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Heterodera circeae sp. n. and *H. scutellariae* sp. n. (Tylenchida: Heteroderidae) from Germany, with notes on the *goettingiana* group

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Summary – Two species of cyst-forming nematodes belonging to the *goettingiana* group are described from woodland in Germany. *Heterodera circeae* sp. n., parasitising *Circaea lutetiana* (Onagraceae), most closely resembles *H. carotae* and *H. cruciferae*, from which it differs by larger cysts (430-720 μm) and longer fenestral length (27-54 μm) and rounded stylet knobs of the second-stage juveniles. *Heterodera scutellariae* sp. n., found on the labiate *Scutellaria galericulata* (Lamiaceae), is distinguished from the other species of this group by shorter body (358-437 μm) and tail (41-53 μm) of the second-stage juveniles. The ITS-rDNA sequences of the new species are very similar and differ from each other by only a few nucleotides. The phylogenetic position of the new species within the group is presented based on analyses of molecular data. A revised diagnosis of the *goettingiana* group is proposed. The species of the group have lemon-shaped, ambifenestrate and abullate (small bullae occasionally present) cysts with egg sac, long vulval slit (>35 μm), thin vulval bridge, low semifenestrae (average fenestral length 30-45 μm), and weak underbridge. Second-stage juveniles are characterised by body length >400 μm, tail length >45 μm, hyaline tail portion >20 μm, stylet length >20 μm, lateral field with four incisures and small phasmids. All known species of the *goettingiana* group exclusively parasitise dicotyledonous plants.

Keywords – cyst nematode, Lamiaceae, new species, Onagraceae, phylogeny, rDNA, woodland.

In 1980, a few juveniles of an unknown *Heterodera* species were isolated from soil collected in a beech forest located close to the town of Münster, Germany. In subsequent years more specimens and a few cysts were isolated from soil from the same site. The common nettle, *Urtica dioica* L., was considered to be a potential host of this cystnematode, which belonged to the *goettingiana* group, but attempts to culture it on this plant in the glasshouse failed. A careful examination of all plants growing at the sampling site in the summer of 2000 finally revealed *Circaea lutetiana* L., a plant of the Onagraceae family common in woodland on heavy soils throughout Germany, to be the host of this heteroderid.

A second *Heterodera* species, resembling *H. cruciferae* Franklin, 1945, was found for the first time in a bulked soil sample collected from a deciduous forest near Bremen in 1989. Subsequent sampling at this site revealed a total of seven heteroderids: *Heterodera ustinovi* Kirjanova, 1969; *H. trifolii* Goffart, 1932; *H. ripae* Subbotin, Sturhan, Rumpenhorst & Moens, 2004; *H. urticae* Cooper, 1955; *Heterodera* sp.; *Punctodera* sp.; *Meloidodera alni* Turkina & Chizhov, 1986. In August 2000, cysts of this unidentified *Heterodera* species were finally found on the roots of *Scutellaria galericulata* L., a labiate plant widely occurring on moist soils, particularly along rivers.

Comparative morphological, morphometric and molecular studies revealed both species to be distinct from the known representatives of the *goettingiana* group and they are described herein as new species, with analyses of their phylogenetic positions within this group based on the ITS-rDNA sequences. A revised diagnosis of the *goettingiana* group is proposed.

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Materials and methods

Nematode populations

The descriptions and molecular studies are based on a population of each *Heterodera* species isolated from soil samples by the sieving-decanting method and the centrifugation-flotation method. In addition, some cysts and males were isolated from roots of *C. lutetiana* and *S. galericulata*, respectively. Juveniles were usually released from crushed cysts. Several populations and ITS-rDNA sequences of species from the *goettingiana* group were included for molecular study (Table 1). Permanent slides of several similar *Heterodera* species were available for morphological comparison from different collections.

Light microscopy

Nematodes were killed and fixed in hot TAF and processed to glycerin by a modified slow evaporation method. Specimens were mounted in dehydrated glycerin on permanent slides and examined, measured and photographed with Zeiss and Leitz light microscopes equipped with Nomarski differential contrast optics. Cyst cones were mostly mounted in glycerin jelly.

DNA extraction, amplification and sequencing

The methods used for DNA extraction and PCR amplification were similar to those described by Subbotin *et al.* (1997). Primers TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) were used in the PCR reaction. Prior to sequencing, PCR products were purified with QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). PCR products were directly sequenced in both directions using TW81, AB28 and internal reverse primer 5.8M5 (5′-GGCGCAATGTGCATTCGA-3′) with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Warrington, Cheshire, UK) according to the manufacturer's instructions using a 377 DNA sequencer (PE Applied Biosystems). Sequences of the new species were submitted to GenBank under accession numbers AY368994 (*H. circeae* sp. n.) and AY368995 (*H. scutellariae* sp. n.).

Sequence alignment and phylogenetic analyses

Original and known sequences of species of the *goettingiana* group (Subbotin *et al.*, 2001; Tanha Maafi *et al.*, 2003; Madani *et al.*, 2004) and sequences of the two outgroup taxa *Meloidodera alni* and *Cryphodera brinkmani* Karssen & van Aelst, 1999 were aligned using ClustalX 1.64 with default options (Thompson *et al.*, 1997). The equally weighted maximum parsimony (MP) and maximum likelihood (ML) analyses of the ITS alignment were performed using PAUP 4b4a (Swofford, 1998). Heuristic search settings were ten random replicates of taxon addition with tree bisection-reconnection (TBR) branch swapping. Gaps were treated as a missing data. For ML analysis, the appropriate substitution model of DNA evolution that best fitted the data set was determined by Akaike Information Criterion with ModelTest 3.04 (Posada & Crandall, 1998). Bootstrap support was estimated by a heuristic search from 100 replicates.

Heterodera circeae [*] **sp. n.**

(Figs 1, 2)

Measurements

See Table 1.

