Chapter 10

Cyst nematodes

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10.1. Introduction

Cyst nematodes are one of the economically important parasitic nematodes. These nematodes are especially adapted to a parasitic mode of life and have sexual dimorphism with vermiform males and swollen sedentary females. Cyst nematodes are taxa defined by their capacity to retain eggs inside the female body, which is transformed into a persistent tanned cyst at the completion of the female life-cycle. The embryonated eggs inside the cysts each contain a dormant second-stage juvenile (Fig. 10.1). Eight genera: *Heterodera* (80 species), *Globodera* (10 species), *Cactodera* (13 species), *Dolichodera*

(1 species), *Paradolichodera* (1 species), *Betulodera* (1 species), *Punctodera* (4 species) and *Vittatidera* (1 species) and total of 111 valid species are presently recognized within the group (Subbotin *et al*., 2010).

10.2. Morphology

The morphology of cyst nematodes is rendered uniquely complex by their sexual dimorphism, parasitic habits, ontogeny and dormant stages (Figs 10.1, 10.2). The specialized morphology of cyst nematodes reflects a complex life history. The morphology of the egg, juvenile stages, male, female and cyst, including features vary among genera and species and used for diagnostics.

10.2.1. Egg

The eggs of cyst nematodes are not readily distinguishable from those of tylenchids. Egg dimensions are often included in species descriptions, suggesting that eggs vary in size and shape among species, although these differences are not often used in diagnostics. In *Cactodera* species light and scanning microscopy revealed distinctive textures on the surface of eggs that may be particular to certain species. For example, the eggshell of *C. cacti*, *C. thornei*, *C. eremica*, *C. milleri* is covered by punctation, whereas *C. weissi*, *C. amaranthi* and *C. salina* have smooth eggshells.

10.2.2. Second-stage juvenile

The second-stage juveniles (J2) of cyst nematodes are vermiform with a rounded head region and tapering tail. After gently killing by heating, the juvenile body becomes slightly curved. Although body length may vary among specimens and populations, average body length is sufficiency stable for species. The overall J2 body length (300-700 μm), length and shape of the tail (30-90 μm) and distinctive hyaline region (20-40 μm) are useful in species diagnosis (Wouts & Weischer, 1977; Wouts & Baldwin, 1998). Additional useful features may include stylet length, position and shape of the stylet knobs and number of incisures. The lateral incisures are informative characters since J2 of most species have four incisures and J2 of species from the *Sacchari* and *Cyperi* groups, *H. bifenestra*, *Betulodera* and *Dolichodera* have three incisures. An important diagnostic feature of the surface cuticle concerning the labial patterns of J2, and their potential for defining taxa was first noted by Stone (1975). Although many cyst nematode J2 retain this basic pattern, in many other species this pattern is modified, often by fusion of adjacent submedial lips with one another and with the labial disc; sometimes the submedial lips further fuse with the anterior labial annuli. The lip patterns are relatively stable and useful in diagnostics. The reproductive system in migratory newly hatched J2, including

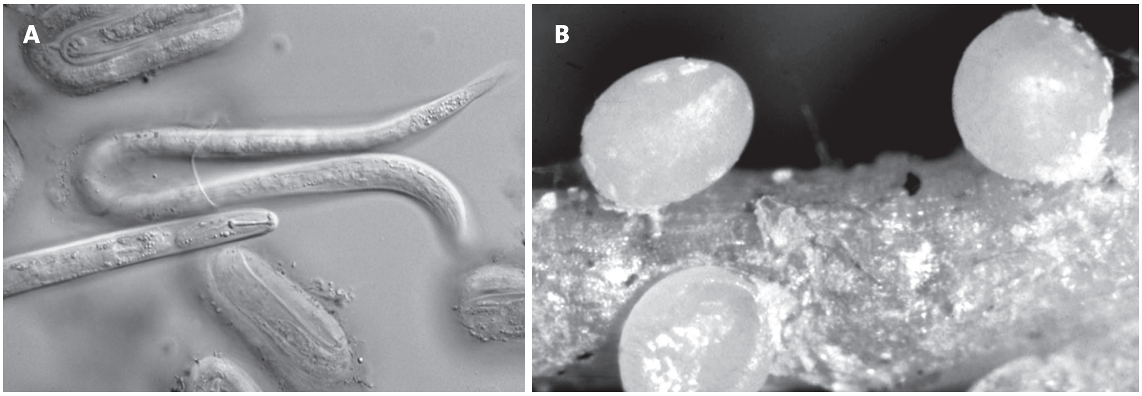

Fig. 10.1. Cyst nematodes, *Globodera pallida*. A: Second-stage juveniles and eggs; B: White females on a root. Courtesy of U. Zunke and T. Vrain.

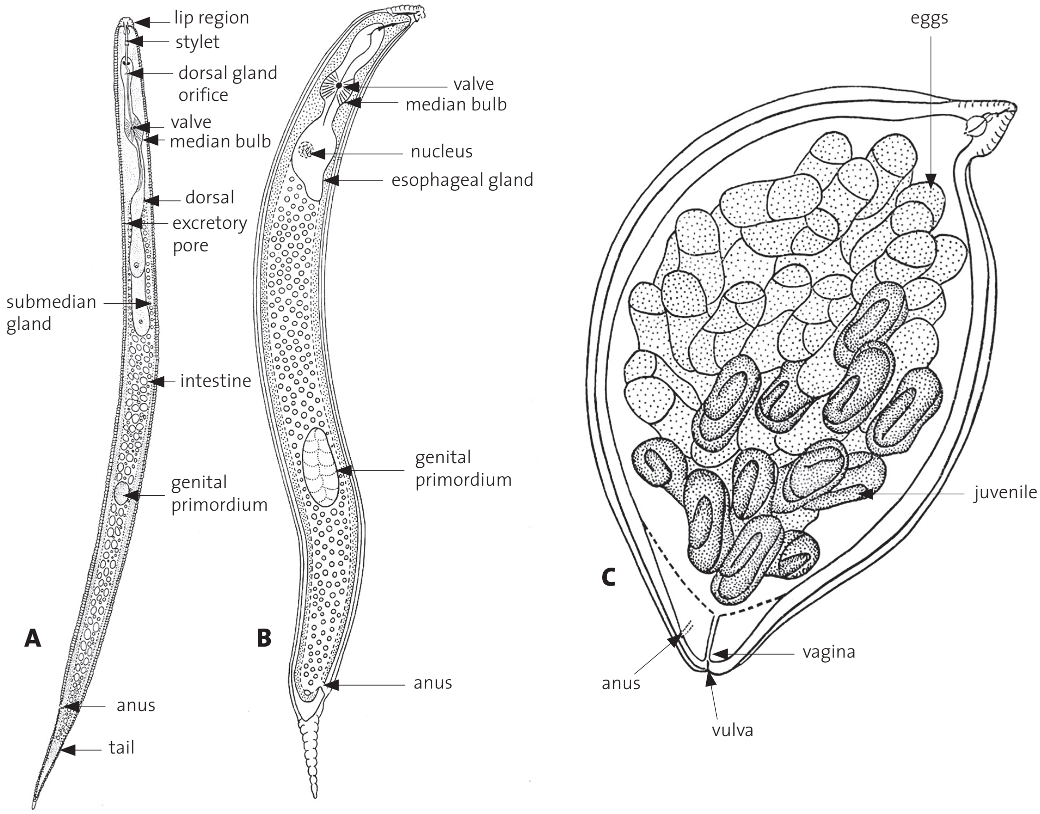

Fig. 10.2. Cyst nematodes, A: Second-stage juvenile; B: Second-stage juvenile molting; C: Mature white female. After Mulvey, (1959).

males and females, is an oval primordium located near the centre of the body and comprises four cells.

10.2.3. Male

Although rare or absent in parthenogenetic forms, males are common for most species of cyst nematodes. They may be found in soil during their life of about 10 days but they are more often found on roots near young females, including within the egg-sac that is attached to the female's posterior region. Often, several males are found with a single female and both sexes can mate several times. Males are usually not used for diagnostics as they have similar morphology and only a few species specific characters, for example, body length, spicule tip and stylet knob structure.

10.2.4. Cyst

The cyst is the stage richest in diagnostic characters (Figs 10.3-10.6). Furthermore, cysts are the stage often most readily available from soil samples. Cysts may be obtained directly from infected roots, or recovered from soil by a range of techniques. All cysts include a narrow anterior protuberance or neck and swollen body, the cyst is swollen and varies considerably in details that may be diagnostic. This includes the presence or absence of a terminal posterior protrusion, the vulval cone, that determines if the female is globose when the cone is absent, as in *Globodera*, *Punctodera*, *Dolichodera* and *Paradolichodera* or lemon-shaped when the cone is present, as in *Heterodera*, *Cactodera*, *Betulodera* and *Vittatidera*. The formation and proportional size of the cone is affected by the relative elasticity of the body wall as the female enlarges and this may be affected by development in relation to cuticle composition, layering and thickness, as well as attachment points and arrangement of vaginal muscles. Thus, size and shape, while reflecting underlying taxon-specific genetics, are variable because they are also subject to environmental factors including nutrition, age, host, physical pressure and crowding. Regardless of limitations, specific trends in size of females and cysts are of some diagnostic value in combination with other characters. Overall cuticle thickness apparently varies among species; for example, a diagnostic feature for *Globodera* mali is the presence of a thin and transparent cuticle that persists in the cyst. Regardless of overall layering, the cuticle structure is distinctively thin-walled and consists of a loose mesh of fibers in a discrete area of the posterior region of the body. In this area, the cuticle ruptures at cyst maturity forming taxon-specific apertures, the fenestrae. These apertures may allow the exit of J2 from the cyst. The absent or present of fenestration and type of fenestration (semifenestrate, bifenestrate or circumfenestrate) is used in generic diagnostics (Figs 10.4, 10.5). Other structures of the vulval cone, such as the vulval slit, vulval bridge, underbridge and bullae are important taxonomic characters (Fig. 10.6).

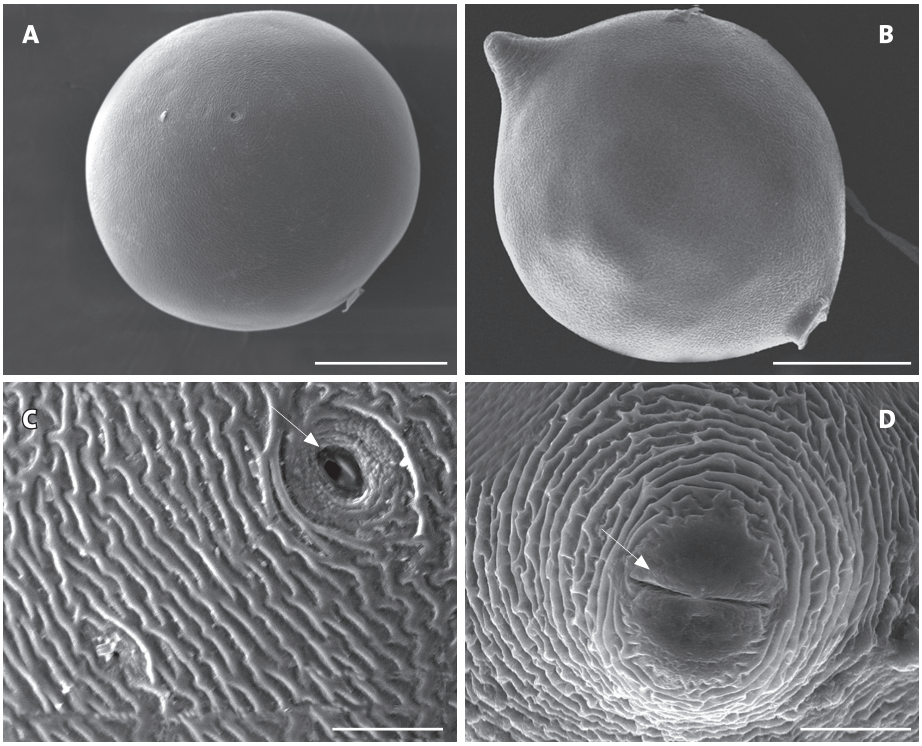

Fig. 10.3. SEM of cyst nematodes. A: Cyst of *Globodera pallida*; B: Cyst of *Heterodera zeae*; D: Terminal region of *Globodera pallida*; C: Vulval cone of *Heterodera glycines* female. Arrows indicate vulval slit. Scale bars: A, B = 200 μm; C = 15 μm; D = 30 μm. Courtesy of T. Powers, USA.

Taxonomic characters associated with fenestrae include the distance from the anus to the nearest edge of the fenestrae. Dividing this measurement by fenestral length gives Granek's ratio, which is useful for differentiation *Globodera* species.

To the outside of the cuticle a subcrystalline layer may be present and, while variable, its occurrence has long been recognized as somewhat taxon-specific. By definition, cysts are tanned and the pigmentation and transitions of color as they mature is diagnostic. Franklin (1951) was among the first to note that cyst color at maturity may vary among species from light tan to nearly black. In *G. rostochiensis*, for example, the cysts are said to pass through a golden stage to brown as they mature, whereas in *G. pallida* they change directly to brown. Cyst color transformations are not limited to *Globodera* and are observed in other genera. For example, females of *H. betae* change from white through yellow to brown, whereas females of *H. schachtii* do not have a distinct yellow color.

