodera* species. A, B: *H. humuli*; C, D: *H. fici*; E, F: *H. turcomanica*; G, H: *H. glycines*; I, J - *H. moths*; K, L: *H. elachista*; M, N: *H. goettingiana*, O, P: *C. cacti*. Scale bars – 20 μ m.

Iran. The soil sample was taken from the soil around an almond tree growing inside in a vineyard. The exact host is not known but the following plants were identified in the sampling area: *Setaria viridis* (Graminae), *Daucus carota* (Umbeliferae), *Thesium arvense* (Santalaceae), *Euphorbia boissieriana* (Euphorbiaceae). *H. turcomanica* has been reported only from Turkmenistan so far.

***Heterodera glycines* Ichinohe, 1952**
(Figs. 1, 3, 6, 7; Tables 1, 3)

Lemon shaped cyst, light to dark brown, with protruding cone. Outer cyst cuticle with rugose pattern of irregular zigzag lines. The fenestra of the vulval cone are divided into two semifenestrae by the vulval bridge (ambifenestrata). The underbridge

is prominent and well developed, bullae prominent, elongated, and located at the level of the underbridge or near to it. Second stage juvenile vermiform, curved ventrally after fixation. Lip region hemispherical, high and set off from the rest of the body, with two annules and a labial disc. Stylet rather robust, with anteriorly directed knobs. Lateral fields with four incisures. Phasmids pore-like and located seven to eight body annules posterior to anus level. Tail tapering uniformly to a nearly bluntly rounded terminus. Hyaline part less than half of tail length. SEM photographs of the lip region of second stage juveniles in en face view reveal fusion of submedial lips with labial disc and extend further to join with the first head annule, but the dorsal and ventral boundaries are incomplete, often with a median division.

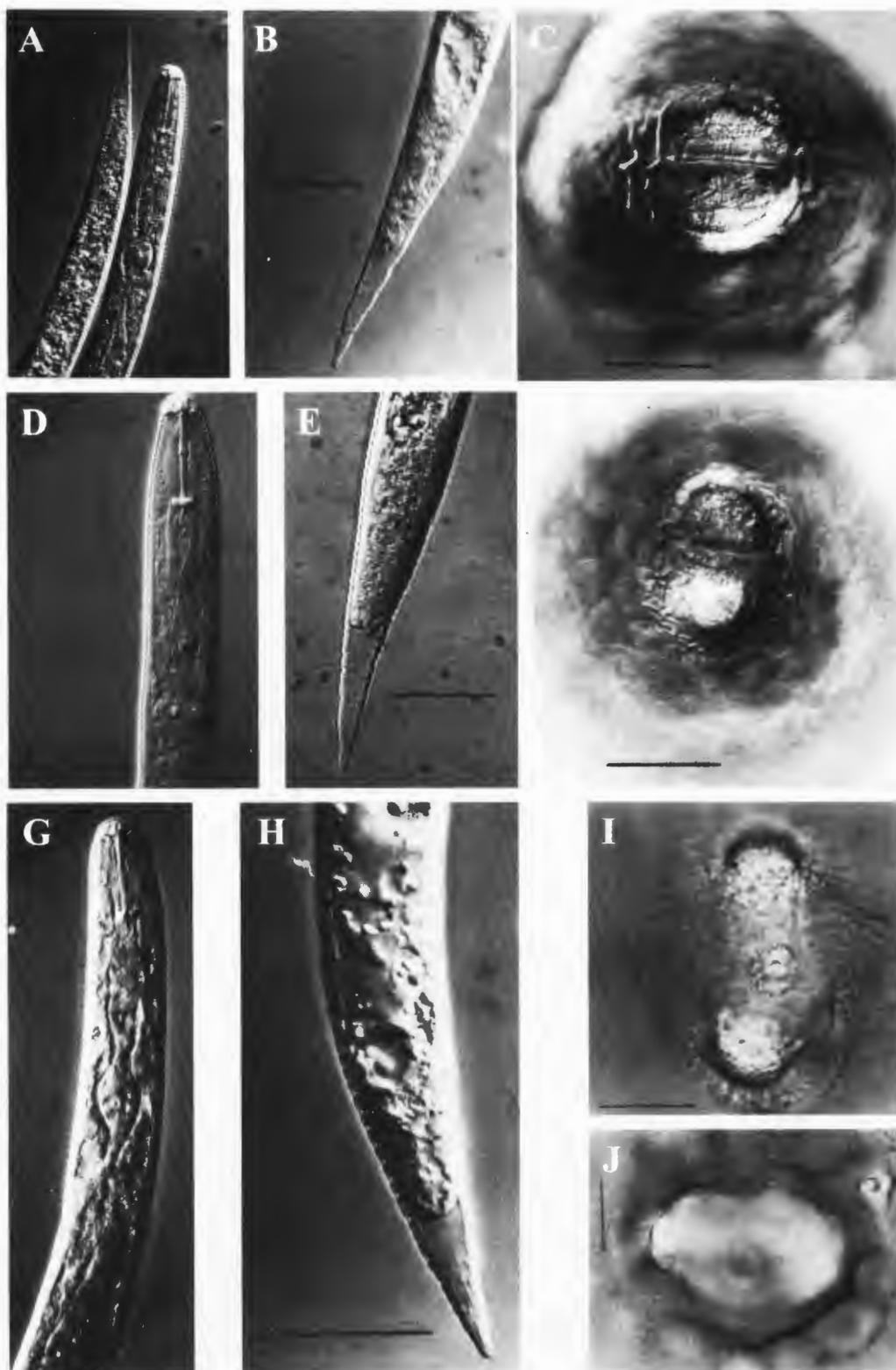


Fig. 2. Photomicrographs of second stage juveniles and cyst vulval cones of *Heterodera*. A, B, C: *H. humuli*. A: anterior end and tail; B: tail; C: end view of vulval-fenestral region showing fenestration and vulval slit; D, E, F: *H. fici*. D: anterior end; E: tail; F: end view of vulval-fenestral region showing fenestration; G, H, I, J: *H. turcomanica*. G: anterior end; H: tail; I, J: end view of vulval-fenestral region showing fenestration, vulval slit and bullae. Scale bars A, B, D, E, G-J – 20 μ m; C, F – 40 μ m.