Description

Cyst

Lemon-shaped with distinct neck and vulval cone, colour changing from white to pale or medium brown without intermediate yellow stage. Cuticle with irregular zig-zag pattern, mostly without remnants of subcrystalline layer. Egg sac large and filled with eggs. Vulval cone ambifenestrate. Bullae absent. Vulval bridge narrow and often broken in old cysts. Vulval slit longer than fenestral width. Underbridge bifurcate, weak, often lost during slide preparation. Anus prominent.

White female

Body lemon-shaped, with thin subcrystalline layer. Cephalic region with anterior lip annule and labial disc *ca* 3 μm wide. Stylet with rounded and backwardly-sloping knobs. Median bulb massive. Egg sac present.

[*] Derived from the genus of the host.

Male

General morphology typical for genus. Body of heat-relaxed specimens C-shaped, posterior part twisted about its longitudinal axis. Lip region hemispherically rounded, set off, with four to six annules and a faint labial disc. Cephalic framework heavily sclerotised. Stylet well developed, knobs rounded with anterior faces sloping posteriorly; stylet base *ca* 5 μm wide. Cephalids two to three and eight to ten annules behind lip region. Median bulb elongated-oval, occupying less than half of body diam.; valve plates well developed, 4 μm long. Dorsal and subventral pharyngeal gland lobes distinct. Hemizonid two annules long and situated five to seven annules anterior to excretory pore. Lateral field with four lines, outer bands irregularly areolated. Cuticle annules 1.5-2.0 μm wide at mid-body. Testis well developed. Spicules arcuate, with *ca* 2 μm wide tridentate tip. Gubernaculum curved, 10-14 μm long. Penial tube present. Tail short and rounded, sometimes appearing smooth at tip. Phasmids not visible.

Second-stage juvenile

Body of heat-relaxed specimens straight or slightly curved ventrad. Lip region slightly set off, rounded, with four to five indistinct annules visible laterally and indistinct labial disc. Cephalic framework strongly sclerotised. Stylet strong, knobs rounded and sloping posteriorly; stylet base 4.5-5.0 μm wide and *ca* 2 μm high. Cephalids indistinct. Median bulb oval, occupying *ca* 50% of corresponding body diam., valve plates 3 μm long. Pharyngeal glands well developed. Hemizonid zero to two annules anterior to excretory pore, one to two body annules long. Lateral field with four lines, only occasionally areolated. Annule width at mid-body 1.5-1.7 μm. Genital primordium situated at 52-63% of body length behind anterior end, with four distinct cells, 10-17 μm long. Tail conoid, gradually tapering to finely rounded terminus; hyaline portion irregularly annulated, occupying 50 (41-53)% of tail length, 3.1-4.8 times longer than its maximum diameter. Phasmids pore-like, eight to 18 annules posterior to anus (*i.e.*, at 25-45% of tail length).

TYPE HOST AND LOCALITY

Collected from rhizosphere and roots of *Circaea lutetiana* L. (Onagraceae, Myrtales, Rosidae), Münster-Nienberge, Germany (UTM grid MC06), in the 'Vorbergshügel' nature reserve, section Bückenbusch, *ca* 60 m east of road Nienberge-Häger in a shallow valley supporting beech forest (*Fagus sylvativa* L.) with young ash (*Fraxinus excelsior* L.) and *Arum maculatum* L., *Urtica dioica* L., *Galium odoratum* (L.), *Primula elatior* (L.), *Hedera helix* L., *Ranunculus ficaria* L., *Ribes silvestre* (Lam.), *Carex* sp., *Allium ursinum* L. *etc*., as subvegetation; heavy clay soil, pH 6.7, rather wet.

No specimens of *H. circeae* sp. n. were isolated from soil samples collected in dense stands of *C. lutetiana* at several sites close to the type locality.

OTHER LOCALITY

Vorwalde near Bramsche, *ca* 50 km north-west of the type locality; *Fagus sylvatica-Fraxinus excelsior-Alnus glutinosa* woodland with *Circaea lutetiana*, *Urtica dioica*, *Ribes silvestre*, *Hedera helix*, grasses *etc*., as subvegetation; sandy loam soil.

TYPE MATERIAL

Holotype cyst, 23 paratype cysts or vulval cones, six females, ten males and 41 second-stage juveniles (J2) deposited in the German Nematode Collection, Biologische Bundesanstalt, Münster, Germany (slides DNST 42/65/1-20). Additional paratypes deposited in the nematode collections of Institute of Parasitology, Russian Academy of Sciences, Moscow (11 cyst cones, five females, one male, 19 J2), and of Rothamsted Research, Harpenden, Herts, UK (five cyst cones, four females, one male, 16 J2).

DIAGNOSIS AND RELATIONSHIPS

Heterodera circeae sp. n. is a member of the *goettingiana* group (see diagnosis below). Diagnostic characters of cysts and J2 juveniles are given in Table 2. Males are characterised by four to six lip annules, a 27-29 μm long stylet with rounded knobs and 25-29 μm long spicules.

The new species is distinguished from *H. urticae* by shorter body (434 *vs* 541 μm) and stylet (25 *vs* 27 μm) in the J2; from *H. goettingiana* Liebscher, 1892 by shorter body (434 *vs* 486 μm), shorter tail (52 *vs* 60 μm) and shorter hyaline tail portion (26 *vs* 37 μm) of the J2. *Heterodera circeae* sp. n. most closely resembles *H. carotae* Jones, 1950 and *H. cruciferae*, from which it differs by larger cysts (555 *vs* 408 and 429 μm, respectively), longer fenestral length (43 *vs* 31 and 34 μm, respectively) and rounded stylet knobs in the J2 (see Table 2).

The ITS sequences of rDNA distinguish *H. circeae* sp. n. from the other species in the *goettingiana* group (see Fig. 5).