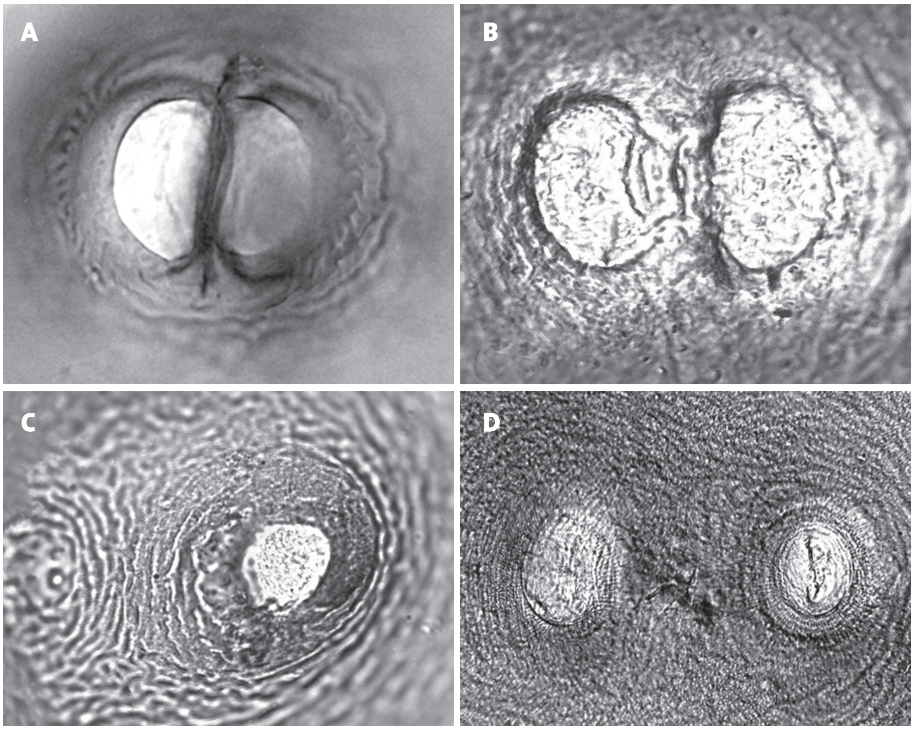

Fig. 10.4. Vulval plates. A: *Heterodera trifolii* (ambifenestrate); B: *Heterodera* sp. (bifenestrate); C: *Cactodera radicale* (circumfenestrate); D: *Punctodera punctata* (circumfenestrate). After Subbotin *et al*. (2010) with modifications.

10.3. Life cycle

The life cycle of cyst nematodes comprises the egg, four juvenile stages and the adults (Fig. 10.7). The first-stage juvenile is limited to the egg; the J2 hatches from the egg while within the cyst or the gelatinous matrix that comprises the egg-sac. Hatching is the result of a complex interplay among external signals and the internal readiness of the J2 to emerge. Hatching of large numbers of J2 for many cyst nematode species occurs in response to host root diffusates. The J2 that hatch within the cyst leave the cyst via either the open fenestral region or the opening created at the neck where the female's head has deteriorated or otherwise broken away. The J2 are then attracted toward the host root, responding to gradients of a variety of stimuli. Once at the invasion site, a J2 enters the root epidermis using repeated thrusts of its protrusible, pointed stylet. Penetration usually occurs in the zone of elongation behind the growing root tip and the J2 migrates intracellularly and crosses the cortical tissue toward the vascular cylinder. Reaching the pericycle, the J2

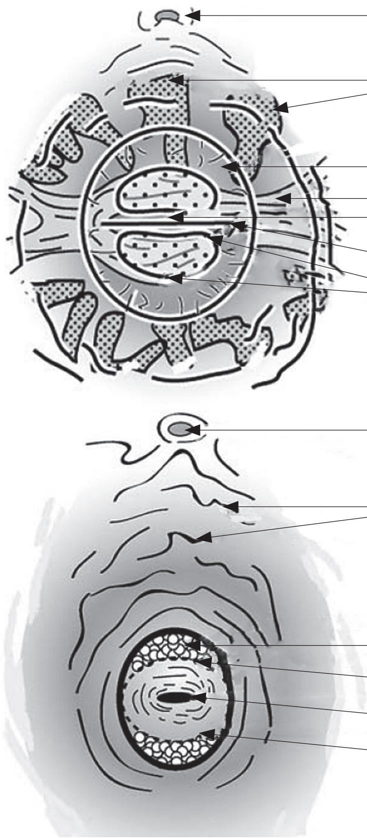

Fig. 10.5. Terminal view of tail region of young cysts (composite of species for genus) showing typical diagnostic features and associated terminology. A: Cone of *Heterodera* (ambifenestrate type); B: Terminal region of *Globodera* (circumfenestrate type). After Subbotin *et al.* (2010).

carefully penetrates a single cell with its stylet and start feeding. After establishing a feeding site, the J2 undergoes three molts to reach the adult stage.

Cyst nematodes are sexually dimorphic. Adults include sedentary obese egg-producing females and vermiform migratory males in most species. Males of amphimictic species are attracted to pheromones which may occur in exudates secreted through the vulva of females, often into a gelatinous matrix. After mating, eggs develop and contain maturing embryos. In most species, the majority of eggs are retained within the female that then dies. The cuticle of the female body is tanned by a polyphenol oxidase to form the tough, leather-like, cyst wall. Females are capable of producing up to 500 to 700 eggs. Females of some species of *Heterodera* can produce a large gelatinous matrix in which some eggs are deposited. The life cycle, from egg to egg, varies depending on temperature and other environmental conditions but is usually completed in about 30 days. The number of generations per year also varies from one to several and depends on species. In some species, eggs within the cyst are especially persistent when conditions are not favorable for plant

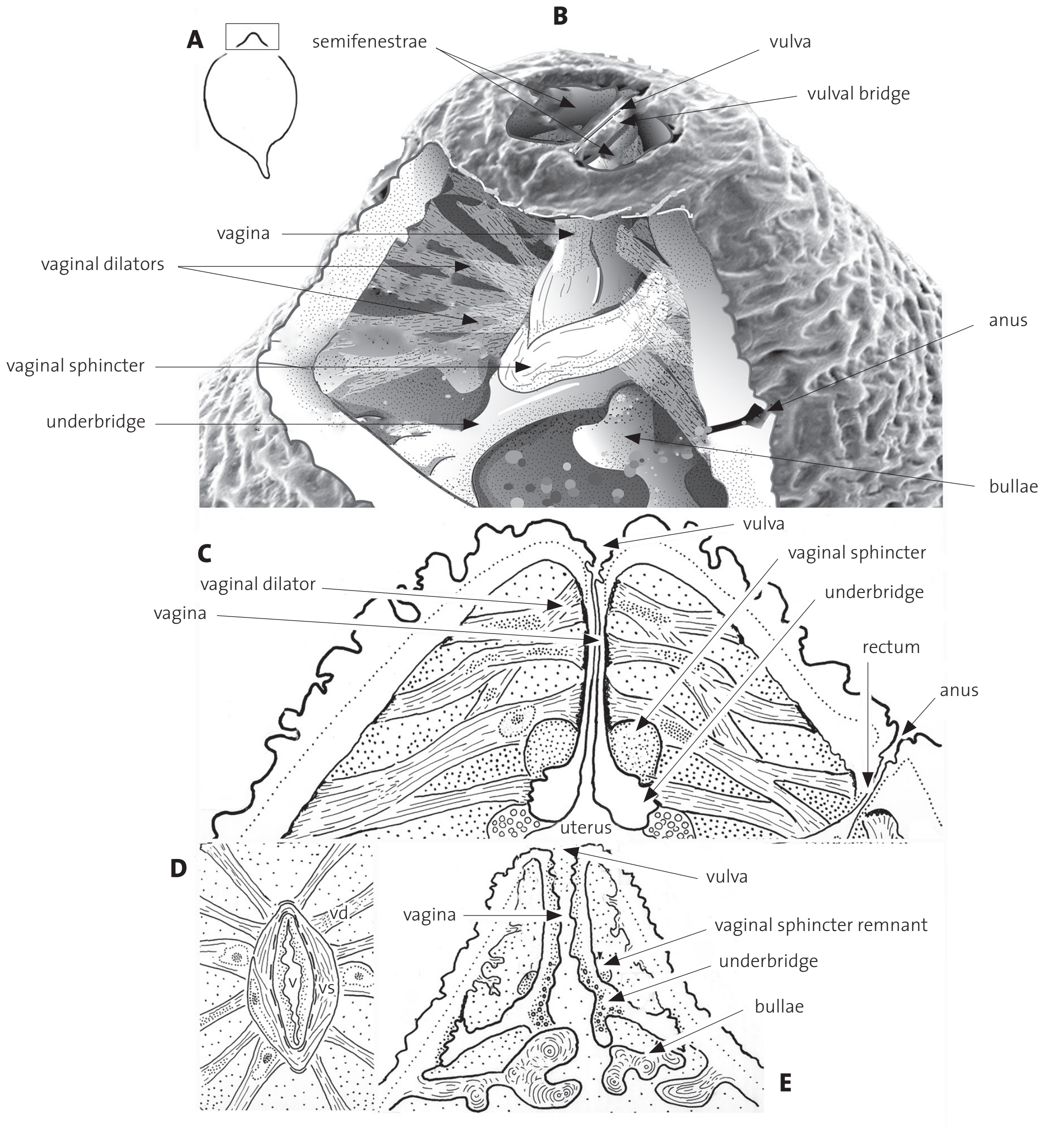

Fig. 10.6. Drawings depicting basic structures of terminal region of cyst nematodes. A: Overview of female. Box indicates terminal region as enlarged in B and C; B: Ventrolateral 3-D view with cut away showing internal structures. Some features that may be exclusive to either female or cyst stages are combined to show a more complete range of possible structures; C: Right lateral view of *Heterodera schachtii*; D: Transverse view through vulva (v) at level of vaginal sphincter (vs) and vaginal dilators (vd) of *H. schachtii*; E: Dorsal view of mature cyst. After Subbotin *et al*. (2010.)

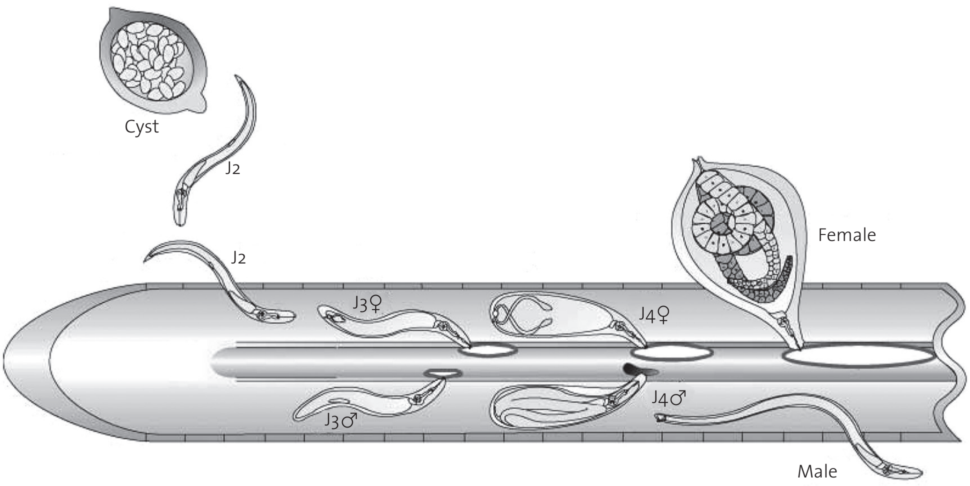

Fig. 10.7. Life cycle of an amphimictic cyst nematode. After Lilley *et al*. (2005) with modification.

growth and infection. Under these conditions they can persist in soil for many years. The combination of diapause and quiescence enables the potato cyst nematodes *G. rostochiensis* and *G. pallida*, for example, to persist in soil for more than 20 years in the absence of a host plant and biological antagonists.

10.4. Plant-host relationships

Cyst nematodes are highly specialized parasites infecting plants from different plant orders. Almost half of the known cyst nematode species were described from plants in the order Poales, one of the largest orders of flowering plants.

Cyst nematode parasitism is a complex process involving the interchange of information between the nematode and plant host. After penetrating a root, the J2 injects secretions of the dorsal pharyngeal gland into this cell. These secretions mediate a massive reprogramming of cell development and induce the formation of a large, multinucleate feeding structure, a syncytium (Fig. 10.8), which is formed by dissolution of walls initially separating adjacent cells. The syncytium contains large metabolically active cells acting as a nutrient sink, and is characterised by multiple enlarged nuclei with nucleoli, dense cytoplasm and a proliferation of mitochondria, endoplasmic reticulum and plastids.

Cyst nematode parasitizing roots lead to remarkable but not specific symptoms. Field symptoms of severe cyst nematode infection often include reduced growth,

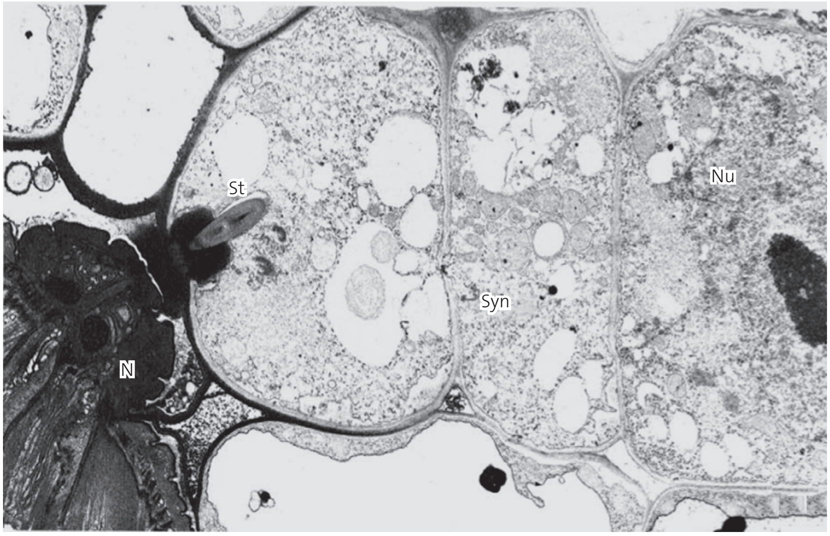

Fig. 10.8. Feeding of *Heterodera glycines* on soybean roots. An infective second-stage juvenile with stylet extended into a syncytium, N = nematode; Syn = syncytium; Nu = nucleus. After Endo, (1978).

stunting, wilting, chlorosis of leaves, and reduction of quality and quantity of yield. Above-ground symptoms of damage are not unique to cyst nematodes. They could be mistaken for nutrient deficiencies, drought stress, herbicide injury, or other plant diseases and often can remain undetected for several years. Symptoms of infection are expressed more fully when mineral nutrition is limiting or root growth is otherwise impeded. Areas of severely affected and symptomatic plants are often round or elliptical in shape. Severely infected plants may die before flowering. Most below-ground symptoms of cyst nematode damage are also not unique. Infected roots are dwarfed or stunted. Extensive branching and local thickening of roots can also be observed. Cyst nematode infections also may make the roots more susceptible to attack by other soil-borne plant pathogens. Many abiotic factors, for example fertility, pH, soil type and organic matter content, influence nematode population development and damage severity. Although worldwide losses to cyst nematodes are considerable, they are difficult to quantify, because they are often influenced by interactions with pathogenic fungi (*Rhizoctonia*, *Fusarium*, and *Pythium*) and bacteria.