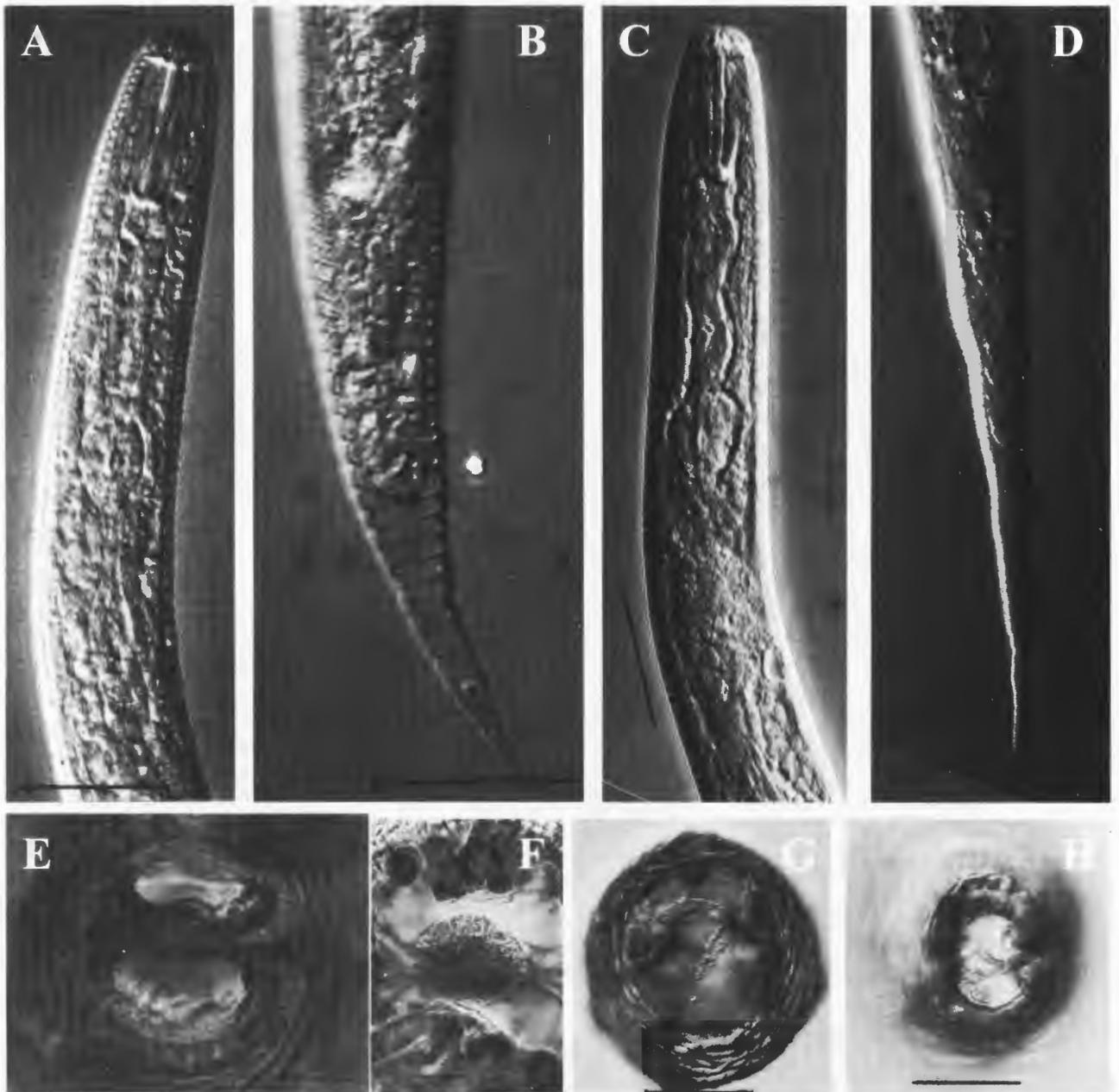


Fig. 3. Photomicrographs of second stage juveniles and cyst vulval cones of *Heterodera* species. A, B, E, F: *H. glycines*. A: anterior end; B: tail; E, F: end view of vulval-fenestral region showing fenestration and bullae; C, D, G, H: *H. mothi*. C: anterior end; D: tail; G, H: end view of vulval-fenestral region showing fenestration and bullae. Scale bars A-F – 20 μm , G, H – 40 μm .