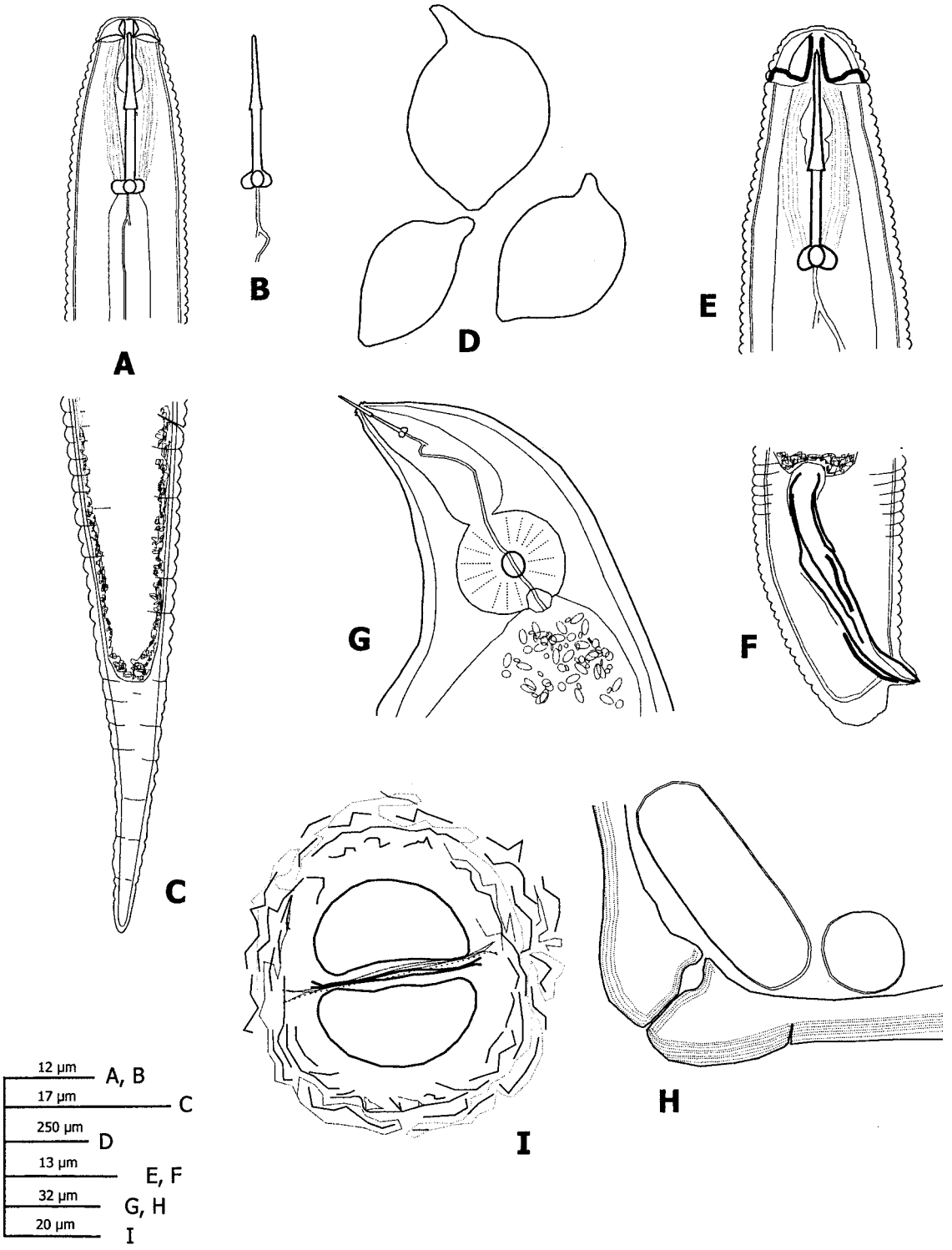

Fig. 1. Heterodera circeae *sp. n. A: Second-stage juvenile head; B: Second-stage juvenile stylet; C: Second-stage juvenile tail; D: Cysts; E: Male head; F: Male tail; G: Anterior end of white female; H: Posterior end of white female; I: Fenestration in vulval cone.*

Heterodera scutellariae[*] sp. n.

(Figs 3, 4)

Measurements

See Table 1.

Description

Cyst

Lemon-shaped with distinct vulval cone, colour changing from pale to medium brown. Several yellow-brown young cysts were observed. Cuticle with irregular zig-zag pattern. Remnants of subcrystalline layer rarely observed. Egg sac present, filled with eggs. Vulval cone ambifenestrate, without bullae. Vulval bridge narrow, basin thick. Vulval slit longer than fenestral width. Underbridge bifurcated, weak and often lost during slide preparation. Anus indistinct.

White female

Body lemon-shaped, covered with subcrystalline layer. Cephalic region with anterior lip annule and labial disc *ca* 2.5 μm in diam. Stylet with rounded and backwardly sloping knobs. Median bulb massive. Egg sac present.

Male

General morphology typical for genus. Body of heat-relaxed specimens C-shaped, posterior part twisted about longitudinal axis. Lip region hemispherically rounded, set off, with four to five annules and faint labial disc. Cephalic framework heavily sclerotised. Stylet well developed, knobs rounded with anterior faces sloping backward; stylet base measuring 5 μm across. Cephalids indistinct, two to three and eight to nine annules behind lip region. Median bulb elongated-oval, occupying less than half of body diam.; valve plates well developed, 4 μm long. Hemizonid 1.5 to two annules long and three to seven annules anterior to excretory pore; hemizonion more than one body diam. behind hemizonid. Lateral field with four lines, outer bands irregularly areolated at both extremities. Cuticle annuli 1.5-2.0 μm wide at mid-body. Testis well developed. Spicules arcuate, with *ca* 2 μm wide, tridentate, tip. Gubernaculum almost linear, about 10-11 μm long. Penial tube present. Tail short and rounded, often appearing smooth at terminus. Phasmids not visible.

[*] Derived from the genus of the host.

Second-stage juvenile

Body of heat-relaxed specimens slightly curved ventrad. Lip region slightly set off, rounded, with four to five indistinct annules and indistinct labial disc. Cephalic framework strongly sclerotised. Stylet strong, knobs slightly concave anteriorly; stylet base 4.2-4.8 μm wide and 2.0-2.5 μm high. Median bulb oval, 15-17 μm long and 11-12 μm wide, occupying about half of corresponding body diam.; valve plates 3 μm long. Pharyngeal glands well developed. Hemizonid zero to one body annule anterior to excretory pore, one to two annules long. Lateral field with four lines, not areolated. Cuticle annules 1.4-1.8 μm wide at mid-body. Genital primordium situated at 60-62% of body length behind anterior end, with four distinct nucleate cells, 11-16 μm long. Tail conoid, gradually tapering to a finely rounded terminus. Hyaline portion of tail irregularly annulated, occupying almost 50% of tail length, three to four times longer than its maximum diam. Phasmids pore-like, six to ten annules posterior to anus (*i.e.*, at 15-33% of tail length).