10.5. Control

Several strategies are used for control of cyst nematodes and rotation with non-host crops and growing resistant cultivars are common management methods.

Two or more years of using non-host crops or resistant varieties may be needed to reduce populations below damaging thresholds. Resistant varieties against several species of cyst nematodes have being developed in many research centers. For example, several cereal cultivars with resistance to *H. avenae* are currently on the market. Cultivar resistance is considered one of the best methods for cereal cyst nematode control and has been found to be successful in several countries such as Australia, Sweden and France on a farm scale. All of the sources of resistance reported to date against cereal cyst nematodes feature single-gene inheritance. Six *Cre* genes for *H. avenae* resistance in wheat were derived from *Aegilops* species. Other resistance genes were derived from *Triticum aestivum* (*Cre1* and *Cre8*) and *Secale cereale* (*CreR*). Several other sources of resistance (*CreX* and *CreY*) are also reported, but their genetic control and gene designation are still unknown. Most of these resistance genes have been introgressed into hexaploid wheat. The *Cre1* gene is highly effective against populations of cereal cyst nematodes from Europe, North Africa, and North America and moderately effective or ineffective to populations in Australia and Asia.

Management of soybean cyst nematode includes rotation to non-host crops such as alfalfa, maize and wheat and planting of SCN-resistant soybean cultivars. Soybean breeding lines named Peking or PI 88788 have been the major source of resistance to SCN for the last 25 years, are very effective against the common races of SCN, and the resistance is easily incorporated into new varieties. The single dominant gene, *H1* from *S. tuberosum* ssp. *andigena* has been widely used in potato breeding programs against *Globodera rostochiensis* pathotype R01 and R04 since its discovery in 1953. Maris Piper , which was released by the Plant Breeding Institute in Cambridge in 1966, was the first cultivar with this gene. Despite sustained efforts to breed for high levels of resistance to the white potato cyst nematode *G. pallida*, none of the varieties on display have complete resistance. Moreover, H1 is ineffective against other *G. rostochiensis* pathotypes and *G. pallida*. In spite of reduced nematode reproduction, resistant cultivars do not prevent root penetration and associated stresses on the plant.

A nematicide application can provide effective control of cyst nematodes. Because of high cost, safety concerns and inconsistent results, nematicides are usually recommended when cyst nematode populations cannot be controlled by other methods. Biological control products are not widely commercially available, but cyst nematode populations in some locations might be maintained below an economic threshold by fungal (*Paecilomyces lilacinus* and *Pochonia*) and bacterial parasites (*Pasteuria*) of eggs and juveniles.

10.6. Pathotypes

A number of studies have shown intraspecific variation in relation to nematode behavior on hosts. Host races and pathotypes have become an important part of an infraspecific classification of some cyst nematodes. They have been recognized and classified for *G. pallida*, *G. rostochiensis*, *H. avenae*, *H. australis*, *H. cajani*, *H. filipjevi*, *H. hordecalis*, *H. schachtii* and *H. zeae*. Potato cyst nematodes are classified into pathotypes on their ability to multiply on different potato clones. However, their pathotype status is still a matter of debate. Kort *et al*. (1977) proposed the International Scheme for *Globodera* pathotypes in which, among the European populations, five pathotypes within *G. rostochiensis* and three within *G. pallida* were recognized. A second approach, the Latin American Scheme (Canto Saenz & de Scurrah, 1977), recognized amongst the South American populations of *G. rostochiensis* and *G. pallida*, four and seven pathotypes, respectively. The distribution of pathotypes of both species according to the Latin American Scheme for South American countries was re-analyzed by Franco *et al*. (1998) (Table 10.1). By using the standard differential clones of Latin and European schemes, several pathotypes of *G. rostochiensis* and *G. pallida* have been identified in potato cyst nematode populations collected in most of the South American countries. This information and the results of other local in-country studies, as in Venezuela (M. Cordero, Tachira, Venezuela, 1996, personal communication), have shown that P4A and P5A pathotypes of *G. pallida* are the most predominant but that P6A pathotype is also present. In the southern region of South America, the situation is complex due to the presence of both species and several pathotypes.

A race test for *H. glycines* was first developed based on variable comparative development of females on four differential soybean lines. This test was proposed by a group of nematologists and soybean breeders (Golden *et al*., 1970; Riggs & Schmitt, 1988) to distinguish 16 races of *H. glycines*. However, the scheme was shown to describe inadequately the extensive genetic diversity of *H. glycines* populations that existed in areas of soybean production and it was noted that the classification could be expanded to include many more groups (or types) by increasing the number of differentials. Thus, Riggs *et al*. (1981) distinguished up to 25 'races' on 12 resistant soybean lines, demonstrating that the set of four differentials used by Golden *et al*. (1970) did not account for all the variability in host compatibility of *H. glycines* (Niblack *et al*., 2002). Niblack *et al*. (2002) proposed an HG Type Test to describe population variation better and to expand the flexibility of the race classification system, with evaluation also based on development of adult females.

Table 10.1. Pathotype groups of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*.

Species and potato accession	*Globodera* species	*G. rostochiensis*					*G. pallida*						
	Virulence group	Ro1		Ro3		Ro5	Pa1			-	-	Pa2/3	
	European pathotypes	Ro1	Ro4	Ro2	Ro3	Ro5	Pa1		-	-	Pa2	Pa3	-
	South. American pathotypes Ploidy, Resistance gene	R1A	R1B	R2A	R3A	-	P1A	P1B	P2A	P3A	P4A	P5A	P6A
Solanum tuberosum ssp. *tuberosum*	4x, (minor)	+/–	+	+	+	+	+	+	+	+	+	+	+
S. tuberosum spp. *andigena* CPC 1673	4x, H1 on chromosome 5	–	–	+	+	+	+	”	“	“	+	+	“
S. kurtzianum KTT 60.21.19	2x, K1 K2 A & B	–	(+)	–	(+)	(+)	+	+	+	+	+	+	+
S. vernei GLKS 58.1642.4	2x .Quantitative	–	+	–	–	+	+	+	+	–	+	+	+
S. vernei Vt 62.33.3	2x. Quantitative		–	–	–	+	–	+	–	–	–	+	+
Ex. *S. multidissectum* hybrid P55/7	2x , 1 + polygenes H2	+	+	+	+	+	–	–/+	–	+	+	+	+
S. t. ssp. *andigena*	H3 + polygenes	+	“	“	“	“	(–)	“	“	“	(–)	(–)	“
CIP 280090.10	Quantitative	“	“	“	“	“	“	“	“	“	–	–	+
S. vernei hybrid 69.1377/94	2x, Polygenes	–	–	–	–	–	–	“	“	“	–	–	“
S. vernei hybrid 63.346/19	2x, Polygenes	–	–	–	–	–	+	“	“	“	+	+	“
S. spegazzinii	2x, Fa = H1	–	–	+	+	+	“	“	“	“	“	“	“
S. spegazzinni	2x, Fb + 2 minor	+	–	+	–	–	“	“	“	“	“	“	“
	Glo1 on chromosome 7	(–)	+	+	+	+	“	“	“	“	“	“	“

+ = compatible interaction: nematode multiplication, susceptible potato;
– = incompatible interaction: nematode no multiplication, resistant potato; ( ) = partial or uncertain interaction; “ = no information.

10.7. Biochemical and molecular identification

Biochemical approaches such as gel electrophoresis for separating protein and enzyme profiles have shown great potential for assisting in identification of cyst nematodes. Isoelectric focusing (IEF) is currently used in many nematology laboratories for routine diagnostics of *G. pallida* and *G. rostochiensis*. By comparison with the biochemical approaches, analysis of DNA for diagnostics has several advantages. DNA does not rely on expressed products and therefore is not influenced by environmental conditions or developmental stage. The DNA diagnostics, being much more sensitive than any biochemical diagnostic technique, can be used with nanograms of DNA extracted from a single specimen or even from a fragment of an individual nematode. For diagnostic purposes, various types of samples can potentially be used as total soil extracts that include a mixture of nematode species and infected plant materials. Dried, alcohol-fixed, formalin-fixed specimens or specimens embedded and mounted on permanent slide, can also be used for diagnostics.

The main DNA regions targeted for diagnostics of cyst nematodes are nuclear ribosomal RNA genes. These include 18S, 28S and especially the internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), which are situated between 18S and 5.8S and 5.8S and 28S rRNA genes, respectively. The flanking 18S and 28S rRNA gene regions contain a rather conservative nucleotide sequences and thus universal primers can be designed and used for amplification of ITS regions. Genes of mitochondrial DNA (mtDNA), with their relatively higher rate of mutations relative to rRNA genes, have great potential for diagnostics of races and populations of cyst nematodes.

PCR-RFLP and PCR with species specific primer presently are used for diagnostics of many cyst nematode species (Subbotin *et al*., 2010). However, the PCR and sequence techniques should be used intelligently and the researcher or advisor must be aware of the peculiarities of each method. Where there is doubt, it is always necessary to confirm identification by several methods, including the use of biochemical methods and traditional morphological features.

10.8. Phylogenetic relationships

The relationship of Heteroderinae to other Tylenchina has long been the subject of speculation and several authors have reviewed the evolution and phylogeny of cyst nematodes. New insight into the phylogeny of cyst nematodes has been provided by analysis of ribosomal RNA gene sequences. Molecular data support the hypothesis of Wouts (1985) that Verutus branches basal to, and as a sister to the clade of, all other heteroderids. Molecular phylogenies also strongly support monophyly of *Cactodera*, *Punctodera*, *Betulodera*, *Globodera* and *Paradolichodera* and they resolve some of the basal relationships within *Heterodera*, usually placing the *Goettingiana* group as branching closest to the origins of the cyst clade (Fig. 10.9). Molecular data also support division of cyst nematodes into several groups: *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii*.

10.9. CLASSIFICATION (according to Chapter 3)

Order Rhabditida Chitwood, 1933
 Suborder Tylenchina Thorne, 1949
  Infraorder Tylenchomorpha De Ley & Blaxter, 2002
   Superfamily Tylenchoidea Örley, 1880
    Family Hoplolaimidae Filipjev, 1934
     Subfamily Heteroderinae Filipjev & Schuurmans Stekhoven, 1941

10.9.1. Subfamily Heteroderinae

Diagnosis (after Siddiqi (2000) with modifications): Hoplolaimidae. *Mature female* spherical, pear or lemon-shaped with a short neck, turning into a tough, hard-walled, yellowish, light to dark brown or black cyst containing eggs and juveniles, in some species eggs may be laid in a gelatinous matrix. Cuticle surface with a zigzag or lace-like pattern, annulation absent on mature female and cyst. Vulva and anus usually close together, almost terminal. Clear translucent vulval fenestra usually present (absent in species of *Afenestrata* group of *Heterodera*), an anal fenestra present only in *Punctodera*. *Male* developed through metamorphosis, labial region annulated, lateral field with four or three incisures, tail short, hemispherical, without bursa. *J2* with three or four incisures in lateral field.

Type genus

***Heterodera* Schmidt, 1871**

Other genera

Betulodera Sturhan, 2002
Cactodera Krall & Krall, 1978
Dolichodera Mulvey & Ebsary, 1980
Globodera Skarbilovich, 1959
Paradolichodera Sturhan, Wouts & Subbotin, 2007
Punctodera Mulvey & Stone, 1976
Vittatidera Bernard, Handoo, Powers, Donald & Heinz, 2010

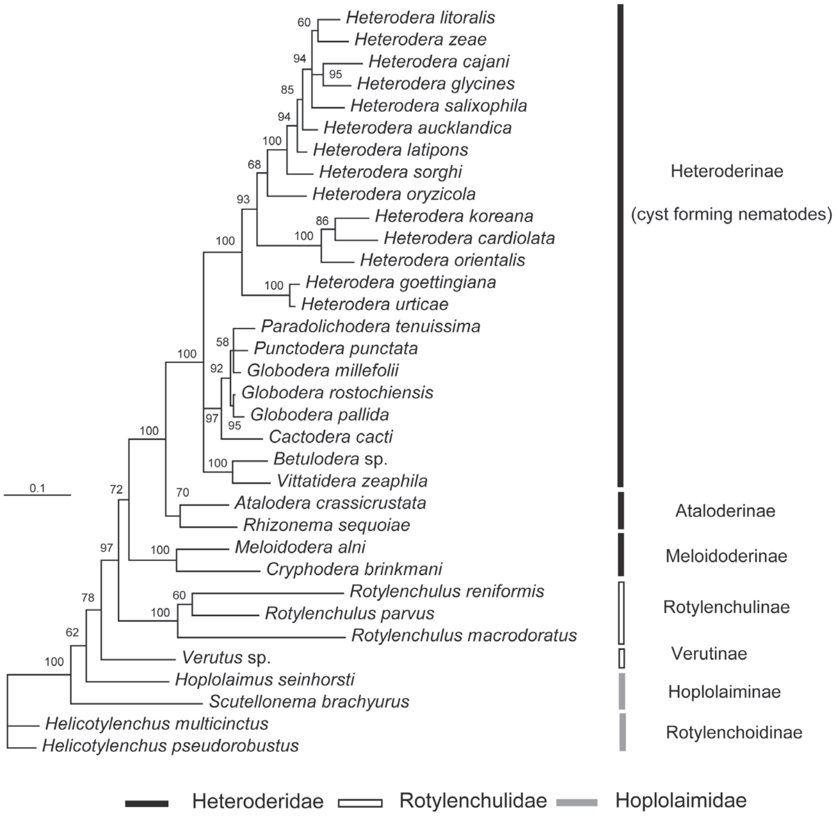

Fig. 10.9. Phylogenetic relationships among Heteroderinae: Bayesian 50% majority rule consensus tree from two runs as inferred from ITS1-5.8S-ITS2 sequences of rRNA gene alignment under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. After Subbotin *et al.* (2010) with modifications.