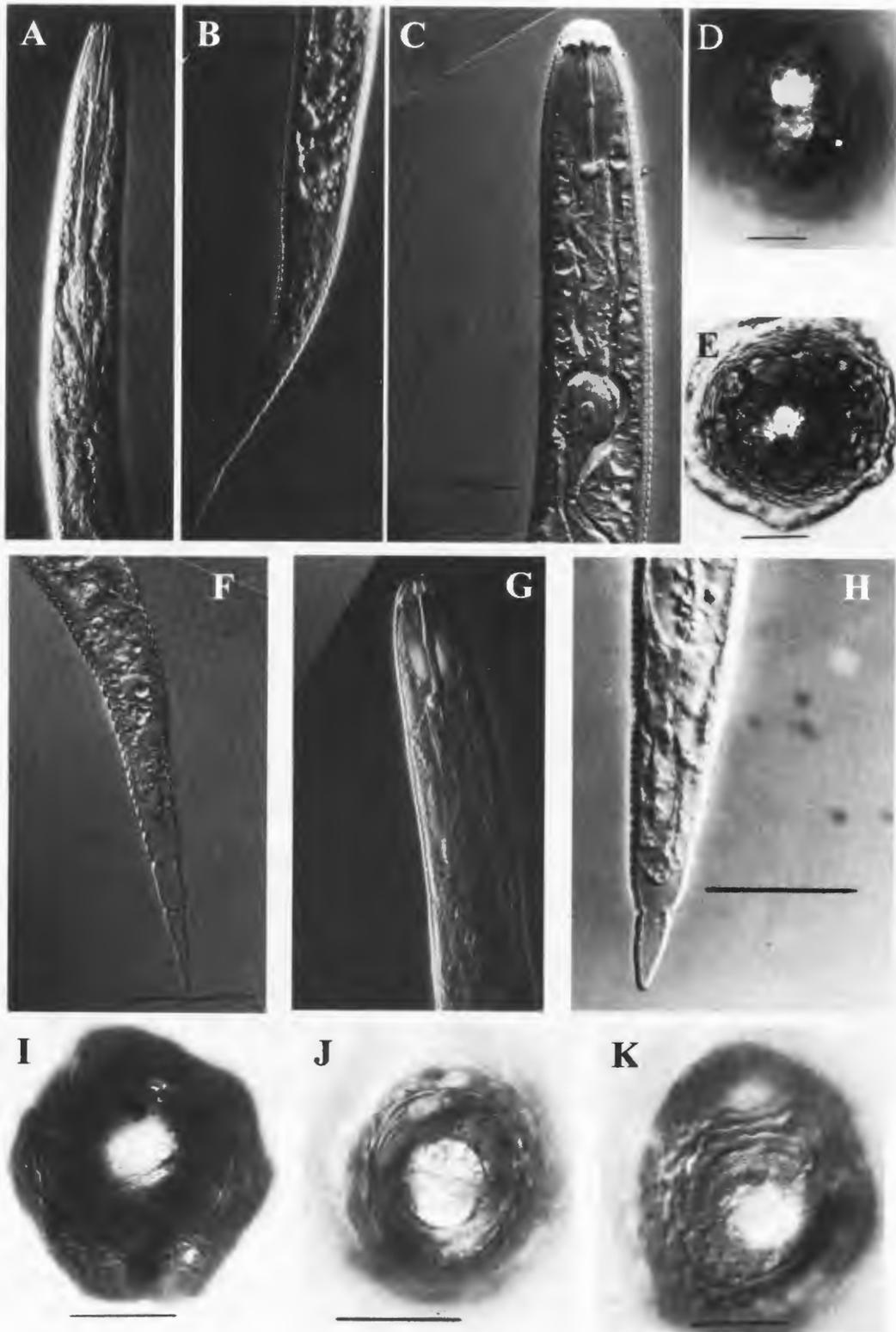


Fig. 4. Photomicrographs of second stage juveniles and cyst vulval cones of *Heterodera* and *Cactodera* species. A, B, I, J: *H. elachista*. A: anterior end; B: tail; I, J: end view of vulval-fenestral region showing fenestration and bullae; C, D, E, F: *H. goettingiana*. C: anterior end; F: tail; D, E: end view of vulval-fenestral region showing fenestration and vulval denticles; G, H, K: *C. cacti*. G: anterior end; H: tail; K: end view of vulval-fenestral region showing fenestration. Scale bar A-C, F-H – 20 µm, D-E, I-K– 40 µm.

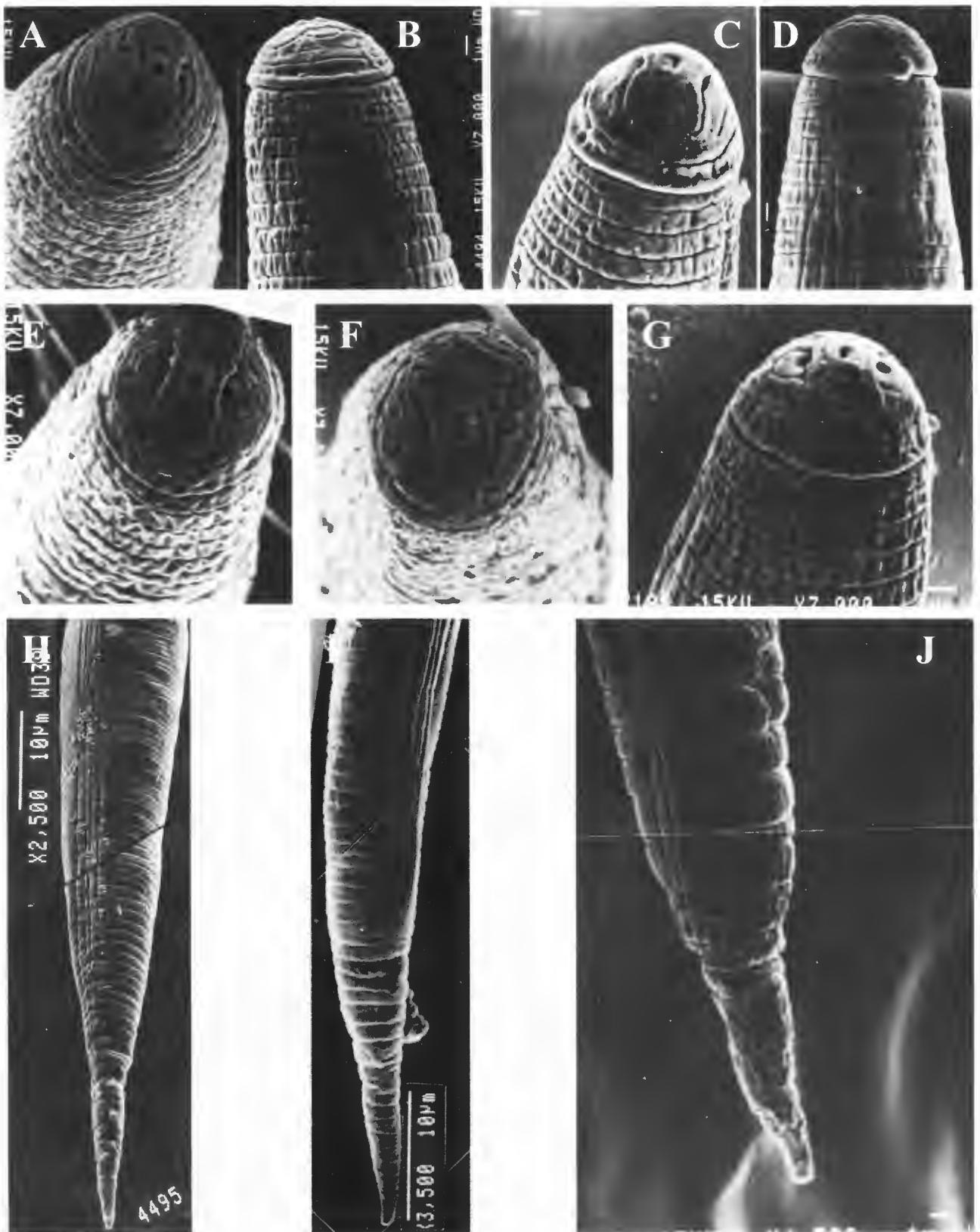


Fig. 5. Scanning electron micrographs of second stage juvenile of *Heterodera* species, lip region and tail. A, B, H: *H. humuli*; C, D, I: *H. fici*; E, F, G, J: *H. turcomanica*. Scale bars A-G – 1 μm, H-I – 10 μm.

Table 2. Morphometric characters of second stage juveniles of some *Heterodera* species from Iran (measurements in μm).