Type host and locality

Collected from the roots and rhizosphere of *Scutellaria galericulata* L. (Lamiaceae, Lamiales, Asteridae), Everinghausen, *ca* 20 km east of Bremen, Germany (UTM grid ND18), in a deciduous *Alnus-Quercus* forest east of the village and on the bank of a small river; humus sand, pH 3.8.

Type material

Holotype cyst, seven paratype cysts or vulval cones, four females, three males and 18 J2 juveniles deposited in the German Nematode Collection, Biologische Bundesanstalt, Münster, Germany (slides DNST 42/66/1-13). Additional paratypes deposited in the nematode collections of Institute of Parasitology, Russian Academy of Sciences, Moscow (four cyst cones, two females, five J2), and of Rothamsted Research, Harpenden, Herts, UK (two cyst cones, two females, 14 J2).

Diagnosis and relationships

Heterodera scutellariae sp. n. has the general characteristics of the *goettingiana* group (see diagnosis below). Additional characters of diagnostic significance of cysts and J2 are given in Table 2. Males have four to five lip annules, a 27-28 μm long stylet with rounded and backwardly sloping knobs and spicules *ca* 29 μm long.

Table 1. *Morphometrics of* Heterodera circeae *sp. n. and* H. scutellariae *sp. n. Measurements are in μm and in form: mean ± standard error (range).*

Stage	Character	*H. circeae* sp. n.		*H. scutellariae* sp. n.	
		Holotype	Paratypes	Holotype	Paratypes
Cyst					
	n	–	40	–	23
	L (excluding neck)	555	555 ± 12 (430-720)	560	560 ± 15 (430-720)
	Diam.	375	397 ± 11.4 (265-550)	430	424 ± 16 (290-550)
	Length/diam.	1.5	1.4 ± 0.02 (1.2-1.8)	1.3	1.3 ± 0.04 (1.1-1.8)
	Neck length	98	–	98	–
Vulval plate					
	n		20		12
	Fenestral length		43 ± 1.7 (27-54)		35 ± 1.8 (27-43)
	Semifenestral width		41 ± 1.2 (31-50)		39 ± 1.3 (33-47)
	Vulval slit length		48 ± 0.8 (43-54)		43 ± 1.5 (35-50)
	Vulval bridge width		6.6 ± 0.3 (4.3-7.8)		7.5 ± 0.6 (5.8-11)
	Underbridge length		83 ± 2.3 (70-97)		86 ± 2.2 (78-97)
	Vulva-anus distance		51 ± 1.1 (43-62)		48 ± 2.2 (35-58)
White female					
	n		35		5
	L (excluding neck)		567 ± 14 (385-670)		456 ± 33 (360-530)
	Diam.		377 ± 12.5 (290-530)		317 ± 41 (240-455)
	Length/diam.		1.5 ± 0.03 (1.2-1.8)		1.5 ± 0.1 (1.2-1.7)
	Stylet length		27 ± 0.8 (25-29)		27 ± 2.5 (25-29)
	Anterior end to median bulb valve		65 ± 3.2 (59-74)		72 ± 0.6 (71-73)
	Median bulb length		34 ± 1.7 (31-37)		34 ± 1.2 (31-35)
	Median bulb diam.		32 ± 2.1 (27-37)		30 ± 0.9 (28-31)
Male					
	n		9		4
	L		1130 ± 18 (1030-1200)		1030 ± 28 (950-1070)
	a		41 ± 1.2 (36-45)		39 ± 0.3 (39-40)
	b		7.2 ± 0.3 (6.5-8.3)		7.3 ± 0.3 (6.7-7.9)
	Max. body diam.		28 ± 0.5 (26-29)		26-27
	Stylet length		27 ± 0.3 (27-29)		27 ± 0.6 (27-28)
	Lip region height		5.8 ± 0.1 (5.1-6.4)		6.4 ± 0.2 (5.9-6.9)
	Lip region diam.		11 ± 0.1 (11-12)		10.1 ± 0.2 (9.8-10.5)
	Opening of dorsal pharyngeal gland from stylet base		6.1 ± 0.3 (4.9-7.8)		4.6 ± 0.6 (2.9-5.9)
	Median bulb from anterior end		91 ± 2.1 (81-102)		94 ± 2.5 (86-104)
	Excretory pore from anterior end		151 ± 2.5 (141-166)		134 ± 4.6 (127-147)
	Cardia from anterior end		155 ± 3.4 (140-171)		142 ± 3.6 (135-157)
	Genital tract length		578 ± 42 (395-705)		562 ± 65 (435-645)
	Spicule length		27 ± 0.6 (25-29)		29
Second-stage juvenile					
	n		28		20
	L		434 ± 4.0 (377-480)		408 ± 5.0 (358-437)
	a		22 ± 0.2 (19-25)		21 ± 0.2 (19-23)
	b		3.9 ± 0.03 (3.5-4.2)		4.2 ± 0.1 (3.9-4.6)
	c		8.4 ± 0.1 (7.7-9.4)		8.7 ± 0.1 (8.0-9.4)
	c′		2.7 ± 0.03 (2.3-2.9)		2.5 ± 0.04 (2.2-2.8)
	Lip region height		4.1 ± 0.05 (3.9-4.6)		4.0 ± 0.04 (3.8-4.4)
	Lip region diam.		8.9 ± 0.06 (8.3-9.8)		9.0 ± 0.1 (8.5-9.8)