10.9.2. Identification key to genera of Heteroderinae

1. Cysts circumfenestrate 2
   - Cysts ambifenestrate or bifenestrate, or without fenestration .... *Heterodera*
2. Cysts with terminal cone 3
   - Cysts without terminal cone 4
3. Vulval slit in cysts > 10 μm long, J2 with four lateral incisures 5
   - Vulval slit in cysts 5-8 μm long, J2 with three lateral incisures .... *Betulodera*
4. J2 with average stylet > 18 μm .... *Cactodera*
   - J2 with stylet = 14-17 μm .... *Vittatidera*
5. Anal region with fenestration .... *Punctodera*

- Anal region without fenestration 6
6. Mature female and cyst spheroidal, perineal tubercles usually present ....
....*Globodera*
- Mature female and cyst elongate-oval shape, perineal tubercles absent 7
7. Bullae absent in cysts, J2 with DGO = 11-15 μm, c = 9-11, stylet < 20 μm ....
....*Paradolichodera*
- Bullae present in cysts, J2 with DGO = 7-8 mm, c = 5-6, stylet = 22-24 μm ...
....*Dolichodera*

10.10. Genera and major species

10.10.1. Genus *Heterodera* Schmidt, 1871

Diagnosis: Mature female and *cyst* body more or less lemon-shaped, with a posterior cone (except apparently secondarily lost in *H. bamboosi*). Vulval slit terminal or sunken into cone, anus on dorsal vulval lip. Vulval slit 6-68 μm in length. Cuticle surface with zigzag or lace-like pattern of ridges. Vulval fenestration ambifenestrate, bifenestrate or absent. D layer in cuticle rudimentary or absent. Subcrystalline layer present or absent. Anus without fenestration. Bullae and underbridge present or absent. *Eggs* retained in body, in some cases egg-sac also present. *J2* with lateral field marked by three or four incisures, phasmids punctiform or lens-like. Several distinct morphological groups: *Afenestrata*, *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii* are distinguished within this genus.

Type species: *Heterodera schachtii*

Total number of species: 80

***Heterodera avenae* Wollenweber, 1924 – the cereal cyst nematode**
(Fig. 10.10)

The cereal cyst nematode was first recorded by Kühn (1874) as a parasite of cereals in Germany and was later found in other countries. *Heterodera avenae* is now found in most wheat growing regions of the world (Meagher, 1977). The cereal cyst nematode is an important pest of cereals and is the principal nematode species on temperate cereals. In Europe, more than 50% of the fields in major cereal growing areas are infected by this nematode (Rivoal & Cook, 1993), annual yield losses reaching £3 million (Nicol & Rivoal, 2008). At least $3.4 million annually was estimated losses in wheat production in the states of Idaho, Oregon, and Washington due to cereal cyst nematodes. The yield losses it causes on wheat ranges from 15-20% in Pakistan, and 40-92% on wheat and 17-77% on barley in Saudi Arabia. In China, yield losses

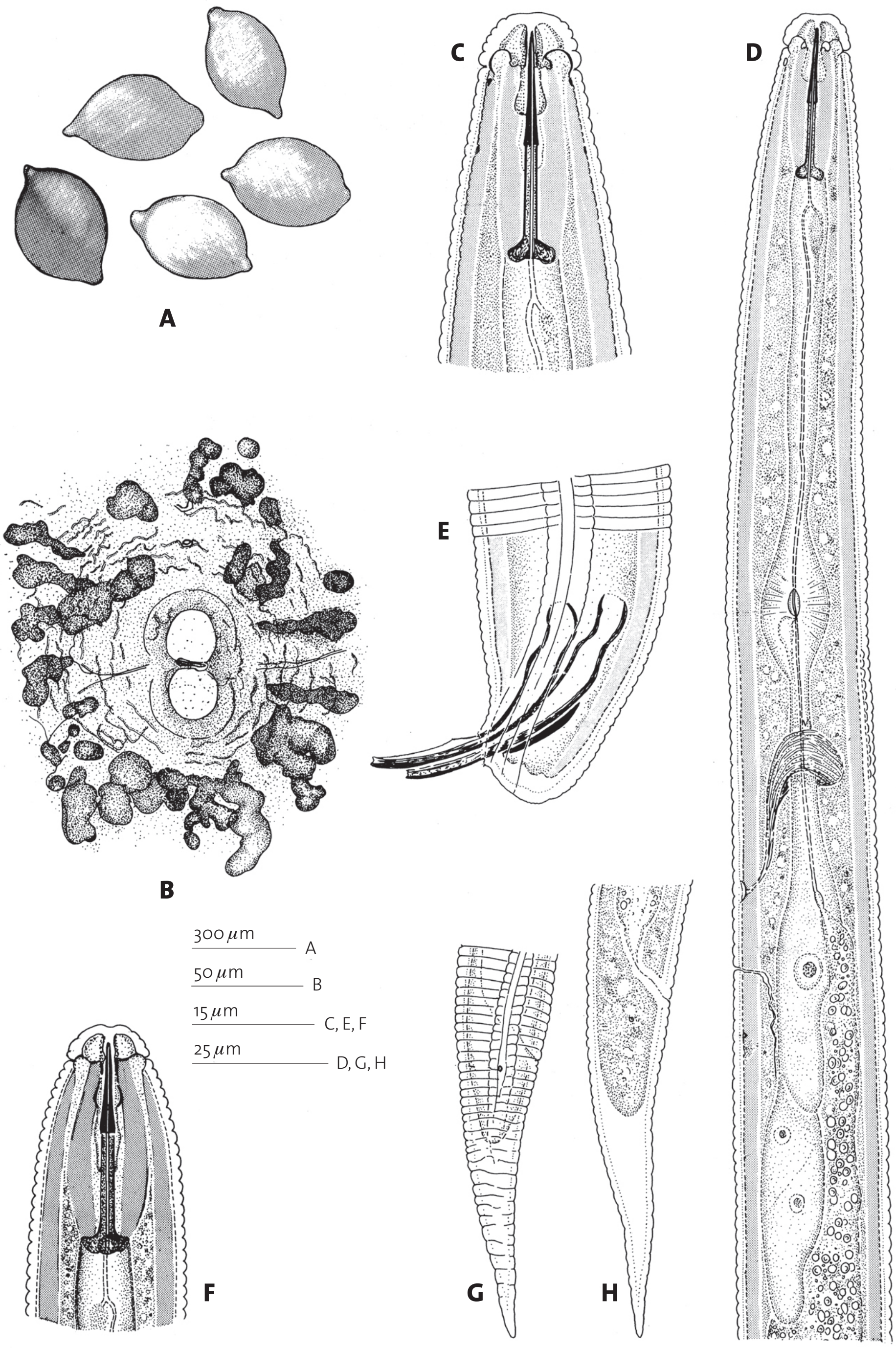

Fig. 10.10. *Heterodera avenae*. A: Cysts; B: Vulval cone; C: Anterior region of male; D: Pharyngeal region of male; E: Tail of male; F: Anterior region of J2; G, H: Tail of J2. After Williams and Siddiqi (1972.)

of wheat crop induced by this nematode can reach 70%. Hosts of *H. avenae* include many species of cereals and grasses (Williams & Siddiqi, 1972). *Heterodera avenae* has only one generation per year, with J2 hatch from the eggs determined largely by temperature (Rivoal & Cook, 1993).

Measurements

Cysts: L = 518-801 μm; W = 432-744 μm; L/W ratio = 0.8-1.8; fenestral length = 32-55 μm; vulval slit = 7-12 μm.

J2: L = 505-598 μm; stylet = 24-27.5 μm; hyaline region = 34-50 μm; tail = 52-79 μm.

Description: *Cyst* lemon-shaped, with prominent neck and vulval cone. Subcrystalline layer conspicuous, sloughing off with formation of dark brown cyst. Bifenestrate (Fig. 10.11A), bullae prominent, crowded beneath vulval cone. J2 vermiform, with a sharply pointed tail. Stylet well developed, with large, anteriorly flattened to concave basal knobs.

Remarks: *Heterodera avenae* belongs to the *Avenae* group and to the *H. avenae* complex. It differs from the similar species *H. australis*, *H. aucklandica*, *H. riparia*, *H.*

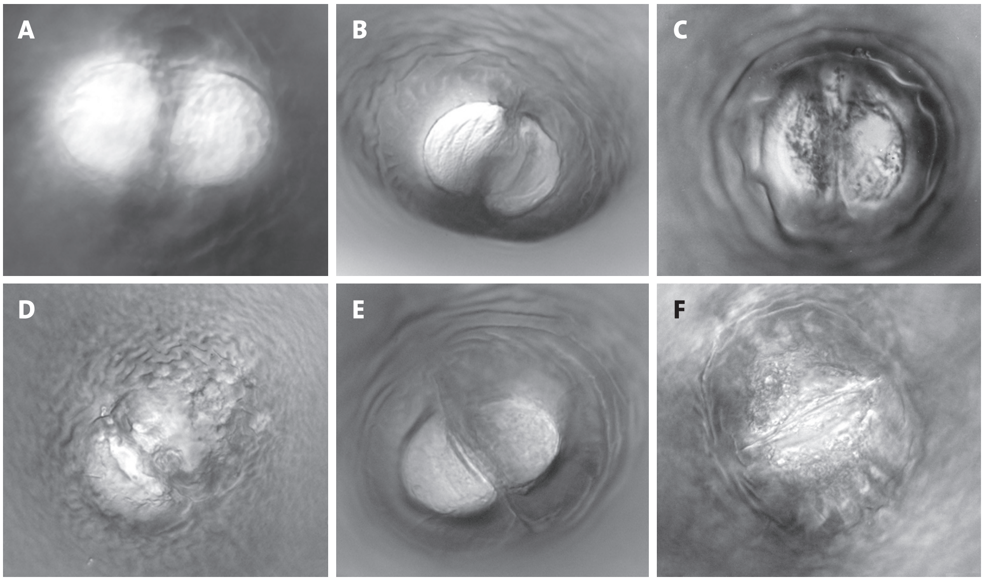

Fig. 10.11. Vulval plates. A: *Heterodera avenae*; B: H. betae; C: H. cajani; D: H. carotae; E: H. ciceri; F: H. cruciferae. After Subbotin *et al*. (2010) with modifications.

pratensis and *H. arenaria* by morphometric characters of J2 and cysts and by PCR-ITS-RFLP and IEF of proteins. Based on the ITS-rRNA gene sequences and PCR-ITS-RFLP, all world populations of H. avenae can be divided into three type: i) *H. avenae*, European populations, type A; ii) *H. avenae*, Asian and African populations, type B; iii) *H. avenae*, several French populations; and iv) *H.* '*avenae*' from China, type C.

Heterodera betae **Wouts, Rumpenhorst & Sturhan, 2001 – the yellow beet cyst nematode**

The yellow beet cyst nematode was observed by Maas *et al*. (1976) during routine examination of samples collected from the southern sugar beet regions of The Netherlands. It was considered a biotype of the clover cyst nematode, *H. trifolii*, and subsequently named *H. trifolii forma specialis* '*betae*', capable of parasitizing sugar beet, a crop not normally affected by the clover cyst nematode. Comparative detailed morphological and molecular analysis of several populations of the yellow beet cyst nematode with closely related species allowed Wouts *et al*. (2001) to report reliable diagnostics features and describe it as a new species, *H. betae*. The yellow beet cyst nematode reproduces on Cruciferae, Chenopodiaceae, Popygonaceae, Caryophyllaceae and a few Leguminosae are good hosts for *H. betae*. Several species of *Beta* and *Brassica* species, rhubarb, radish, spinach, common chickweed, pinks, garden pea, cabbage and broad beans are hosts for yellow beet cyst nematode. In the field, the threshold level at which damage occurs varies considerably between host species. Damage occurs in sugar beet at a level of *ca* 500 J2 and eggs per 100 cm^3 soil at the beginning of the season, whereas in peas and beans the threshold is over 800 J2. The yellow beet cyst nematode reaches epidemic levels only in lighter soils.

Measurements

Cysts: L = 475-1160 μm; W = 225-702 μm; L/W = 1.5-2.5; fenestral length = 30.3-75 μm; vulval slit = 36-67 μm.

J2: L = 525-672 μm; stylet = 26-33 μm; hyaline region = 32-50 μm; tail = 65-84 μm.

Description: *Cyst* lemon-shaped, often asymmetrical. Vulval cone high and wide, ambifenestrate (Fig. 10.11B), in young cysts surrounded by gelatinous matter containing up to 30 eggs. Vulval bridge narrow. Heavily pigmented bullae present, mainly anterior to underbridge, globular, some with basal stem, close to cyst wall. *J2* body slightly curved ventrally. Stylet robust, knobs heavy, deeply concave anteriorly, convex posteriorly. Tail gradually tapering to slender rounded terminus, hyaline region forming 50-60% of total tail length.