Species	<i>H. mothi</i>		<i>H. goettingiana</i>		<i>C. cacti</i>	
	Khuzestan population	Iranshahr population	Doroud population	Akbar Abad population	Mahalat 1 population	Mahalat 2 population
n	12	20	20	11	20	10
L	449 \pm 17.5 (424-486)	465 \pm 20.01 (431-501)	456 \pm 25.96 (408-486)	459 \pm 33.9 (409-519)	447 \pm 18.06 (416-479)	448 \pm 38.63 (402-513)
a	27.90 \pm 1.46 (25.94-30.4)	30.10 \pm 1.61 (27.56-33.40)	22.68 \pm 1.22 (20.4-24.3)	22.16 \pm 1.30 (20.35-24.24)	23.06 \pm 1 (21.25-24.36)	25.40 \pm 1.70 (22.33-27.35)
b			6.6 \pm 0.38 (3.93-5.64)	4.42 \pm 0.15 (4.18-4.68)	4.02 \pm 0.23 (3.56-4.56)	
c	6.18 \pm 0.41 (5.73-7)	6.55 \pm 0.29 (6.21-7.26)	7.24 \pm 0.66 (6-9.33)	7.12 \pm 0.58 (6.3-8.24)	9.52 \pm 0.44 (8.81-10.4)	10.29 \pm 0.84 (9.13-11.15)
c'	6.77 \pm 0.57 (5.73-7.4)	6.68 \pm 0.45 (6-7.5)	5.08 \pm 0.45 (4-5.83)	5.18 \pm 0.38 (4.65-5.85)	3.76 \pm 0.26 (3.3-4.08)	3.59 \pm 0.31 (3.25-4)
Stylet Length	17.8 \pm 0.42 (17-18)	17.6 \pm 0.50 (17-18)	25 \pm 0.75 (24-26)	24.8 \pm 0.94 (22.84-25.7)	24.3 \pm 0.57 (23-25)	24.3 \pm 1.03 (23-26)
Lip region height	3	3.05 \pm 0.22 (3-4)	3.7 \pm 0.37 (3-4)	4.3 \pm 0.23 (3.8-4.76)	4	4
Lip region width	6.8 \pm 0.42 (6-7)	6.4 \pm 0.51 (6-7)	8.7 \pm 0.52 (8-10)	8.9 \pm 0.27 (8.56-9.52)	9	8.5 \pm 0.5 (8-9)
D.G.O.	4.5 \pm 0.93 (4-6)	5.4 \pm 0.68 (4-7)	7.2 \pm 1.10 (6-9)	6.2 \pm 0.76 (4.76-7.14)	4 \pm 0.51 (3-5)	4.5 \pm 0.5 (4-5)
Anterior to exc. pore	83 \pm 4.32 (77-92)	90 \pm 3.23 (83-95)	96 \pm 7.11 (84-106)	100 \pm 7.16 (89.5-118.4)	100 \pm 4.08 (92-107)	101 \pm 10 (91-117)
Median bulb length	56 \pm 2.57 (53-60)	59 \pm 1.69 (56-63)	67 \pm 4.44 (58-75)	72 \pm 5.56 (63.8-81.4)	68 \pm 4.04 (58-73)	70 \pm 4.6 (64-76)
Body width at mid body	16 \pm 0.57 (15-17)	15.4 \pm 0.51 (15-16)	20 \pm 0.4 (19-21)	21 \pm 0.94 (19-22.37)	19.4 \pm 0.5 (19-20)	17.6 \pm 1.4 (16-20)
Body width at anus	10.8 \pm 0.63 (10-12)	10.6 \pm 0.49 (10-11)	12.5 \pm 0.63 (11-13.5)	12.5 \pm 0.78 (11-13)	12.5 \pm 0.51 (12-13)	12 \pm 0.41 (12-13)
Hyaline part	36 \pm 4.67 (31-45)	32 \pm 2.58 (28-37)	33 \pm 3.17 (27-38)	38 \pm 4.25 (31-43.8)	18 \pm 2.79 (12-24)	16 \pm 2.5 (12-19)
Tail length	73 \pm 5.43 (63-81)	71 \pm 3.02 (66-76)	63 \pm 5.88 (54-70)	65 \pm 6.01 (53.8-74.25)	47 \pm 2.96 (40-52)	44 \pm 3.8 (39-48)
Hyaline / Stylet	2.00 \pm 0.25 (1.72-2.5)	1.84 \pm 0.14 (1.56-2.18)	1.32 \pm 0.1 (1.13-1.48)	1.55 \pm 0.15 (1.75-1.55)	0.75 \pm 0.11 (0.5-0.96)	0.66 \pm 0.12 (0.46-0.79)
L / Median bulb length	8.00 \pm 0.40 (7.07- 8.43)	7.92 \pm 0.33 (7.43-8.95)	6.82 \pm 0.42 (5.56-7.53)	6.40 \pm 0.14 (6.18-6.66)	5.22 \pm 0.32 (4.44-5.77)	6.43 \pm 0.54 (5.74-7.13)