Table 1. *(Continued).*

Stage	Character	*H. circeae* sp. n.		*H. scutellariae* sp. n.	
		Holotype	Paratypes	Holotype	Paratypes
	Stylet length		25 ± 0.1 (24-26)		24 ± 0.2 (23-26)
	Opening of dorsal pharyngeal gland from stylet base		5.5 ± 0.2 (4.6-6.9)		5.1 ± 0.1 (4.9-5.9)
	Median bulb from anterior end (MB)		70 ± 0.8 (59-76)		62 ± 1.5 (52-70)
	Excretory pore from anterior end		101 ± 1.0 (87-110)		89 ± 1.3 (77-98)
	Cardia from anterior end		111 ± 1.1 (100-121)		98 ± 1.8 (88-108)
	Diam. at mid-body		20 ± 0.1 (19-21)		19 ± 0.1 (18-20)
	Diam. at anus		13 ± 0.1 (12-15)		12 ± 0.1 (12-13)
	Tail length (TL)		52 ± 0.4 (47-57)		47 ± 0.7 (41-53)
	Hyaline portion of tail (H)		26 ± 0.3 (23-29)		25 ± 0.5 (20-29)
	L/MB		6.2 ± 0.1 (5.8-6.6)		6.6 ± 0.2 (5.7-7.9)
	TL/H		2.0 ± 0.02 (1.7-2.3)		1.9 ± 0.04 (1.5-2.3)
Egg					
	n		15		14
	L		100 ± 0.8 (96-106)		97 ± 0.5 (93-100)
	Diam.		47 ± 0.8 (41-52)		45 ± 1.1 (40-53)
	Length/diam.		2.2 ± 0.05 (1.9-2.6)		2.2 ± 0.05 (1.9-2.5)

Morphological characters distinguishing *H. scutellariae* sp. n. from other species in the *goettingiana* group are given in Table 2. From *H. urticae* and *H. goettingiana* it is mainly distinguished by shorter body (408 *vs* 541 and 486 μm, respectively), shorter tail (47 *vs* 58 and 60 μm, respectively) and shorter hyaline tail portion (25 *vs* 29 and 37 μm, respectively) of the J2; from *H. carotae* and *H. cruciferae* by larger cysts (560 *vs* 408 and 429 μm, respectively) and higher number of lip annules (five *vs* three to four) in the J2; and from *H. circeae* in shape of the stylet knobs (rounded *vs* slightly concave anteriorly).

The ITS sequence of rDNA differentiates *H. scutellariae* from other species of the *goettingiana* group (see Fig. 5).

Molecular characterisation and phylogenetic relationships within the *goettingiana* group

The ITS region sequence length for both species is 954 bp, which is the shortest amongst the species of this group. Sequences of *H. circeae* sp. n. and *H. scutellariae* sp. n. are very similar and differ from each other by only three nucleotides situated in the ITS1. Observed sequence divergence (0.3%) between these species is half that revealed in the present study between populations of *H. goettingiana* (six nucleotides, 0.6%) or *H. carotae* (six nucleotides, 0.6%). Although very low ITS sequence divergence is a good indicator as to specific identity of studied organisms, it is not always correct. Identical ITS sequences have been found in other pairs of clearly morphologically distinct cyst-forming nematode species such as *H. avenae* Wollenweber, 1924 and *H. arenaria* Cooper, 1955 (Subbotin *et al*., 2001), and *H. trifolii* and *H. daverti* Wouts & Sturhan, 1979 (Subbotin, unpubl.).

A phylogenetic study based on analyses of sequences from the ITS-rDNA revealed a basal position of the *goettingiana* group (*H. goettingiana*, *H. carotae*, *H. cruciferae*, *H. urticae*) within the genus *Heterodera* (Subbotin *et al.*, 2001). Positions of the new species within the *goettingiana* group yielded by two methods are shown in Figure 5. These species formed a separate clade and are closely related to an undescribed species from Iran.

Notes on the *goettingiana* group

Baldwin and Mundo-Ocampo (1991) listed a total of 23 *Heterodera* species in the *goettingiana* group, to which *H. carotae*, *H. kirjanovae* Narbaev, 1988 and *H. turangae* Narbaev, 1988, previously overlooked species, have to be added. Sturhan (1998) and Subbotin *et al*. (2001) proposed combining those *Heterodera* species having J2 with three incisures in the lateral field and grass