Remarks: The yellow beet cyst nematode can be easily distinguished morphologically from the bisexual sugar beet nematode, *H. schachtii*, by the larger fenestrae, longer body, stylet and length of the hyaline part of the J2 tail.

Heterodera cajani **Koshy, 1967 – the pigeon pea cyst nematode**

The pigeon pea cyst nematode was first briefly described by Koshy (1967) from roots of pigeon pea, *Cajanus cajan* (L.) Mill. in India. Surveys indicate that *Heterodera cajani* is the most widespread cyst nematode in India and is now present in all major pigeon pea growing regions of India and Pakistan. The list includes several dozen species of Fabaceae and Pedaliaceae. More than nine generations per year have been reported for a vegetation season. Nematode damage has been reported on many agronomic crops. This nematode is one of the major limiting factors in the production of pulses, *viz.*, pigeonpea, cowpea, green gram, black gram, which causes stunting of plants, reduction in number of nodules on roots with heavy crop yield losses to the tune of 30 to 67%.

Measurements

Cysts: L = 390-690 μm; W = 175-510 μm; L/W = 1.0-2.5; fenestral length = 21-69 μm; vulval slit = 39-60 μm.

J2: L = 324-515 μm; stylet = 20-33 =m; hyaline region = 17-40 μm; tail = 32-64 μm.

Description: *Cyst* light to dark brown, typically lemon-shaped with protruding neck and vulva. Subcrystalline layer present on young cysts. Ambifenestrate (Fig. 10.11C) with strong underbridge. Bullae prominent dark-brown, located beneath underbridge. *J2* body tapering anteriorly and posteriorly but more so posteriorly. Labial region offset, bearing four annuli. Stylet strong, well developed with anteriorly directed or rounded knobs. Tail with bluntly rounded and narrow terminus.

Remarks: *Heterodera cajani* belongs to the *Schachtii* group. It differs from several species of this group by a smaller cyst size. Nematode morphologically resembles *H. medicaginis* and *H. mediterranea*, and differs from *H. medicaginis* by a longer vulval slit and from *H. mediterranea* by a longer J2 body and tail.

Heterodera carotae **Jones, 1950 – the carrot cyst nematode**

Heterodera carotae occurs throughout the carrot-growing areas of European and other countries. It was found that only *D. carota* ssp. *sativa*, *D. carota* ssp. *carota* and *D. pulcherrimus* (Willd.), several wild Umbelliferae, such as *Torilis leptophylla*, *T. arvensis* and *T. japanica* are hosts. Up to four generation may occur in favorable growing conditions. In infected fields, irregular plant growth was observed, the patches enlarging over time. Foliage is stunted, reddish and may dry out when infestations are heavy. Carrots are usually small, abnormally developed and have numerous radicles which gives the roots a characteristic bearded appearance, referred to as 'hairy root'. Carrot cyst nematodes have caused up to a 50% decrease in crop yield.

Measurements

Cysts: L = 218-625 μm; W = 165-500 μm; L/W ratio = 1.9-2.8; fenestral length = 27-36 μm; vulval slit = 43-51 μm.

J2: L = 375-452 μm; stylet = 22-25 μm; hyaline region = 20-31.8 μm; tail = 43.5-59 μm.

Description: *Mature cysts* small, lemon-shaped. Bullae absent. Underbridge bifurcate, slender, unsclerotized. Fenestration indistinct, ambifenestrate (Fig. 10.11D). Vulval bridge frequently broken in older specimens. *J2* labial region slightly offset with four indistinct annuli. Stylet knobs with concave anterior faces. Most common variation is presence of 1-3 spherical refractive bodies within tail, sometimes with associated swelling.

Remarks: *Heterodera carotae* belongs to the *Goettingiana* group. It most closely resembles *H. cruciferae* and *H. goettingiana*; from the first species it differs on average by a longer J2 hyaline region and a longer vulval in cysts. From the second species it differs by the shorter J2 tail and shorter cyst length.

Heterodera ciceri Vovlas, Greco & Di Vito, 1985 – the chickpea cyst nematode

This nematode was found in several fields of chickpea (*Cicer arientinum* L.) showing a marked decline of the plants (Vovlas *et al*., 1985). It is abundant and widespread in Northern Syria, several localities in Turkey and Lebanon, where it causes severe yield loss of chickpea and lentil in fields. A host range study demonstrated that females and cysts of *H. ciceri* occurred in large numbers in the roots of chickpea, lentil, grass pea and pea. Few females were found in the roots of haricot bean and lupin (*Lupinus albus* L.), but no reproduction occurred on sugar beet (*Beta vulgaris* L.), curled dock (*Rumex crispus* L.) or *Trifolium* spp. Under field conditions, it is inferred that there is only one generation of the nematode during the growing season of spring sown chickpea. The tolerance limit of chickpea is around 1 egg/cm^3 of soil. Yield losses of 20% and 50% can be expected in fields infected with 8 or 16 egg/cm^3

of soil respectively, and complete crop failure can occur in fields infested with more than 60 egg/cm^3.

Measurements

Cysts: L = 570-930 μm; W = 350-550 μm; L/W ratio = 1.6-2.4; fenestral length = 32-52 μm; vulval slit = 32-60 μm.

J2: L = 440-585 μm; stylet = 27-30 μm; hyaline region = 31-42 μm; tail = 53-72 μm.

Description: *Cyst* lemon-shaped with a distinct neck and a prominent cone. Cyst covered with white subcrystalline layer. Ambifenestrate (Fig. 10.11H) with numerous prominent, dark-brown bullae irregularly distributed at periphery of vulval cone and, in most cysts, beneath underbridge level. Underbridge well developed with furcate ends and central thickening. *J2* labial region hemispherical slightly offset with three labial annuli, oral disc plate dorsoventrally elongated and two rounded lateral sectors bearing large, semilunar-shaped, amphidial apertures. Lateral field with four incisures. Tail irregularly annulated, tapering uniformly, rather abruptly ending in a finally rounded terminus.

Remarks: *Heterodera ciceri* belongs to the *Schachtii* group. It resembles *H. trifolii*, *H. rosii*, and *H. daverti* in its general morphology. It differs from *H. trifolii* by the presence of males, the different host-range (none of the *Trifolium* spp. tested were hosts of *H. ciceri*) and by the fenestral measurements.

Heterodera cruciferae **Franklin, 1945 – the cabbage cyst nematode**

Heterodera cruciferae has a narrower host range than *H. schachtii*, but apparently infects all species of *Brassica*, including common agricultural crops such as cabbage, Brussels sprouts, cauliflower, broccoli and radish (Baldwin & Mundo-Ocampo, 1991). Franklin (1951) reported several Brassica species as experimental hosts of *H. cruciferae*. Crop losses induced by *H. cruciferae* were reported in yield of Brussels sprouts in California. *Heterodera cruciferae* parasitizes cool-weather or winter-grown crops, so that the number of generations completed in a season depends on the growing period up to three generations might occur in Europe (Stone & Rowe, 1976).

Measurements

Cysts: L = 355-690 μm; W = 288-571 μm; L/W ratio = 0.6-1,8; fenestral length = 22-59 μm; vulval slit = 29-55 μm.

J2: L = 333-504 µm; stylet = 20-25 =m; hyaline region = 16-30 µm; tail = 26-58 µm.

Description: Cyst broad, almost spherical to lemon-shaped. Ambifenestrate (Fig. 10.11F), abullate, with very low semifenestral arches separated by a narrow vulval bridge, semifenestrae unobstructed in mature cysts, but in newly formed cysts body wall may still be intact. *J2* labial region offset with three or four annuli and a dorsoventrally elongated oral disc flanked by lateral lips bearing amphidial apertures. Stylet knobs anterior face flat, rounded or slightly concave. Tail tapering uniformly to a fine rounded terminus.

Remarks: *Heterodera cruciferae* belongs to the *Goettingiana* group and closely related with *H. carotae*.

***Heterodera elachista* Ohshima, 1974 – the Japanese cyst nematode**

This nematode species has been discovered widely distributed in Japan, mainly on upland rice fields. Based on a series of experiments, this nematode was shown to be the major factor causing continuous cropping failure of upland rice and was tentatively identified as *H. oryzae* (Ohshima, 1974). *Heterodera elachista* was also identified from a rice field in Iran and on roots of *Carex* sp. from China. The yield damage induced by this nematode ranges from 7-19% (Bridge *et al*., 1990). Greater yield losses were observed when the roots were invaded by the nematodes before tillering. In a field experiment, negative correlations between yields and final soil cyst populations were more evident in early, relative to later, sown crops. Nematodes had the most severe impact during the later stages of plant growth.

Measurements

Cysts: L = 328-557 µm; W = 229-540 µm; L/W ratio = 1.1-1.9; fenestral length = 23-50 µm; vulval slit = 26-46 µm.

J2: L = 330-450 µm; stylet = 16-21 µm; hyaline region = 25-39 µm; tail = 44-70 µm.

Description: *Cyst* light to dark brown, spherical to lemon-shaped. Subcrystalline layer present. Ambifenestra low (Fig. 10.12A) and rounded, ca as wide as long. Vulval bridge narrow, medium sized underbridge; cone terminus with a few large, peripheral, dark brown, bullae lacking finger-like projections. *J2* body slightly curved ventrally when killed by gentle heat. Stylet well developed with rounded or anteriorly concave knobs. Tail long, narrowly tapering to a very fine, rounded terminus.

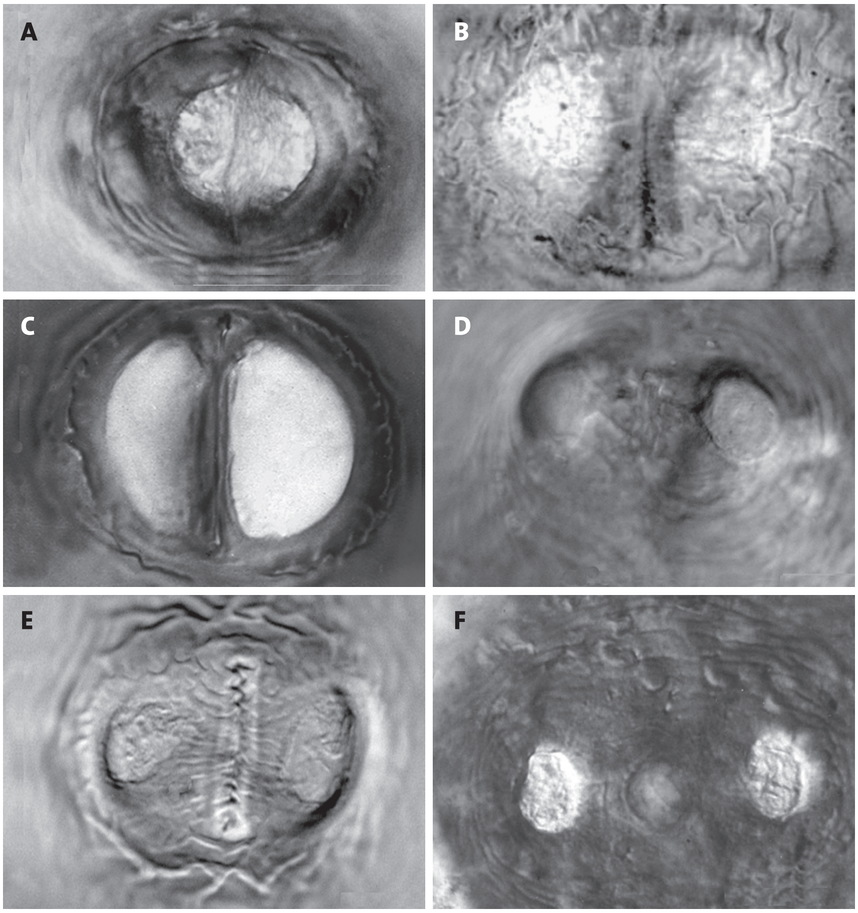

Fig. 10.12. Vulval plates. A: *Heterodera elachista*; B: H. fici; C: *H. glycines*; D: *H. hordecalis*; E: *H. humuli*; F: *H. latipons*. After Subbotin *et al*. (2010) with modifications.

Remarks: *Heterodera elachista* belongs to the *Cyperi* group and is morphologically close to *H. oryzae*, *H. sacchari* and *H. leuceilyma*. It differs from *H. sacchari* and *H. leuceilyma* by lacking finger-shaped projections on the slender underbridge of the cysts and from *H. oryzae* by smaller cysts and shorter J2.

Heterodera fici **Kirjanova, 1954 – the fig cyst nematode**

The fig cyst nematode, *H. fici*, was first described from roots of plant *Ficus elastica* Roxb. growing in a pot, which was imported from Harbin, China to Russia. Subsequent surveys revealed that this nematode is widely distributed in natural conditions in Mediterranean other countries, where it infects figs, and in glasshouses from many countries where it parasitizes ornamental plants from the genus *Ficus*. Under field condition in the Mediterranean region, this nematode develops more than one generation per year. The nematode can be considered a potential threat in fig nurseries, where fig rootstocks are often obtained from seeds. It is also noteworthy that the nematode has caused considerable damage to potted plants. Poor plant growth and leaf chlorosis have been reported on both edible and ornamental figs infected with *H. fici*. The host range of *H. fici* is restricted to the species of the genus *Ficus*, including *F. carica* L., *F. benghalensis* L., *F. lyrata* Warb, *F. australis* Willd. and *F. rubiginosa* Desf. (Kirjanova & Krall, 1971).

Measurements

Cysts: L = 340-697 μm; W = 272-560 μm; L/W = 1.0-1.7; fenestral length = 42-74 μm; vulval slit = 35-56 μm.

J2: L = 328-470 μm; stylet = 20-23 μm; hyaline region = 18-33 μm; tail = 40-61 μm.