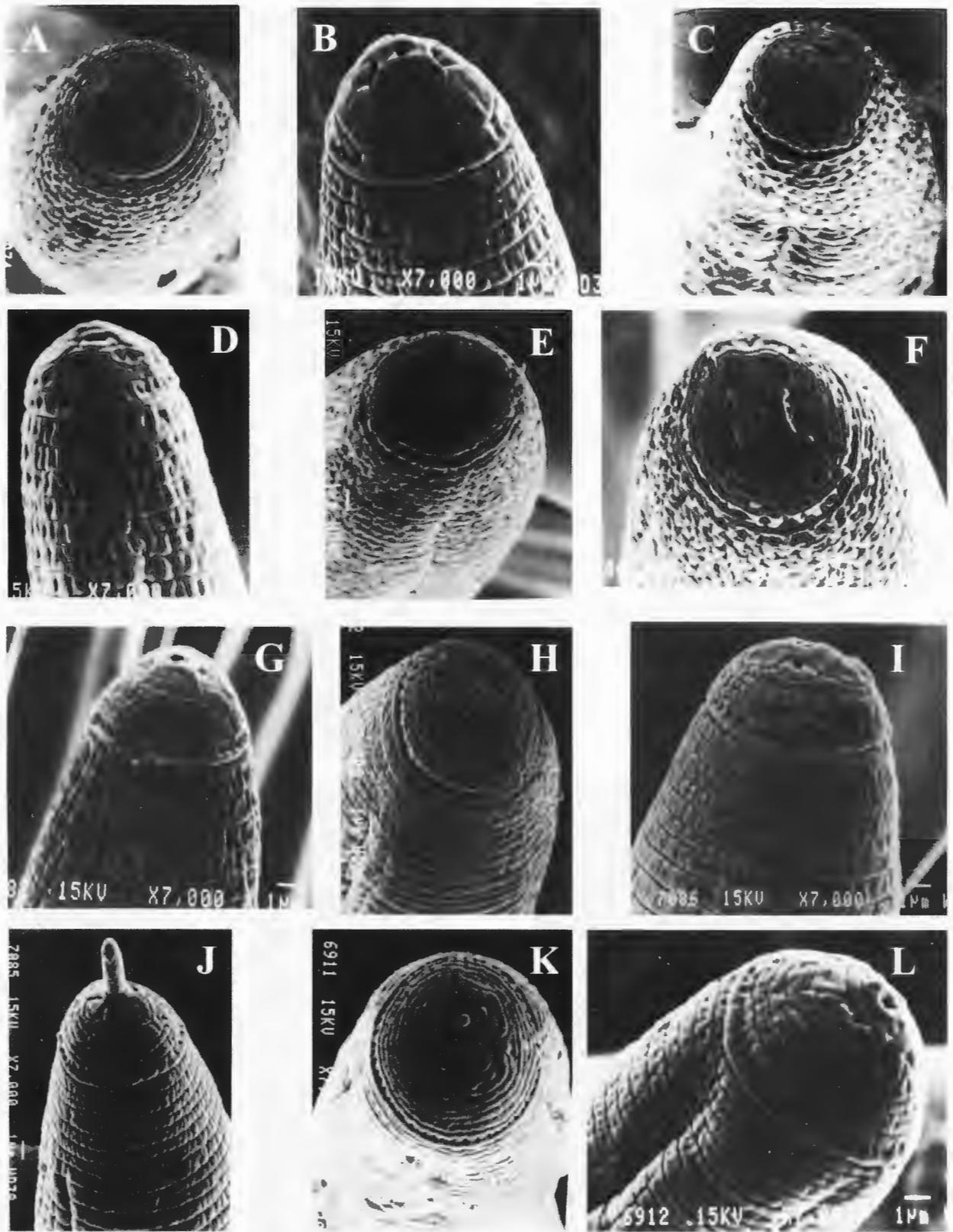


Fig. 6. Scanning electron micrographs of second stage juvenile of *Heterodera* and *Cactodera* species, lip region and tail. A, B,; *H. glycines*; C, D: *H. mothi*; E, F, G: *H. elachista*. H, I, J: *H. goettingiana*; K, L: *C. cacti*. Scale bars – 1 μ m.

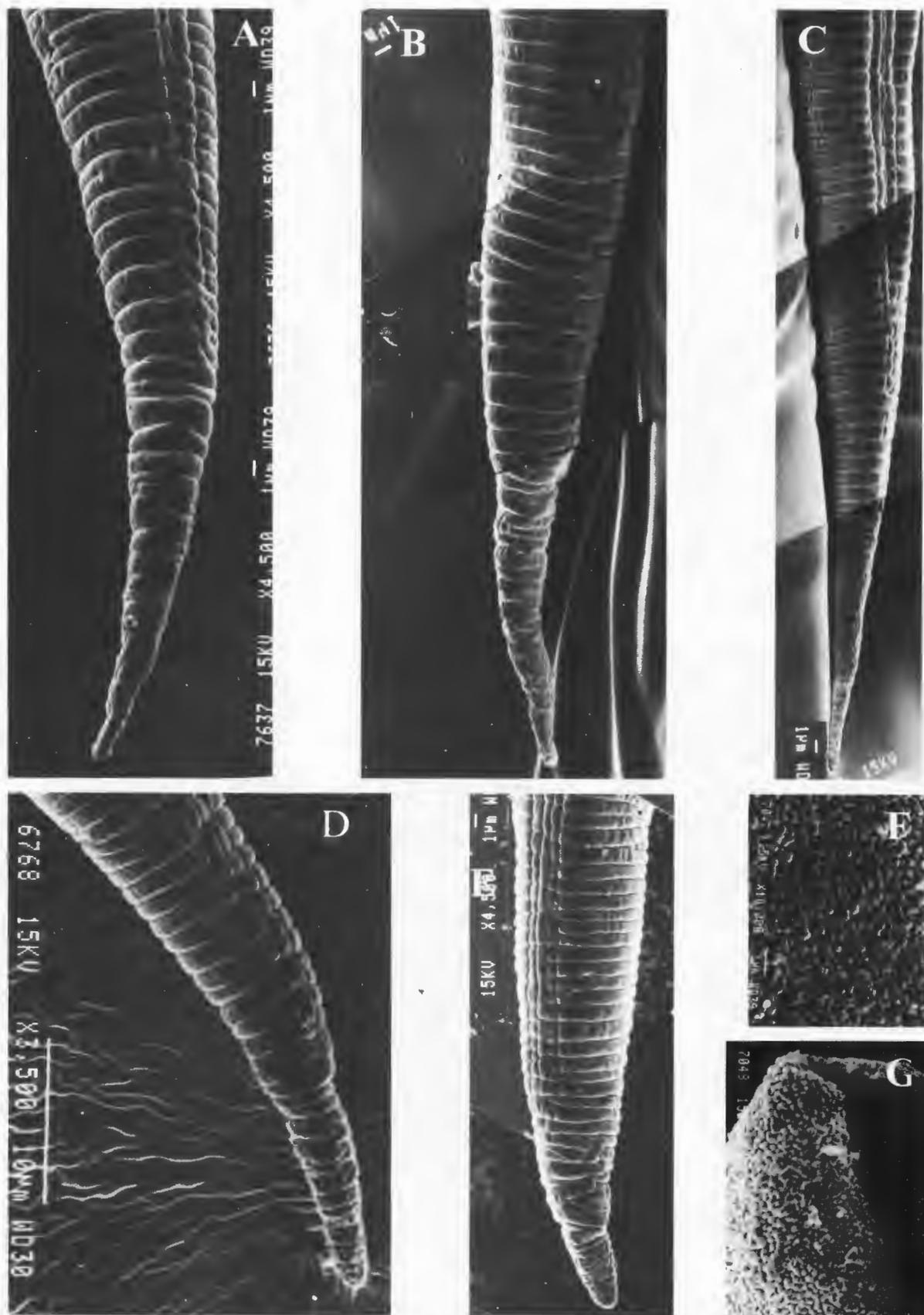


Fig. 7. Scanning electron micrographs of second stage juvenile of *Heterodera* and *Cactodera* species, tail and eggshell surface. A: *H. goettingiana*; B: *H. elachista*; C: *H. mothi*; D: *H. glycines*; E, F, G: *C. cacti*. Scale bars A-C, E-G- 1 µm, D - 10 µm.

Table 3. Morphometric characters of cysts and cyst vulval areas of some *Heterodera* species found in Iran (measurements in μm).