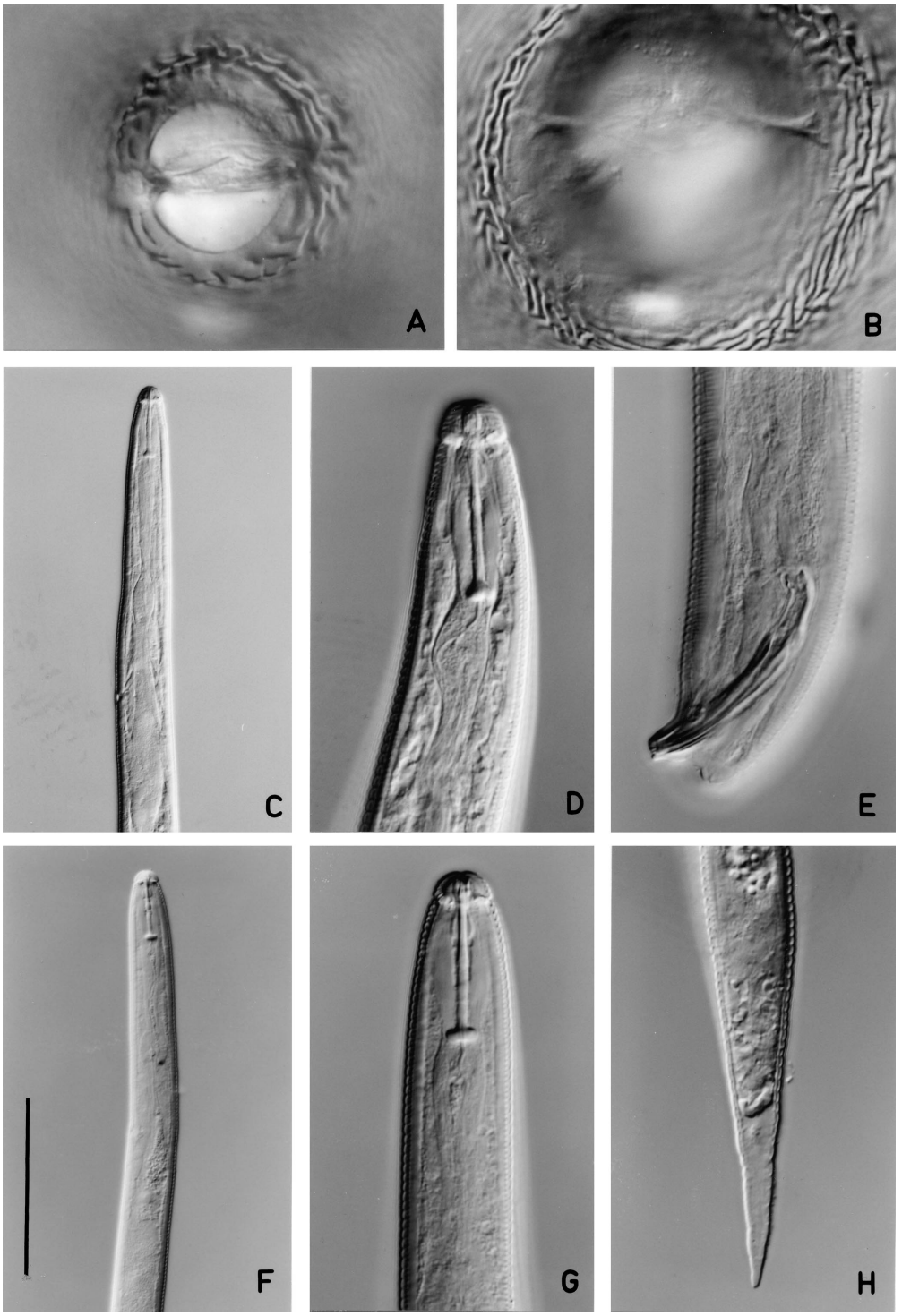

Fig. 2. Heterodera circeae *sp. n. Light micrographs. A: Fenestration in vulval cone; B: Same as A, deeper level, with underbridge; C: Male, anterior region; D: Male, cephalic region; E: Male, posterior region; F: Second-stage juvenile, anterior region; G: Second-stage juvenile, cephalic region; H: Second-stage juvenile, posterior region. (Scale bar: A, B = 50 μm; C, F = 70 μm; D, G, H = 25 μm; E =20 μm.)*

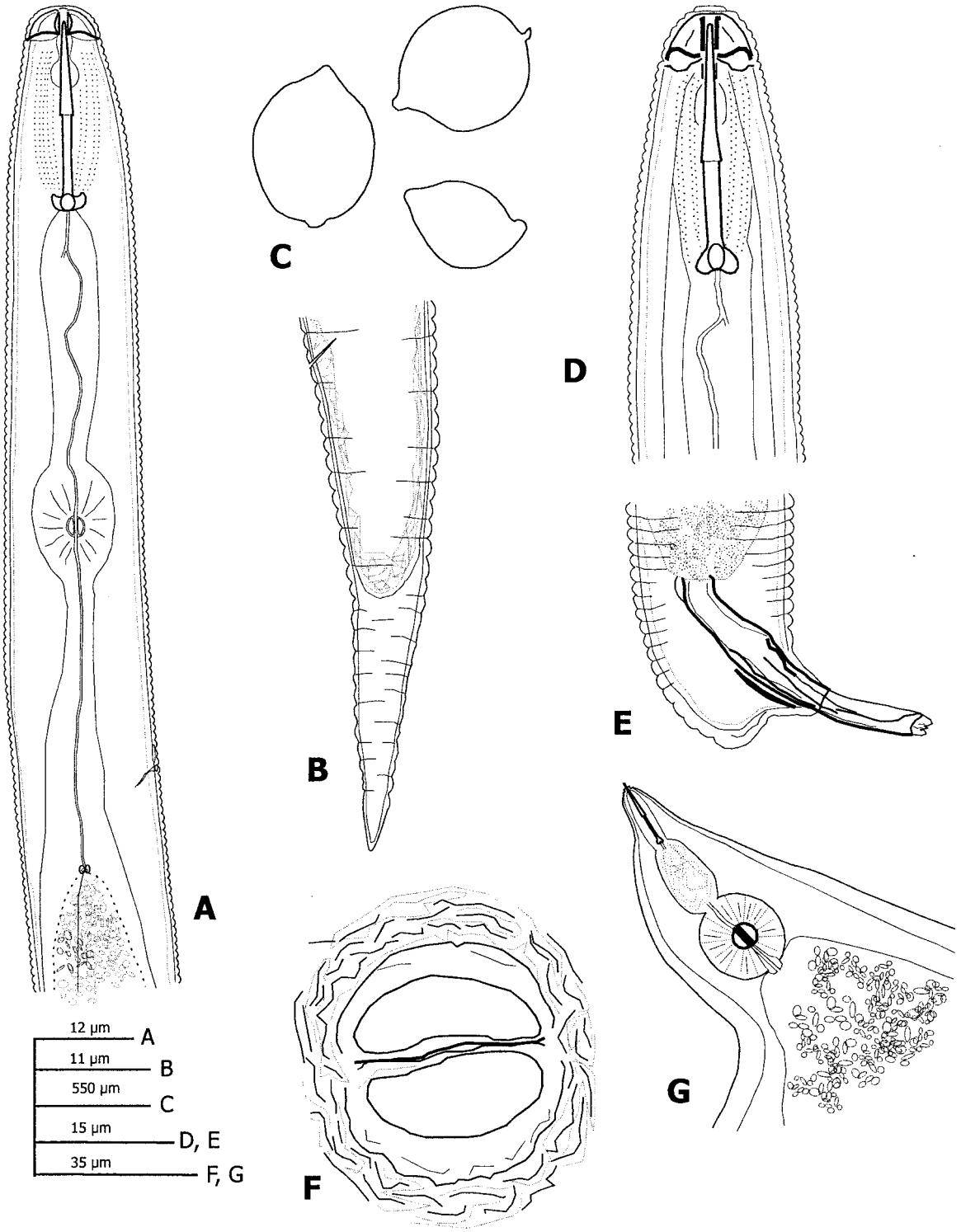

Fig. 3. Heterodera scutellariae *sp. n. A: Anterior end of second-stage juvenile; B: Second-stage juvenile tail; C: Cysts; D: Male head; E: Male tail; F: Fenestration in vulval cone; G: Anterior end of white female.*