Description: *Cyst* body light to dark brown, basically lemon-shaped, neck and vulval cone distinct. Neck protruding, curved posteriorly. Fenestra ambifenestrate, sometimes top of cone appearing bifenestrate (Fig. 10.12B). This bifenestrate appearance could be misleading unless many vulval cones are examined to reveal the true ambifenestrate vulval cone of this species. Semifenestra symmetrical, separated by vulval bridge, surrounded by well developed basin. Bullae dome shaped, small, scattered around underbridge plane. Underbridge weakly developed, with furcate ends. J2 body vermiform; tapering at both extremities, more so posteriorly. Stylet well developed, basal knobs rounded, directed slightly anteriorly. Tail long, tapering, terminus rounded.

Remarks: *Heterodera fici* belongs to the *Humuli* group. It differs from other members of the *Humuli* group (*H. humuli*, *H. ripae*, *H. vallicola* and *H. litoralis*) by rather ambifenestrate than bifenestrate cysts and longer vulval slit.

Heterodera glycines Ichinohe, 1952 – the soybean cyst nematode
(Fig. 10.13)

A cyst nematode parasitizing soybean plants, *Glycine max* L. and causing ‘yellow dwarf’ symptoms, was recorded from Shirakawa, Fukushima Prefecture, Japan, in 1915. Ichinohe (1952) was the first to make careful morphological comparisons with other *Heterodera* species and to give a specific name and brief description of this nematode. In Japan, yield losses have been estimated at 10-70% (Ichinohe, 1988). Presently, *H. glycines* occurs in most countries of the world where soybean

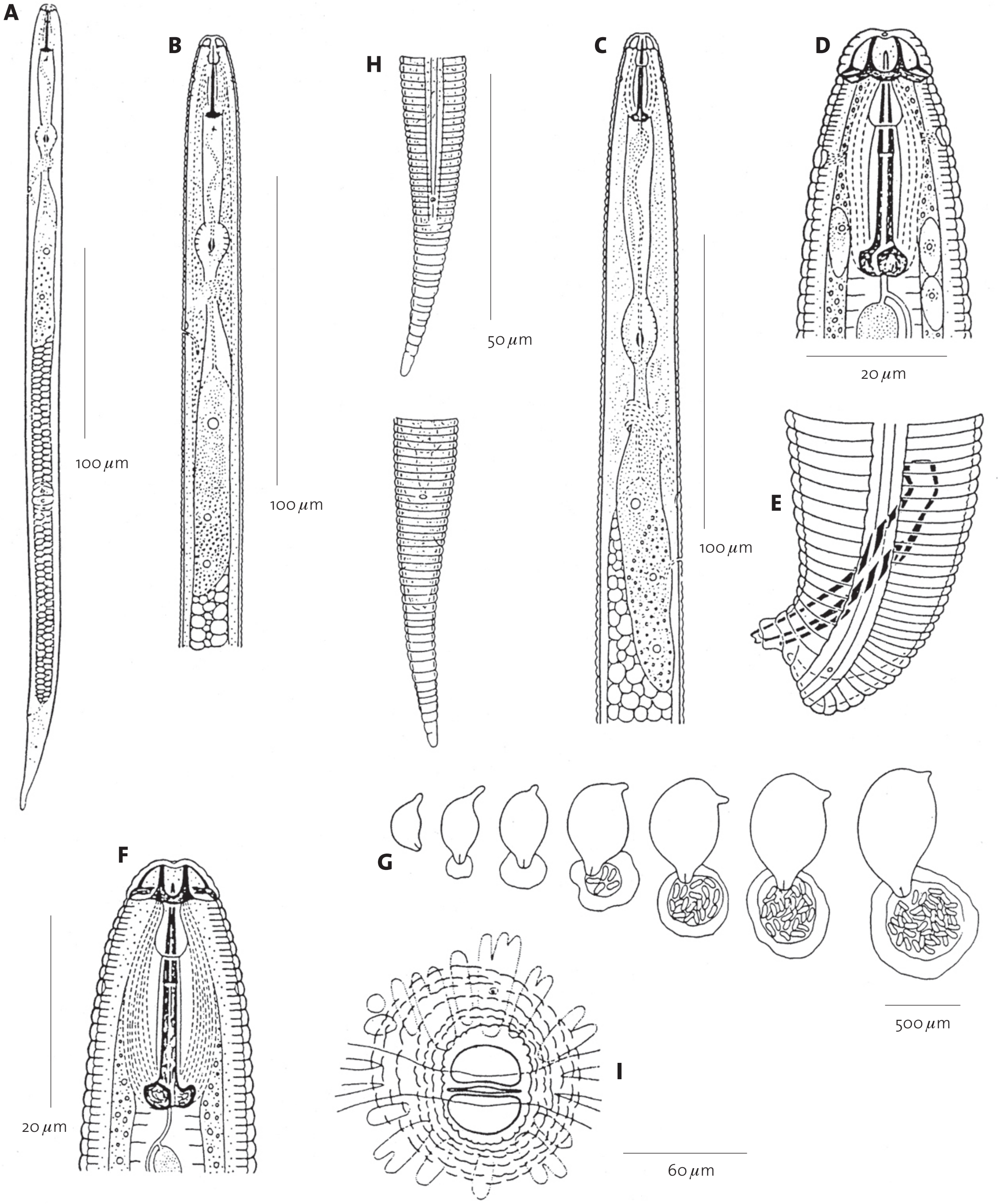

Fig. 10.13. *Heterodera glycines*. A-B, F-H: J2. A: Entire body; B: Pharyngeal region; H: Tails; F: Anterior region; C-E: Male. C: Pharyngeal region; D: Anterior region; E: Tail; G: Cysts with egg-sacs; I: Vulval cone. After Burrows and Stone, (1985).

is produced. In a study of losses predicted in ten soybean-producing countries, together accounting for 97% of the world crop, *H. glycines* appeared to be the most important constraint (losses of 8,969,400 t) on yield and estimated at US$1,960 million (Wrather *et al*., 2001). In these countries, total yield losses attributed to *H. glycines* were greater than those for any other pest of the crop. The soybean cyst nematode (SCN) is widely distributed throughout the north-central United States where different maturity groups with the same source of SCN resistance are grown. State surveys in the region report from 14% to 63% of fields are infested with SCN.

Heterodera glycines has a broad host range, especially Fabaceae, but also occurs on other families. More than 66 weed species of nine families are suitable hosts. Riggs (1992) provided a list of non-fabaceous hosts comprising 63 species in 50 genera from 22 families (*e.g*. Boraginaceae, Capparaceae, Caryophyllaceae, Chenopodiaceae, Brassicaceae, Lamiaceae, Fabaceae, Scrophulariaceae, Solanaceae). In field conditions, *H. glycines* was also found in several other plants, including henbit (*Lamium amplexicaule*), purple deadnettle (*Lamium purpureum*), mouse-ear chickweed (*Cerastium holosteoides*) and common chickweed (*Stellaria media*) (Riggs, 1992).

Three to five generations develop during the cropping season. Optimum temperature is 23-28°C; development stops below 14°C and above 34°C. In the absence of a host, J2 and eggs in cysts may remain viable in soil for 6-8 years.

Heterodera glycines disturbs root growth, interferes with nodulation and causes early yellowing of soybean plants. The above-ground symptoms of damage on individual plants and appearance of infested fields are usually not sufficiently specific to allow direct identification. Infected plants are predisposed to *Fusarium* wilt. Sudden death syndrome is a soil-borne disease of soybean caused by the fungus *Fusarium solani* in association with *H. glycines*.

Measurements

Cysts: L = 340-920 μm; W = 200-688 μm; L/W ratio = 1.0-2.4; fenestral length = 35-72 μm; vulval slit = 36-60 μm.

J2: L = 345-504 μm; stylet = 21-25 μm; hyaline region = 18-36 μm; tail = 35-59 μm.

Description: *Cyst* mainly lemon-shaped, sometimes round with a protruding neck and cone. Ambifenestrate (Fig. 10.12C), bullae prominent, located at or anterior to underbridge, extending into vulval cone from interior of body wall cuticle. Shape varying from round to finger-like, round bullae differently sized, finger-like bullae of variable length and thickness. Underbridge well developed. *J2* body vermiform with regularly annulated cuticle. Stylet robust with anteriorly protruding knobs. Tail tapering uniformly to a finely rounded terminus.

Remarks: *Heterodera glycines* belongs to the *Schachtii* group and is distinguished from similar species by a combination of morphological and morphometric characteristics. It differs from H. schachtii by shape of the J2 stylet knobs (slightly convex *vs* moderately or strongly concave), shorter average J2 stylet and longer average fenestral length.

Races and HG types: The race test for *H. glycines* was first developed based on comparative development of females on four differential soybean lines and was proposed by a group of nematologists and soybean breeders (Riggs & Schmitt, 1988). The proposed system of races is based on the reaction of four host differentials for identification of 16 races of *H. glycines*. Twelve races are known to occur in the USA, three races (1, 3 and 5) are present in Japan, six in Argentina (1, 3, 5, 6, 9 and 14) and seven in China. Niblack *et al*. (2002) proposed an HG Type Test to better describe population variation and to expand the flexibility of the race classification system. The HG Type system uses three of the four resistant soybean genotypes currently used as indicator hosts (Peking [= PI 548402], PI 88788, and PI 90763).

***Heterodera goettingiana* Liebscher, 1892 – the pea cyst nematode**

In 1890, G. Liebscher reported infection and yield lost of pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) by nematodes identified as *H. schachtii* at fields of the Agricultural Institute at Göttingen, Germany. Two years later he described this species as *H. goettingiana*. Several researchers have reported diseases of peas, primarily in European countries, caused by this nematode, however, little was known about the biology and pathogenicity of *H. goettingiana* until the mid-1900s (Franklin, 1951). Infected pea fields show sharply delineated patches with dwarfed, poorly branched and yellowing plants that die prematurely. Infected plants either fail to flower or flower too early. The root system is poorly developed. Development takes 3-15 weeks depending on soil temperature and moisture as well as host species. One or two generations occur during the growing season in the UK, and three generations may develop in southern Italy. Cysts with eggs can remain viable in the absence of a host for 12 years.

Measurements

Cysts: L = 400-780 μm; W = 310-540 μm; L/W ratio = 1.3-2.2; fenestral length = 43-71 μm; vulval slit = 43-61 μm.

J2: L = 408-519 μm; stylet = 23-26 μm; hyaline region = 27-38 μm; tail = 54-74 μm.

Description: *Cyst* lemon-shaped with light to dark brown cyst wall. Subcrystalline layer not visible. Vulval cone ambifenestrate. In some old cysts, vulval bridge ruptured, fenestrae joining to form a large oval fenestrum. Bullae absent, although bullae-like structures and vulval denticles present. Underbridge weak. *J2* body vermiform, curved ventrally after fixation. Labial region hemispherical, with 2-5 annuli, slightly offset from body. Lateral field with four incisures, not areolated. Stylet knobs rounded, slightly projecting anteriorly. Tail tapering uniformly to a finely rounded terminus.

Remarks: *Heterodera goettingiana* belongs to the *Goettingiana* group. From several other representatives of the *Goettingiana* group (*H. cruciferae*, *H. carotae*, *H. ciceae*, *H. scutellariae*, *H. persica*) it differs by average longer J2 body, longer tail and longer hyaline region.

Heterodera hordecalis Andersson, 1974 – the barley cyst nematode

In 1967, heavy infestations by a cyst nematode were found on barley in a field in Halland province, Sweden. This nematode was later described by Andersson (1974) as a new species. *Heterodera hordecalis* is reported from several European and Asian countries. It also infects wheat, rye and many grasses.

Measurements

Cysts: L = 330-950 μm; W = 255-680 μm; L/W ratio = 1.0-1.5; fenestral length = 47-80 μm; vulval slit = 14-33 μm.

J2: L = 410-550 μm; stylet = 21-26 μm; hyaline region = 29-46 μm; tail = 44-60 μm.

Description: *Cyst* body ovoid, with distinct neck and vulval cone. Newly formed cysts with remnants of subcrystalline layer. Bifenestral (Fig. 10.12D), semifenestrae widely separated. Semifenestrae usually appearing oval in focal plane at surface but round or horseshoe-shaped when focused at mid-bridge level. In centre of bridge, a rigid, often dumb-bell like structure, surrounding anterior end of vagina. From narrow part of this structure, fine ridges usually extending through weaker parts of bridge toward semifenestrae. Underbridge extremely strong, sometimes with pronounced thickening in middle, and with ends basically bifurcate, each branch consisting of a number of cords, although often irregularly splayed. Bullae most commonly absent. J2 labial region slightly offset with three or four annuli. Lateral field with four incisures. Tail tip finely rounded.

Remarks: *Heterodera hordecalis* belongs to the *Avenae* group and is most similar to *H. latipons*. The most striking distinguishing features are those of the vulval slit, which is much longer in *H. hordecalis* than in *H. latipons*. The structures of the middle part of the bridge are much more rigid in *H. hordecalis* than in *H. latipons*. The stylet knobs of the *J2* are more anteriorly projecting in *H. hordecalis* than in *H. latipons*, although this can be variable in the latter, and the tail and hyaline region are slightly longer in *H. hordecalis* than in *H. latipons*.

Heterodera humuli **Filipjev, 1934 – the hop cyst nematode**

Heterodera humuli was reported from many European countries, the USA and Canada. Only one generation is produced per year. In an experimental study, the nematode significantly reduced plant height and fresh and dry weight of shoots. Infected plants showed more severe nutrient deficiency symptoms. A negative correlation has been demonstrated between the numbers of J2 in soil and cone yield in hop plantations. Hosts include *Humulus lupulus*, *Urtica dioica* and *U. urens*.

Measurements

Cysts: L = 290-610 μm; W = 245-450 μm; L/W ratio = 1.2-1.7; fenestral length = 49-76 μm; vulval slit = 33-43 μm.

J2: L = 336-468 μm; stylet = 21-25 μm; hyaline region = 22-30 μm; tail = 42-53 μm.