Species	<i>H. humuli</i>	<i>H. fici</i>	<i>H. turcomanica</i>	<i>H. glycines</i>	<i>H. elachista</i>	<i>H. mothi</i>		<i>H. goettingiana</i>	
Locality	Amol	Sanandaj	Meshkinshahr	Gorgan	Tonekabon	Khuzestan	Iranshahr	Akbarabad	Doroud
Cysts									
n	35	39	10	61	39	38	14	52	25
Length (excluding neck)	418 \pm 33 (370-500)	503 \pm 52 (340-530)	416 \pm 81.8 (280-570)	575 \pm 59 (480-720)	431 \pm 48 (340-530)	636 \pm 74 (510-770)	694 \pm 72.9 (600-820)	650 \pm 75.6 (500-700)	640 \pm 68.4 (510-780)
Width	310 \pm 32.9 (250-390)	377 \pm 54.6 (280-510)	355 \pm 75 (250-480)	411 \pm 53 (320-540)	311 \pm 48 (250-460)	324 \pm 48.2 (230-430)	336 \pm 57.4 (270-430)	420 \pm 49.2 (330-520)	382 \pm 42.8 (310-480)
Length / Width	1.35 \pm 0.12 (1.17-1.68)	1.33 \pm 0.15 (1.04-1.65)	1.19 \pm 0.2 (0.76-1.52)	1.41 \pm 0.16 (1.08-1.97)	1.39 \pm 0.15 (1.14-1.9)	1.98 \pm 0.23 (1.53-2.73)	2.09 \pm 0.23 (1.83-2.62)	1.55 \pm 0.15 (1.28-1.92)	1.68 \pm 0.2 (1.44-2.23)
Vulval area									
n	10	10	10	15	10	8	10	10	15
Vulval slit length	37.5 \pm 4 (30-43)	46.5 \pm 3 (42-50)	4.8 \pm 0.8 (4-6)	46 \pm 2.6 (42.5-50)	40 \pm 3.8 (32-45)	40 \pm 2.62 (36-44)	39 \pm 3.22 (34-45)	53 \pm 5.9 (44-61)	46 \pm 5 (35-55)
Fenestral length	48 \pm 5.6 (41-58)	46.5 \pm 3 (42-50)	58 \pm 4.6 (52-65)	44 \pm 7.9 (35-55)	40 \pm 6.7 (30-50)	44 \pm 4.50 (38-51)	40 \pm 4.53 (33-49)	61 \pm 4.6 (55-70)	58 \pm 8.3 (43-71)
Fenestral width	24.3 \pm 2.6 (20-27)	24.7 \pm 2.8 (22-30)	16.5 \pm 2.4 (13-20)	35 \pm 3.74 (30-42.5)	31.2 \pm 4.7 (26-42)	30 \pm 2.14 (27-33)	29 \pm 2.51 (25-33)	43 \pm 3.6 (36-48)	38 \pm 4.9 (30-45)
Underbridge length	80 \pm 4.5 (75-90)	92 \pm 10.65 (80-110)		115 \pm 8 (110-125)	85	80 \pm 2.62 (45-52)	55 \pm 4 (50-60)	107 \pm 6.35 (98-115)	97 \pm 10.1 (78.5-105)
Vulva- anus distance	44 \pm 5.6 (38-50)					80 \pm 2.44 (78-85)	78 \pm 2.3 (75-82)		
Width of vulval bridge	11.7 \pm 3.8 (8-18)		27.1 \pm 3.41 (20-32)						
Semifenestra length			19.2 \pm 1.7 (17-22)						

All the morphological characters and morphometrics of the Iranian populations correspond well with the descriptions reported for *H. glycines* Ichinohe, 1952 (Hirschmann, 1956; Burrows & Stone, 1985). *H. glycines* is characterised by ambifenestrate cysts, long vulval slit, strong underbridge, robust and numerous bullae in vulval cone. It belongs to the '*schachtii*' group according to Mulvey (1972) and Stone (1975).

Based on vulval cone characteristics, *H. glycines* resembles *H. trifolii*, *H. daverti*, and to some extent *H. schachtii*, but differs from the first species by the presence of male and a shorter body length (average 435-454 vs 517 μm in Wouts & Sturhan, 1978), shorter stylet of second stage juvenile (average 22.6-22.7 vs 28 μm), shorter distance of the opening of dorsal pharyngeal gland from stylet knobs (average 4-4.3 vs 5.14 μm), shorter tail length (average 50-51 vs 65.3 μm), and shorter hyaline part of the tail (29.7-29.8 vs 37.5 μm). Second stage juveniles of *H. glycines* further differ from *H. trifolii* by having a shorter tail with bluntly rounded terminus compared with the longer tail with very fine rounded terminus in *H. trifolii*. Second stage juveniles of *H. glycines* differ from *H. daverti* by the shorter stylet (average 22.6-22.7 vs 25 μm), and relatively short hyaline part of the tail (29.7-29.8 vs 33.3 μm). *H. glycines* is differentiated from *H. schachtii* by some characteristics of second stage juveniles and the cyst vulval area. The second stage juveniles of *H. glycines* differ from those of *H. schachtii* by having a shorter stylet length (22.6-22.7 vs 25.6 μm in Wouts & Weischer, 1977), shorter distance of the dorsal pharyngeal gland opening from stylet knobs (average 4-4.3 vs 5.4 μm). The cysts of *H. glycines* possess longer and wider fenestrae compared with those of *H. schachtii* (35-55 vs 24-38 μm and 30-42.5 vs 18-30 μm , respectively). Moreover, cysts of *H. glycines* lack the molar shaped bullae which is considered as a diagnostic character for discrimination of *H. schachtii* from similar species.

In Iran, *H. glycines* was first reported from soybean fields by Tanha Maafi *et al.* (1999). It was found in two of the most important soybean growing regions, the Mazandaran and Golestan provinces in the northern regions. Before the present study the soybean cyst nematode was catalogued as a quarantine nematode in Iran. The occurrence of this highly pathogenic nematode in the most important soybean producing areas is a serious threat for this crop. The high numbers of *H. glycines* in soil and root samples collected from two different soybean cultivating areas suggest the establishment of the soybean cyst nematode in the

country occurred a long time ago.

***Heterodera elachista* Oshima, 1974 (Figs. 1, 4, 6, 7; Tables 1, 3)**

Spherical to elongated cyst with distinct vulval cone, light to dark brown, subcrystalline layer present, but detached in older cysts; cuticle with irregular zigzag pattern. Vulval cone ambifenestrate, vulval slit long (average: 40 μm), few scattered but large, dark brown and prominent bullae present; generally a single large one situated near the anus. Vulval bridge narrow, underbridge moderate. Second stage juvenile vermiform, slender, body curved ventrally after fixation. Lip region low, hemispherical with three fine annules and a labial disc, slightly offset from the rest of the body, stylet moderate, cone distinctly shorter than the posterior part. Stylet knobs small, rounded or slightly concave anteriorly. Lateral field with three incisures, starting seven to eight annules below the lip region and ending mid-way along the tail. Phasmids small, six to eight annules posterior to anus level. Tail long, narrowly tapering to a very fine, rounded terminus. SEM photographs of the lip region of second stage juveniles in en face view show fusion of submedian lips with labial disc and the ventral and dorsal boundaries of the fused oral disc showing a depression in the median position.