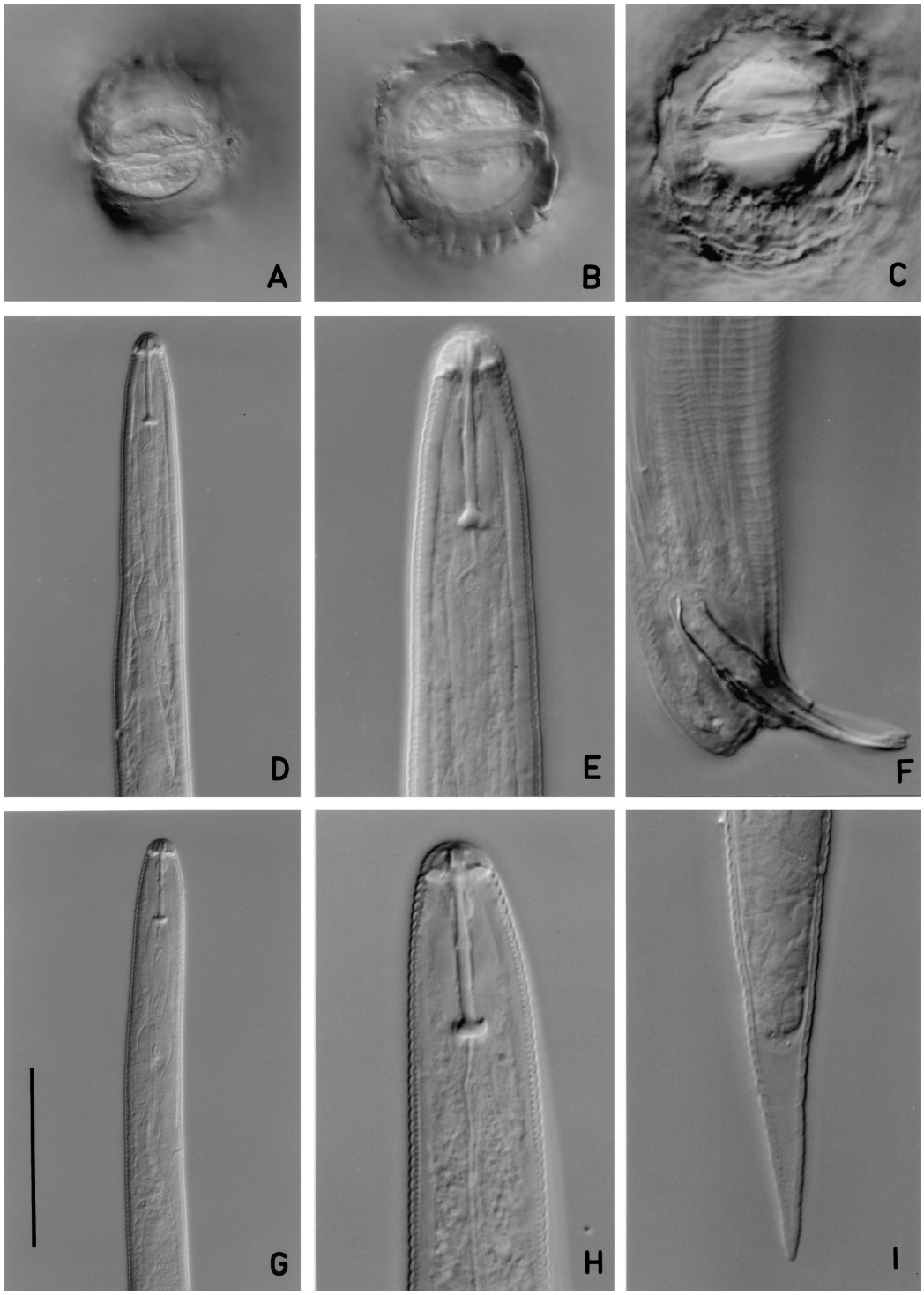

Fig. 4. Heterodera scutellariae *sp. n. Light micrographs. A: Fenestration in vulval cone; B: Same as A at deeper level; C: Vulval cone at level of underbridge; D: Male, anterior region; E: Cephalic region; F: Male, posterior region; G: Second-stage juvenile, anterior region; H: Second-stage juvenile, cephalic region; I: Second-stage juvenile, posterior region. (Scale bar: A-D, G = 50 μm; E, H, I = 25 μm; F = 20 μm.)*

Table 2. *Morphological characters of species from the* goettingiana *group (measurements of means given in μm).*

Species	Cyst				Second-stage juvenile				
	Size	Fenestral length	Underbridge length	Body length	Lip annules	Stylet length	Stylet knobs	Tail length	Hyaline portion of tail length
H. goettingiana[1]	521 × 372	35	117	486	2-3	25	smoothly rounded to slightly hooked shaped with recurved anterior surface	60	37
H. carotae[2]	408 × 309	31	90	422	4	24	concave anterior face	52	28
H. cruciferae[3]	429 × 333	34	85	431	3-4	24	anterior face flat to concave	50	25
H. urticae[4]	492 × 435	38	weak	541	5	27	slightly concave anteriorly	58	29
H. circeae sp. n.	555 × 397	43	83	434	4-5	25	rounded and slightly sloping posteriorly	52	26
H. scutellariae sp. n.	560 × 424	35	86	408	5	24	slightly concave anteriorly	47	25

Data from: [1]Stone and Course (1975); [2]Mathews (1975); [3]Stone and Rowe (1976); [4]Mathews (1971).