Description: *Cyst* lemon-shaped, occasionally nearly spherical. Abullate, thin walled, light colored, darkening with age. Bifenestrate (Fig. 10.12E), vulval bridge broad, semifenestrae circular or subcircular, often obscured by thin membrane with a fine fingerprint-like pattern. Underbridge slender, weak, with furcate ends. *J2* labial region rounded, offset with 2-4 annuli. Lateral field with four incisures, reducing to three anteriorly and posteriorly, not areolated. Tail tapering, terminus often constricted and irregularly shaped.

Remarks: *Heterodera humuli* belongs to the *Humuli* group and is similar to *H. ripae* and *H. vallicola*. It differs from *H. ripae* by a longer J2 tail and hyaline region and from H. vallicola by more slender cyst, longer fenestra.

Heterodera latipons **Franklin, 1969 – the Mediterranean cereal cyst nematode**

In the early 1960s, a cyst nematode similar to *H. avenae* was detected in Israel and Libya on the roots of stunted wheat and barley plants. It was morphologically studied and described by Franklin (1969) as a new species under the name *H. latipons* based on characteristics of the Israel population. This nematode was later recorded in many countries, mainly from the Mediterranean and the Orient. *Heterodera latipons* often occurs in mixed populations with *H. avenae* in cereal cropping systems. Re-examination of *H. latipons* type specimens made by Mor and Sturhan (2000) showed that the type material consists of two morphological types which are mainly distinguished by cyst characteristics, one type agreeing with that figured in the original description of *H. latipons* and the other resembling the related species *H. hordecalis*, which is mainly known in northern and central Europe.

Hosts include barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), *Phalaris minor*, *P. paradoxa* and *Elytrigia repens*. *Triticum durum* Desf. was considered to be a poor host of this nematode. Yield losses as high as 50% were reported on barley in Cyprus. In Syria, the nematode causes average yield losses of 20 and 30% in barley and durum wheat, respectively, and the nematode was more damaging under water stress conditions. Moreover, damage is more severe in fields infested concomitantly with *H. latipons* and the fungus *Bipolaris sorokiniana* (Sacc.) Shoemaker, the causal agent of the common root rot and seedling blight of barley, *i.e.*, the presence of the nematode increases the aggressiveness of the fungus (Scholz, 2001). In all areas studied, *H. latipons* completed only one life cycle during the growing season (Mor *et al.*, 1992).

Measurements

Cysts: L = 300-700 μm; W = 320-560 μm; L/W ratio = 0.6-1.7; fenestral length = 52-76 μm; vulval slit = 6-11 μm.

J2: L = 401-559 μm; stylet = 22-25 μm; hyaline region = 20-36 μm; tail = 42-68 μm.

Description: Cyst dark to mid-brown covered with white subcrystalline layer. Bifenestrate (Fig. 10.12H), semifenestrae separated by a distance greater than fenestral width, vulval slit short. Strong underbridge with pronounced thickening in middle and with ends splayed. Bullae usually absent, sometimes present at underbridge level. *J2* body slightly curved dorso ventrally when killed by heat. Stylet with well developed, anteriorly concave, knobs.

Remarks: *Heterodera latipons* belongs to the *Avenae* group and closely resembles *H. hordecalis* and *H. turcomanica*. These nematodes share similar circular semifenestrae separated by a distance longer than the semifenestra diameter and a rather typical underbridge but with a pronounced enlargement underlying the vulval slit. The most important differentiating character between *H. latipons* and *H. hordecalis* is the vulval slit which in *H. latipons* is much shorter.

***Heterodera oryzae* Luc & Brizuela, 1961 – the rice cyst nematode**

Heterodera oryzae was described by Luc and Brizuela (1961) from swamp rice fields in the central part of the Ivory Coast. It was also found parasitizing banana, *Musa* sp., in Senegal. In experimental tests, J2 of *H. oryzae* penetrated into roots of *Mariscus umbellatus* and *Zea mays* and developed into numerous females. In these hosts populations can be increased, or at least maintained. In roots of *Glycine hispida*, *Pueraria phaseoloides* and *Solanum lycopersicum* only a few females developed. *Cyperus umbellatus* and elephant grass (*Pennisetum purpureum*) are considered as hosts for *H. oryzae*. Host yield loss induced by this nematode has not been clearly shown. It is considered to be less aggressive than other cyst nematodes infecting rice (Luc, 1986).

Measurements

Cysts: L = 310-999 μm; W = 220-690 μm; L/W ratio = 0.9-2.3; fenestral length = 19-46 μm; vulval slit = 34-65 μm.

J2: L = 350-589 μm; stylet = 16-25 μm; hyaline region = 22-45 μm; tail = 46-69 μm.

Description: *Cyst* mature cysts dark brown to black, lemon-shaped with prominent vulval cone. Ambifenestrate (Fig. 10.14A), underbridge long with dendroid-like extremities attaching to cyst wall but without finger-like projections. Bullae present but number, size, shape and location varying, although usually small, rather spherical and scattered inside vulval cone. *J2* body straight or very slightly ventrally curved when heat-relaxed. Labial region with three or four annuli. Lateral field with three incisures. Tail conical, with elongated terminus and pointed tip.

Remarks: *Heterodera oryzae* belongs to the *Cyperi* group and is morphologically similar to *H. oryzicola* and *H. elachista*. It differs from *H. oryzicola* by having a larger cyst, longer J2 stylet, longer J2 hyaline region and anteriorly projecting J2 stylet knobs.

***Heterodera sacchari* Luc & Merny, 1963 – the sugar cane cyst nematode**

This species was originally described from sugar cane in the Congo, Africa (Luc & Merny, 1963). Rice (*Oryza sativa*) and sugar cane are the major field crops infected by this nematode. *Oryza glaberrima* is also infected, but cysts are smaller and contain fewer eggs. Pot experiments showed that maize, millet and sorghum, commonly cultivated in cropping systems with rice in West Africa, were poor hosts of *H. sacchari*. Infected sugar cane plants were stunted and thin, and secondary roots are less abundant than in healthy plants. Rice plants attacked by *H. sacchari* showed

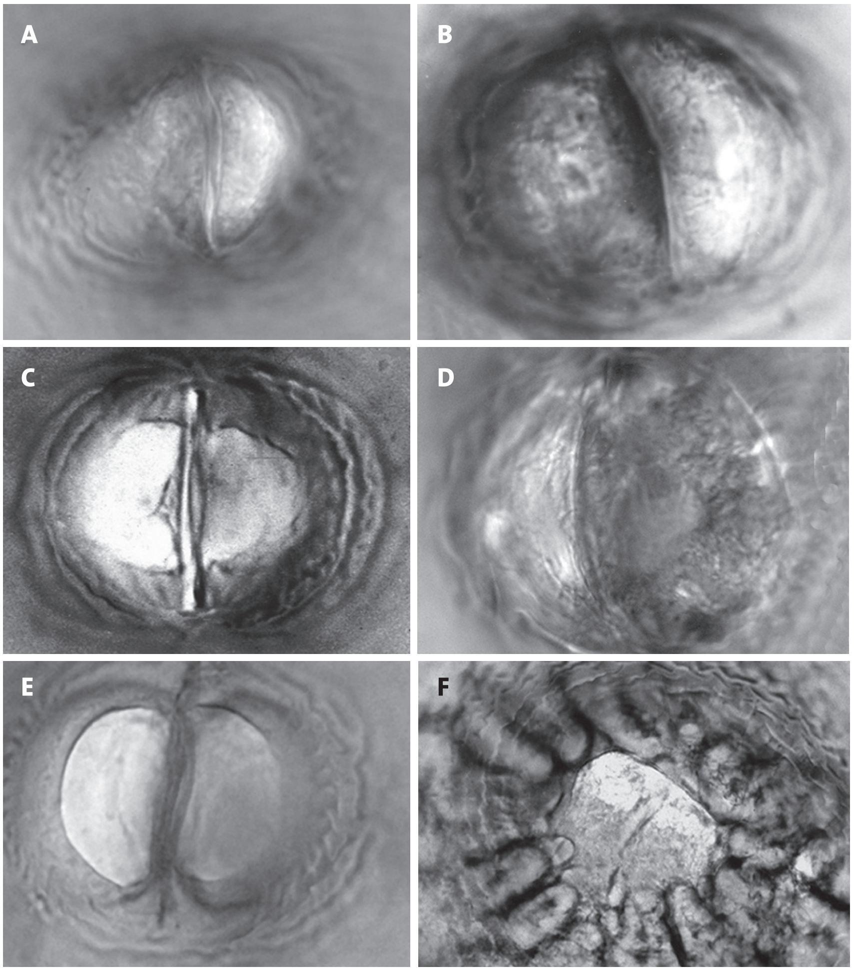

Fig. 10.14. Vulval plates. A: *Heterodera oryzae*; B: *H. sacchari*; C: *H. schachtii*; D: *H. sorghi*; E: *H. trifolii*; F: *H. zeae*. After Subbotin *et al*. (2010) with modifications and courtesy of U. Zunke)

intense chlorosis, delayed and reduced tillering and reduced grain yield. The roots of attacked plants were twiggy, very necrotic and blackened. A field study conducted during 1997 at the West Africa Rice Development Association in Cote d'Ivoire showed that under low water availability in sandy soil, damage and reduced upland rice grain yield was significant (Coyne & Plowright, 2000).

Measurements

Cysts: L = 380-1030 μm; W = 280-830 μm; L/W ratio = 1.0-2.0; fenestral length = 29-60 μm; vulval slit = 44-62 μm.

J2: L = 360-560 μm; stylet = 19-26 μm; hyaline region = 20-36 μm; tail = 46-66 μm.

Description: *Mature cyst* brown to dark brown, lemon-shaped with prominent vulval cone and neck of medium size. Cuticle with a lace-like pattern, numerous 'perforations' evenly but irregularly distributed on inner layer. Cyst cone ambifenestrate (Fig. 10.14B), fenestrae rather obscurely demarcated, underbridge strongly developed with finger-like projections, few peripheral bullae. *J2* body straight or slightly ventrally curved when heat-relaxed, slightly tapering at anterior end, more attenuated at posterior end. Labial region dome-shaped, with three annuli, labial framework heavily sclerotized. Lateral field composed of three lines, generally irregularly areolated. Stylet strong with knobs anteriorly concave. Tail elongated, conical, with pointed terminus.

Remarks: *Heterodera sacchari* belongs to the *Sacchari* group and to the *H. sacchari* species complex together with *H. leuceilyma* and *H. goldeni* (Tanha Maafi *et al*., 2007b). The three species are very similar in morphometrics and other morphological characters and are not easily distinguished.

Heterodera schachtii **Schmidt, 1871 – the sugar beet cyst nematode**

The sugar beet cyst nematode has been recognized as a plant pathogen since 1859 when it was associated with stunted and declining sugar beet in Germany. In the following years it became recognized as a pest of great importance in beet growing areas of several European countries. *Heterodera schachtii* is found in all major sugar beet production areas of the world, favoring temperate regions but apparently tolerating a broad range of climates. It is widespread in Europe, USA and Canada (Baldwin & Mundo-Ocampo, 1991). Annual yield loss in the EU countries, based upon world market sugar prices, was estimated in 1999 at up to €90 million (Müller, 1999). The optimum temperature for development is *ca* 25°C. In some climates, 3-5 generations may complete development on sugar beet in one season (Franklin, 1972). *Heterodera schachtii* was described from *Beta vulgaris* L. and it parasitizes mainly plants of the families Amaranthaceae (Caryophyllales) (many species of *Beta* and *Chenopodium*) and Brassicaceae (Brassicales) (*Brassica oleracea*, *B. napus*, *B. rapa*, *Rhaphanus sativus* and many others including diversity of common weeds) (Franklin, 1972). Some plants from Polygonaceae, Scrophulariaceae, Caryophyllaceae and Solanaceae are susceptible to nematode infection.

Measurements

Cysts: L = 480-960 μm; W = 396-696 μm; L/W ratio = 0.9-2.0; fenestral length = 28-48 μm; vulval slit = 33-54 μm.

J2: L = 400-512 μm; stylet = 23-28 μm; hyaline region = 17-33 μm; tail = 40-56 μm.

Description: *Cyst* color light to dark brown. Ambifenestrate (Fig. 10.14C), within cone, remnants of vagina attached to side walls by underbridge and a number of irregularly arranged, dark brown molar-shaped bullae situated a short distance beneath vulval bridge. *J2* labial region offset, hemispherical, with four indistinct annuli. Stylet moderately heavy with prominent, forwardly-directed knobs. Tail acutely conical with rounded tip, distinct hyaline terminal section 1-1.25 stylet lengths long.

Remarks: *Heterodera schachtii* belongs to the *Schachtii* group and is distinguished from closely related species (*H. trifolii*, *H. glycines*, *H. betae* and others by a combination of morphological and morphometric characteristics. PCR-ITS-RFLP profile generated by MvaI is unique for *H. schachtii*. Diagnostics of this species using PCR with species specific primers was also developed.

Heterodera sorghi Jain, Sethi,
Swarup & Srivastava, 1982 – the sorghum cyst nematode

Heterodera sorghi was first recorded and described by Jain *et al*. (1982) from the roots of sorghum in Uttar Pradesh, India, Subsequently, it was found in other states of India. The life cycle studies of *H. sorghi* carried out on maize revealed that the nematode was able to complete its life cycle from J2 to J2 in 24 days at 28-36° C. More than four generations may be completed in a maize cropping season of 100-110 days duration. Significant reduction in the shoot length and fresh shoot and root weights of maize was observed at an initial inoculum level of 4 J2/cm^3 of soil and hence this inoculum level was considered as the minimum damaging threshold level for this host.

Measurements

Cysts: L = 550-910 μm; W = 400-600 μm; L/W ratio = 1.2-1.8; fenestral length = 40-56 μm; vulval slit = 28-39 μm.