The morphological characters and morphometrics of second stage juveniles and cysts of Iranian *H. elachista* populations agreed well with the original description except for the longer fenestral length (40 vs 29.3 μm) (Oshima, 1974; Nobbs *et al.*, 1992). The lemon shaped, bullate and ambifenestrate cysts place *H. elachista* in the '*schachtii* group' or 'group 4' of Mulvey (1972). Baldwin & Mundo-Ocampo (1991) came to the same conclusion. According to Stone's grouping of *Heterodera* species based on the lip region (Stone, 1975), *H. elachista* should be placed within the '*goettingiana* group' as Nobbs *et al.* (1992) concluded earlier. However, these latter authors also mentioned the necessity of an additional grouping for some of the *Heterodera* species with bullae and weak to absent underbridge and classified within the '*schachtii* group' or '*goettingiana* group', such as *H. cyperi*, *H. elachista*, *H. oryzicola* and *H. moths*. Based on molecular and morphological characters, Subbotin *et al.* (2001) erected the '*cyperi* group' to include all of these species. In view of the morphological characters and morphometrics of its second stage juveniles as well as the characteristics of the vulval cone and the phylogenetic relationships (Tanha Maafi *et al.*, 2003), *H. elachista* is to be placed in the '*cyperi*

group'.

Having second stage juveniles with a rather shorter body length and three lateral lines, ambifenestrate cysts, and especially their occurrence in rice fields made it necessary to compare *H. elachista* populations with other species earlier recorded either from rice or from weeds in rice fields viz, *H. oryzicola*, *H. oryzae*, *H. sacchari*, and *H. mothi*. Amongst these species, *H. elachista* possesses significantly the smallest cyst size while the last three species have larger cyst sizes. The underbridge in both *H. oryzae* and *H. sacchari* is more prominent with the central section enlarged into a plate like structure, while *H. elachista* and *H. oryzicola* possess a thinner and fragile underbridge. *H. elachista* can be further differentiated from *H. oryzicola* and *H. oryzae* by: (i) the lip region pattern of second stage juveniles in which the fused oral disc with submedial lips is much shorter and wider than in the two latter species, as well as the existence of a slight indentation between adjacent submedial lips and a distinct depression between the submedial and lateral lips, (ii) few bullae (vs many bullae in *H. oryzicola* and *H. oryzae*), (iii) very weak underbridge (vs well developed in *H. oryzae*) (Oshima, 1974; Nobbs *et al.*, 1992).

Heterodera elachista differs from *H. mothi* in having more rounded and smaller cysts (average: 406-446.3 vs 630 μm), a much longer stylet length of second stage juveniles (average: 18.6-19.2 vs 16-17 μm ; Khan & Husain, 1965), and the presence of three incisures in the lateral field of second stage juveniles against four (Shahina & Maqbool, 1991). *H. elachista* is also close to *H. cyperi* but can be separated from this species by having larger cysts (430.7 vs 640 μm) and possessing bullae in the vulval cone (Golden *et al.*, 1962).

Heterodera elachista was found in rice fields in two different areas in Tonekabon region (Mazandaran province). *H. elachista* has only been reported from Japan so far (Oshima, 1974; Nobbs *et al.*, 1992).

***Heterodera mothi* Khan and Husain, 1965 (Figs. 1, 3, 6, 7; Tables 2, 3)**

Lemon shaped to elongated cyst, some specimens fully elongated ($L/W > 2$), light brown to slightly dark. Subcrystalline layer present, cuticle with irregular zigzag pattern. Vulval cone distinct, ambifenestrate; vulval slit long, a few but well pigmented bullae present, underbridge short and weak. Second stage juvenile vermiform, slender, body curved ventrally after fixation, lip region dome shaped low with three fine annules and a

labial disc, continuous or slightly set off the rest of the body. Stylet developed, stylet knobs rounded, somewhat forwarded anteriorly. Lateral field with four incisures starting seven to nine annules below the lip region and ending at the second half of the tail. Phasmids small, 10-13 annules posterior to anus level, tail long, tapering to a nearly fine, rounded to acute terminus. SEM photographs of the lip region of second stage juveniles in en face view show fusion of submedial lips with labial disc; the ventral and dorsal boundaries of the fused structure show a distinct division in the median position.

The characteristics of cyst vulval cone as well as the morphological features and morphometrics of second stage juveniles agree well with redescription of *H. mothi* by Shahina & Maqbool (1991). Khan & Husain (1965) described *H. mothi* with three lateral lines in the lateral field of second stage juveniles and placed it within the '*schachtii* group'. Mathews (1971) also considered it as a member of the '*schachtii* group', but Mulvey (1972) and Mulvey & Golden (1983) included *H. mothi* in the '*goettingiana* group'. Shahina & Maqbool (1991) redescribed *H. mothi* with four incisures in the lateral field of the second stage juveniles and classified it in the '*schachtii* group' because of the presence of both strongly developed bullae and an underbridge in their studied materials. We consider *H. mothi* to be a member of the '*cyperi* group'.

Amongst the cyst-forming nematodes reported on graminaceous plants, viz. *Cyperus rotundus* and *Cynodon dactylon* four species are similar to *H. mothi*, i.e. *H. cyperi*, *H. graminis*, *H. delvii* and *H. elachista*. *H. mothi* differs from *H. cyperi* by having (i) bullae, a longer vulval slit (39.2-40.4 vs 30 μm), (ii) four incisures in the lateral field of second stage juveniles, (iii) a longer tail (average 70.9-72.9 vs 60 μm) and (iv) a shorter hyaline part of the tail (average 32.3-35.60 vs 24.7 μm). Cysts of *H. mothi* can be distinguished from *H. graminis* and *H. delvii* by (i) being more elongated (average $L/W = 2$ vs 1.3 and 1-1.2, respectively), and (ii) having bullae in the vulval area. The second stage juveniles of *H. mothi* differ from those of *H. graminis* and *H. delvii* by having (i) four incisures in the lateral field (against three in *H. graminis* and *H. delvii*), (ii) a longer tail length (average 70.9-72.9 vs 56 and 49-60 μm , respectively). They further differ from *H. graminis* in having a longer hyaline part of the tail (average 32.3-35.6 vs 29 μm) and a shorter stylet length (average 17.6-17.8 vs 22 μm).

Heterodera mothi was recovered from the soil around banana plants in Iranshahr (Systann and Blouchesta province) and *Cyperus rotundus* in

Khuzestan province. As no cyst was observed on the banana roots in first sample, it is assumed that the cysts were associated with the graminaceous weeds present in the banana plantations. *H. mothi* was previously reported from Iran based on the morphological characters of cyst vulval cone (Tatchian *et al.*, 1976).