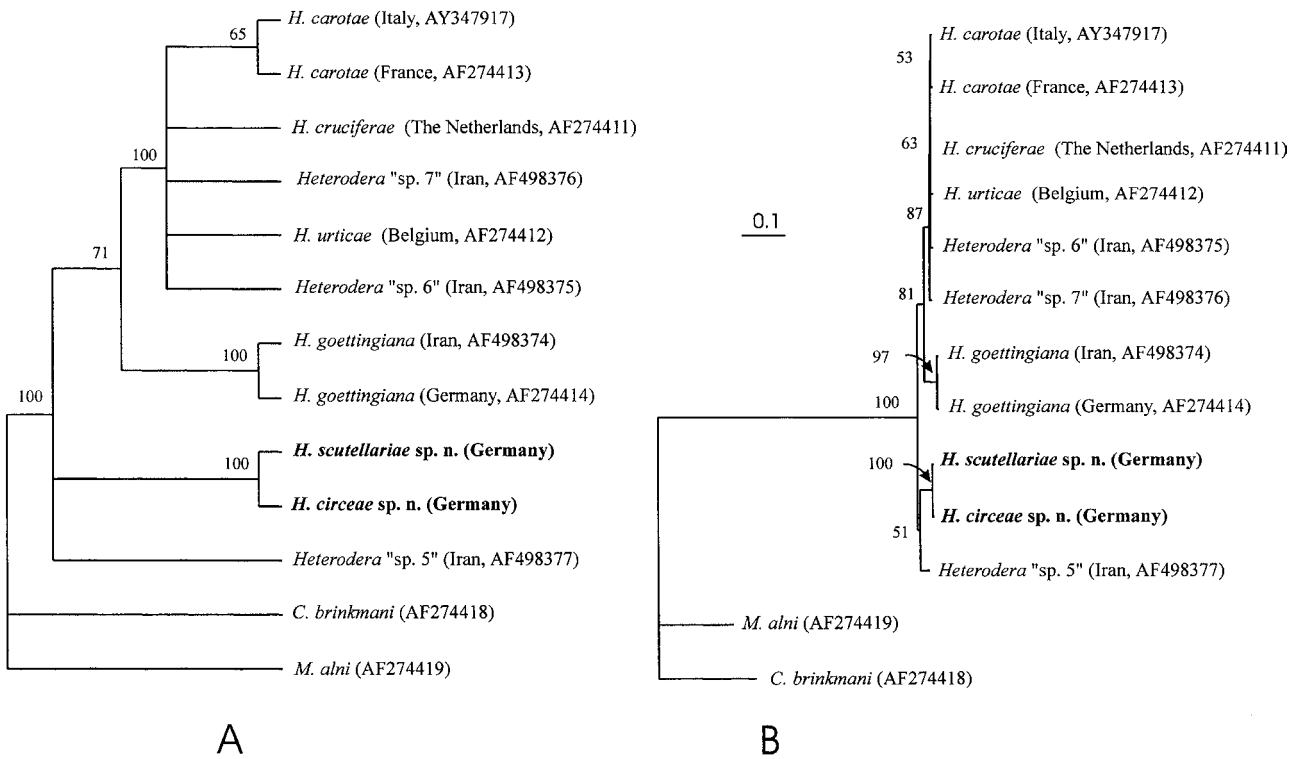

Fig. 5. *Phylogenetic relationships within the* goettingiana *group as inferred from analyses of 13 ITS sequences of rDNA. A: Strict consensus of five most parsimonious trees after analyses (Tree length = 636; CI = 0.9292; HI = 0.0708; RI = 0.8747; RC = 0.8128); B: Single maximum likelihood tree (lnL = −3961.65718; TVM + G model of DNA evolution). Bootstrap values more than 50% are given on appropriate clades.*

hosts, into the *bifenestra* group or the *cyperi* group, most having previously been placed in the *goettingiana* group. Several other species appear to be members of the *humuli* group.

Subbotin *et al*. (2001), using analyses of sequences from the ITS-rDNA, demonstrated that only four valid species (*H. goettingiana*, *H. cruciferae*, *H. carotae*, *H. urticae*) could be retained in the *goettingiana* group (*sensu stricto*). Placement of *H. bergeniae* Maqbool & Shahina, 1988 in this group and its relationship to the species presently in the *goettingiana* group has still to be evaluated, as has the possible placement in this group of *H. johanseni* (Sharma, Kaushal, Singh, Pande, Pokharel & Upreti, 2001) Sturhan, 2002. Recently, several undescribed species belonging to the *goettingiana* group were reported from Iran (Tanha Maafi *et al*., 2003). The description of *H. circeae* sp. n. and *H. scutellariae* sp. n. increases the number of known species in this group to six.

Diagnosis of the *goettingiana* group

Cysts lemon-shaped, ambifenestrate and abullate (small bullae occasionally present), with egg sac, vulval slit long (>35 μm), vulval bridge thin, semifenestrae low (average fenestral length 30-45 μm), underbridge weak. Second-stage juveniles with body length >400 μm, tail length >45 μm, hyaline tail portion >20 μm, stylet length >20 μm, lateral field with four incisures and small phasmids. All species exclusively parasitise dicotyledonous plants.

Morphometrical and morphological characters useful for identification of species from this group are given in Table 2.

Only one species of the *goettingiana* group, *H. cruciferae*, is assumed to be polyphagous with hosts in different plant orders. Since the records of a few *Lamium* and *Stachys* species as hosts of *H. cruciferae* are based on field observations (Winslow, 1954; Hesling, 1963), and as *H. scutellariae* sp. n., a cyst-nematode resembling *H. cruciferae*, is described from a labiate, the status of Lamiaceae species as hosts of *H. cruciferae* should be confirmed with precisely identified nematode populations. In our glasshouse experiments, *H. scutellariae* failed to reproduce on the crucifer *Raphanus napus* L.

Heterodera circeae sp. n. appears to be the first *Heterodera* species reported from a host in the family Onagraceae in subclass Rosidae, although *Globodera zelandica* Wouts, 1984 has *Fuchsia excorticata*, also in Onagraceae, as its type host.

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