***Heterodera goettingiana* Liebscher, 1892 (Figs. 1, 4, 6, 7; Tables 2, 3)**

Lemon shaped cyst with small but distinct vulval cone, light to dark brown; a very thin subcrystalline layer present, cuticle with irregular zigzag pattern and fine punctuation. Vulval cone ambifenestrate, vulval slit long (44-61 μm); in some older cysts the vulval bridge is ruptured and the two fenestrae are joined and form a large oval fenestrum. In all specimens bullae-like structures, so called vulval denticles, are present; in some specimens these denticles resemble bullae. Underbridge weak. Second stage juvenile vermiform, body curved ventrally after fixation. Lip region hemispherical, high with four fine annules and a labial disc, slightly set off from the rest of the body. Stylet strong, stylet knobs large, rounded, slightly projecting anteriorly. Lateral field with four incisures starting seven to nine annules below the lip region and ending at the second half of the tail. Phasmids small indistinct, 9-12 annules posterior to anus level. Tail narrowly tapering to a fine, rounded terminus. SEM photographs of the lip region of second stage juveniles in en face view show fusion of submedian lips with labial disc and ventral and dorsal boundaries of the fused oral disc with a partial division in the median boundaries position.

The morphometrics and morphological features of second stage juveniles and males of the Iranian populations of *H. goettingiana* agree well with those given by Stone & Course (1974); the characters of cysts and the vulval area of cysts, however, are slightly different. The cysts differ by having a larger body length (650 vs 521 μm), and a longer fenestral length (61.10 vs 35.3 μm). In all Iranian specimens vulval denticles are located below the fenestration, and in many cases they seem quite large bullae (vs without denticles). The cyst cuticle and egg shell possess fine punctuation, visible by light microscope and scanning electron microscope (no information about the cuticle and egg shell in the description of *H. goettingiana* given by Stone & Course, 1974). The lip region of second stage juveniles differs from the description by Stone & Course (1974) by having four (sometimes five) distinct annules (vs two to three

annules). Some second stage juveniles of *H. goettingiana* deposited at the Institute of Nematology, Biologische Bundesanstalt, Münster were also studied. Those specimens also possess four to five distinct annules in the lip region. Based on the morphometrics and the morphological features, as well as on SEM observations of lip region patterns of second stage juveniles and earlier molecular studies (Tanha Maafi *et al.*, 2003), we consider the Iranian specimens as *H. goettingiana*.

Heterodera goettingiana generally resembles *H. cruciferae*, *H. carotae*, and *H. urticae* in the 'goettingiana group', but differs from *H. cruciferae* and *H. carotae* in having larger cysts (521 \pm 53 vs 429 \pm 67 and 408 μm , respectively) and in second stage juveniles having longer tail (60 \pm 5.3 vs 50 \pm 2.7 and 51.8 μm , respectively) and a longer hyaline part of the tail (37 \pm 3.2 vs 25.2 \pm 2.7 and 28.3 μm , respectively). It is distinguished from *H. urticae* by having larger and more elongated cysts (521 \pm 53 vs 492 μm and length/width (average 1.55-1.68 vs 1.1) and in second stage juveniles by having a shorter stylet and shorter hyaline part of the tail (24.6 \pm 0.8 vs 27 μm and 37 \pm 3.2 vs 29 μm , respectively) (Mathews, 1971; Mathews, 1975; Stone & Course, 1974; Stone & Rowe, 1976).

Heterodera goettingiana was found in two different regions in the Lorestan province including Akbar abad (Doroud) and Doroud in a clover and fallow field, respectively. The material sampled from Doroud was tested on pea (*Pisum sativum*) in the greenhouse and multiplied successfully.

***Cactodera cacti* (Filipjev and Schuurmans Stekhoven, 1941) Krall and Krall, 1978 (Figs. 1, 4, 6, 7; Tables 2, 3)**

Mahalat 2 Population. Cyst (n=21): Length (excluding neck) = 490 \pm 62 μm (380-600); width = 382 \pm 59 μm (280-490); length / width ratio = 27.70 \pm 3.3 (23-35). Vulva area (n=10): Fenestra diameter (dorsal-ventral) = 28 \pm 3.3 μm (23-35); fenestra diameter (lateral) = 28 \pm 2.06 μm (25-30); distance of anus to vulval slit = 41 \pm 6.37 μm (35-55).

Lemon shaped generally rounded cyst with distinct vulval cone, light brown to brown, rarely dark brown. Subcrystalline layer not distinct, cuticle with irregular zigzag pattern, with distinct punctuation. Vulval area circumfenestrate, abulate. Second stage juvenile vermiform, body curved ventrally after fixation, lip region high, hemispherical with four distinct annules and a labial disc, set off from the rest of the body. Stylet well developed, stylet knobs large, fully rounded

rarely slightly concave anteriorly. Lateral field with four incisures starting seven to eight annules below the lip region and ending at the second half of the tail. Phasmids small, 10-13 annules posterior to anus level, tail conical with a fine, rounded terminus. SEM photographs of the lip region of second stage juveniles in en face view indicate existence of six lips which are separated from one another and lateral lips reduced in size. In some cases the fusion of lateral lips with submedian lips on one side of labial disc is observed, the labial disc is elongated dorso-ventrally. This pattern agrees with type 1 lip region pattern of Stone (1975), which is characteristic for *Globodera* and *Cactodera*.

By comparison of the morphological features and morphometrics of the Iranian populations with similar species of the genus, the Iranian specimens of *Cactodera* correspond with *C. cacti* and all the data agree well with previously reported data (Graney & Bird, 1990; Mulk, 1977; Mulvey and Golden, 1983). Furthermore, there is host range similarity between the Iranian *C. cacti* populations and the original description. *C. cacti* is morphologically closely similar to *C. milleri*, but differs from it in various characters. The second stage juveniles of *C. cacti* differ from those of *C. milleri* by having a longer stylet length (average 24.3 vs 21.8 μm) and a longer body length (average 447.25-448.17 vs 426 μm). The two species also show some differences in the dimensions of vulval area, the fenestra diameter measured dorso-ventrally and laterally in *C. cacti* are larger than those of *C. milleri* (27.70 vs 13.3 and 27.70 vs 18.7 μm , respectively). *Cactus* plants are common host for *C. cacti* whereas *C. milleri* reproduces only on *Chenopodium album*, *C. amaranticolor* and *C. quinoa* and not on five tested cactus species (Graney & Bird, 1990; Mulk, 1977). We found *C. cacti* on cactus plants in two different greenhouses in Mahalat (Markazi province), one of the most important producing areas for ornamental plants.

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Tanha Maafi Z., Sturhan S., Kheiri A., Geraert E., Subbotin S., Moens M. Морфология некоторых цистообразующих нематод Ирана.

Резюме. Проведенное определение цистообразующих нематод в Иране выявило присутствие нескольких видов родов *Heterodera* and *Cactodera*. Впервые приводятся описания *Heterodera elachista*, *H. fici*, *H. glycines*, *H. goettingiana*, *H. humuli*, *H. turcomanica* и *Cactodera cacti*, обнаруженных на различных растениях в различных регионах страны. Для всех этих видов, а также для *H. mothi* даны описания морфологии и морфометрические данные.