J2: L = 420-525 μm; stylet = 20-22 μm; hyaline region = 25-35 μm; tail = 42-60 μm.

Description: *Cyst* light to dark brown, lemon-shaped with prominent neck and vulval cone. Cyst cone ambifenestrate (Fig. 10.14D), semifenestrae separated by narrow vulval bridge. Underbridge strongly developed without finger-like projections in middle. Bullae dark brown, numerous, occurring at level of underbridge, varying in size, shape and number. J2 body slightly curved ventrally when heat relaxed. Lateral field with three incisures, not areolated. Stylet strong, cone and shaft almost equal length, knobs with concave anterior surfaces. Tai1 tapering posteriorly, conical.

Remarks: *Heterodera sorghi* belongs to the *Sacchari* group and is most closely related to *H. sinensis*. It differs from *H. sinensis* by shorter average J2 body and shorter hyaline region.

***Heterodera trifolii* Goffart, 1932 – the clover cyst nematode**

The clover cyst nematode is an important pest of diverse agricultural crops and many pasture plants, especially parasitizing white clover, *Trifolium repens*. *Heterodera trifolii* is a cosmopolitan species found in all continents. Some authors propose considering *H. trifolii* within a conglomerate of independently evolved mitotic parthenogenetic populations comprising polyploidy and aneuploid forms with 3n = 24 to 28 and 4n = 34-35 chromosomes and host races with more or less extended host ranges. Good hosts within Fabaceae for *H. trifolii* are also: *T. repens*, *Trifolium* spp., *Lotus oroboides*, *Medicago falcata*, *Melilotus officinalis*, *Melilotus albus*, *Melilotus* spp., *Kummerowia stipulacea*, *K. striata*, *Vicia villosa*, *Phaseolus vulgaris* and *Sesbania macrocarpa*. Several generations may occur during a year, so population density may increase rapidly (Mulvey, 1959). At optimal temperature of about 25°C. the life cycle is completed in 17 days (Baldwin & Mundo-Ocampo, 1991). This nematode causes reduction in yield, nitrogen fixation and persistence of this plant in pastures. Several studies have shown yield losses and growth suppression of white clover due to *H. trifolii* infection.

Measurements

Cysts: L = 360-1020 μm; W = 195-680 μm; L/W = 1.2-2.7; fenestral length = 40-80 μm; vulval slit = 39-66 μm.

J2: L = 461-678 μm; stylet = 23-31 μm; hyaline region = 27-45 μm; tail = 49-78 μm.

Description: *Cyst* brown to dark brown containing a few to several hundred eggs. Vulval cone distinct. Ambifenestrate cysts (Fig. 10.14E) with underbridge and bullae strong and elongated. Long vulval slit and strongly pigmented underbridge with bifurcate ends. *J2* labial region offset, with three or four annuli, labial framework heavily sclerotized. Lateral field with four incisures, not areolated, outer incisures crenate. Stylet robust, anterior surfaces of knobs concave. Tail conoid, tapering uniformly to a finely rounded terminus.

Remarks: *Heterodera trifolii* is a member of the *H. trifolii* species complex including *H. betae*, *H. lespedezae* and *H. galeopsidis* and belongs to the *Schachtii* group. It differs from similar species *H. betae* by a shorter J2 hyaline region and shorter stylet.

Heterodera zeae Koshy, Swarup & Sethi, 1971 – the corn or maize cyst nematode

Heterodera zeae is be widely distributed and economically important (Koshy & Swarup, 1971). This species has also been found in Egypt and Pakistan. In 1981, *H. zeae* was reported for the first time in the Western Hemisphere in maize fields in Kent County, Maryland, USA (Sardanelli *et al*., 1981). Many researchers have evaluated the host range for different populations of *H. zeae*. Populations from India and the USA apparently differ in their host range, although these have not yet been compared under identical conditions. Among 15 species of weeds collected from fields infested with *H. zeae* in India, only *Cyperus rotundus*, *Parthenium hysterophorus*, *Trianthema portulacastrum*, *Urochloa panicoides*, *Achyranthes aspera*, *Digera muricata* and *Eclipta prostrata* were infected and of the 32 crop plants tested, only *Lagenaria siceraria*, wheat, barley, maize, *Pennisetum americanum* and sorghum were infected. *Setaria italica* was a good host of *H. zeae* in India. Host range tests show that the Egyptian populations reproduced on maize, barley, millet, Sudan grass and wheat, but not on rice, peanut, lupin, snapbean, pigeon pea, broad bean, pea, cowpea, sesame, tomato, sunflower, sugar beet, cotton, kenaf, flax, lime, sour orange, onion, leek or watermelon. Populations from Pakistan are reported to be widespread on peach, almond, citrus, garlic, mango, strawberry and many other crops, but these may be field associations and reproduction on these hosts has not been established.

Heterodera zeae is widely distributed in the maize growing regions in India causing substantial damage for this crop. Infected maize plants are stunted, leaf emergence is retarded and fresh and dry weights are reduced. Suppression of maize plant growth by the Maryland population of *H. zeae* was demonstrated in tests in field microplots and in plant growth chambers. Maize growth (dry weight) and yield were suppressed by 13 to 73% in 4 out of 5 years in the presence of *H. zeae* (Krusberg *et al*., 1997). However, maize yield suppression has not been demonstrated in maize fields in Maryland. The high soil temperatures in India and Egypt may be a primary reason why *H. zeae* is economically important to maize in those countries (Hashmi *et al*., 1993).

Measurements

Cysts: L = 342-805 μm; W = 245-551 μm; L/W ratio = 1.0-2.2 ; fenestral length = 35-58 μm; vulval slit = 29-58 μm.

J2: L = 350-484 μm; stylet = 19-25 μm; hyaline region = 17-30 μm; tail = 32-50 μm.

Description: *Cyst* light brown, basically lemon-shaped, cuticle thin walled, lacking subcrystalline layer. Thin subcrystalline layer discernible in young cysts only. Fenestra ambifenestrate (Fig. 10.14H), semifenestra separated by fairly wide vulval bridge, fenestral length and width variable, basin wide but generally poorly defined. Bullae prominent, immediately beneath (anteriad) to underbridge and characteristically arranged as four finger-like bullae in a distinct formation (observed in all but a few of the several hundred cysts examined). Immediately beneath finger-like bullae are a number of randomly arranged bullae. Underbridge simple, short, and thin, found in all but a few of cysts examined, lacking forking at ends. J2 body typically vermiform, tapering at both ends. Labial region slightly offset, rounded, with low profile, with 3-5 annuli, labial framework moderately developed. Lateral field with four distinct lines. Stylet strongly developed with round or slightly anteriorly directed knobs. Tail short, tapering conically, with acutely rounded terminus.

Remarks: *Heterodera zeae* differs from other species by the location of the bullae at two levels, *i.e.*, four, finger-like, bullae located immediately beneath a short, thin, underbridge and with many randomly located bullae further below.

10.10.2. Genus *Globodera* Skarbilovich, 1959

Diagnosis (after Baldwin & Mundo-Ocampo, 1991 and Siddiqi, 2000): *Mature female* and *cyst* spheroid, lacking terminal cone. Vulval area circumfenestrate. Vulva located in a cavity beneath outline of body, vulval slit $< 15\ \mu$m. No anal fenestra. Vaginal remnants, underbridge and bullae rarely present. Cuticle with distinct D-layer. All eggs retained in body, egg-sac absent. Egg surface smooth. Male lateral field with four lines, spicules $> 30\ \mu$m, distally pointed. *J2* with four incisures in lateral field. Tail conical, pointed, phasmids punctiform. En face pattern typically with six separate lips, sometimes with fusion of adjacent submedial lips.

Type species: ***Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959**

Total number of species: 10

Potato cyst nematodes (PCN) include two species: *G. rostochiensis* and *G. pallida*, which have been reported from many countries and are jointly considered to be one of the most economically important pest of potato. Molecular analysis however indicates presence of several still undescribed species. It is now generally accepted that PCN are native to South America (Stone, 1979), where they are the principal pest of Andean potato crops. The two species of PCN have different distributions in the

Andes. Factors which may be responsible include day length, temperature, altitude, rainfall or the interaction of any of them with the host potato. Human activities over centuries may also have influenced distribution. In South America, PCN is mainly found between 2000 m and 4000 m above sea level, with the heaviest infestations between 2900 m and 3800 m above sea level (Franco, 1977). Evans *et al*., (1975) mapped the distribution of *G. rostochiensis* and *G. pallida* in some of the Andean countries, and showed that most Andean populations north of Lake Titicaca are *G. pallida*. The distribution of *G. rostochiensis* is mainly south of Lake Titicaca. Later on, similar studies confirmed the view of Evans *et al*. (1975), that the two species occupy different zones in the Andes. The demarcation line between the two species is near 15.6 °S. With few exceptions, populations north of this line are mainly *G. pallida*. Those from areas around Lake Titicaca and further south are predominantly *G. rostochiensis* with few *G. pallida* or mixtures of both species. The most southerly population from the east side of the Andes in Bolivia are mixtures of *G. rostochiensis* and *G. pallida*. PCN (*G. rostochiensis* and *G. pallida*) have been introduced in many parts of the world, particularly to Europe, and also to the USA, Canada, New-Zealand and numerous other countries where potatoes are grown.

Globodera rostochiensis **(Wollenweber, 1923) Skarbilovich, 1959 – the golden potato cyst nematode**
(Figs 10.15, 10.16)

The golden cyst nematode, associated with potato plants, *Solanum tuberosum*, from Rostock, Germany, was first reported in 1881 and was initially considered to be *H. schachtii*, this being the only known species of cyst nematode at that time. During the early 1900s, it became more widely known throughout Europe and was formally described in 1923. In temperate regions, *G. rostochiensis* usually completes only one generation, although a second generation may be initiated but not completed – J2 hatch from first generation eggs, but are unable to reach the adult stage (Jones, 1950; Evans, 1969). In subtropical regions, two generations might occur. Development of one generation requires 6-10 weeks. The J2 can go into diapause and remain viable for many years, hatching continuing for 25 years or more. Heavily infected plants become yellow and stunted. Infected plants have reduced root systems which are abnormally branched and brownish in color. Symptoms in the field first appear in small patches. At low nematode densities tuber sizes are reduced, whereas at higher densities both number and size of tubers can be reduced. At eight and 64 eggs/g of soil, yield losses of about 20 and 70%, respectively, can be expected. Seinhorst (1965, 1982), Brown (1969) and Elston *et al*. (1991) proposed models that described the relationships of potato cyst nematode population densities before planting with potato yield and post-harvest nematode populations. The damaging effect of potato cyst nematodes is not only determined by nematode density, but also by

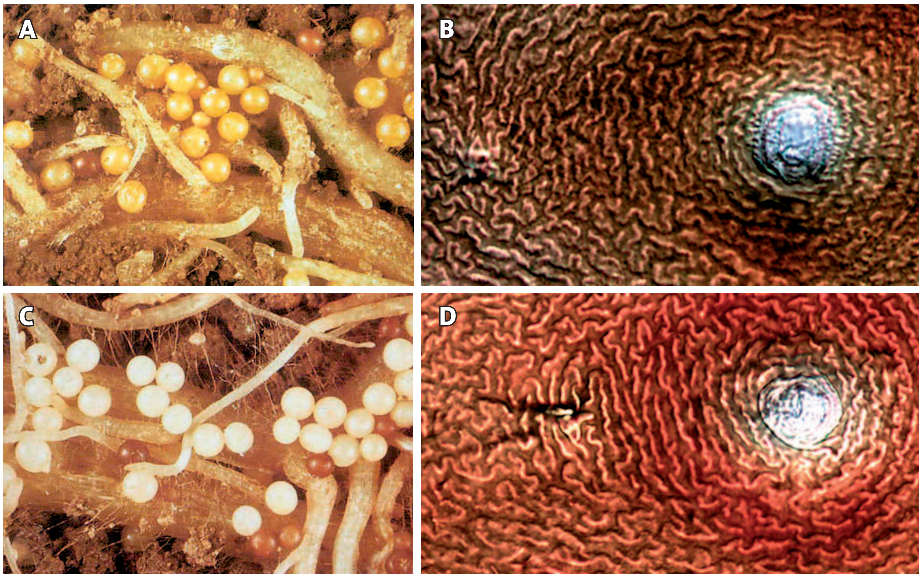

Fig. 10.15. Potato cyst nematodes. A, B: *Globodera rostochiensis*, C, D: *G. pallida*. A, C: Females and cysts on roots; B, D: Vulval plates. A, D - courtesy of I. Cid Del Prado; B, D - courtesy of V.N. Chizhov.

such factors as cultivar, crop husbandry and environmental conditions. Potato cyst nematodes are responsible for annual potato tuber losses of up to 9% in Europe (Evans & Stone, 1977), information on economic importance in some South American countries is little known or unavailable, although yield losses in Bolivia and Perú have been estimated to be ca US$ 13 000,000 and US$ 128 000,000, respectively (Franco and Gonzalez, 2010).

Hosts include potato, *Solanum tuberosum* (Solanaceae, Solanales), tomato, *Solanum lycopersicum* L. and eggplant, *S. melongena* L. Other hosts include many *Solanum* spp., *Datura* spp. *Hyoscyamus niger* L., *Nicotiana acuminata* Hook., *Physalis* spp. *Physochlaina orientalis* G. Don, *Salpiglossis* spp., *Capsicum annuum* L., *Saracha jaltomata* Schlecht.

Measurements

Cysts: L = 450-990 μm; W = 250-810 μm; L/W ratio = 0.9-1.8; fenestral diam. = 14-21 μm; number of ridges between anus and fenestra = 16-22; Granek's ratio = 2.3-4.6.

J2: L = 366-502 μm; stylet = 19-23 μm; DGO = 2.4-6.7 μm; hyaline region = 18-30 μm; tail = 37-57 μm.

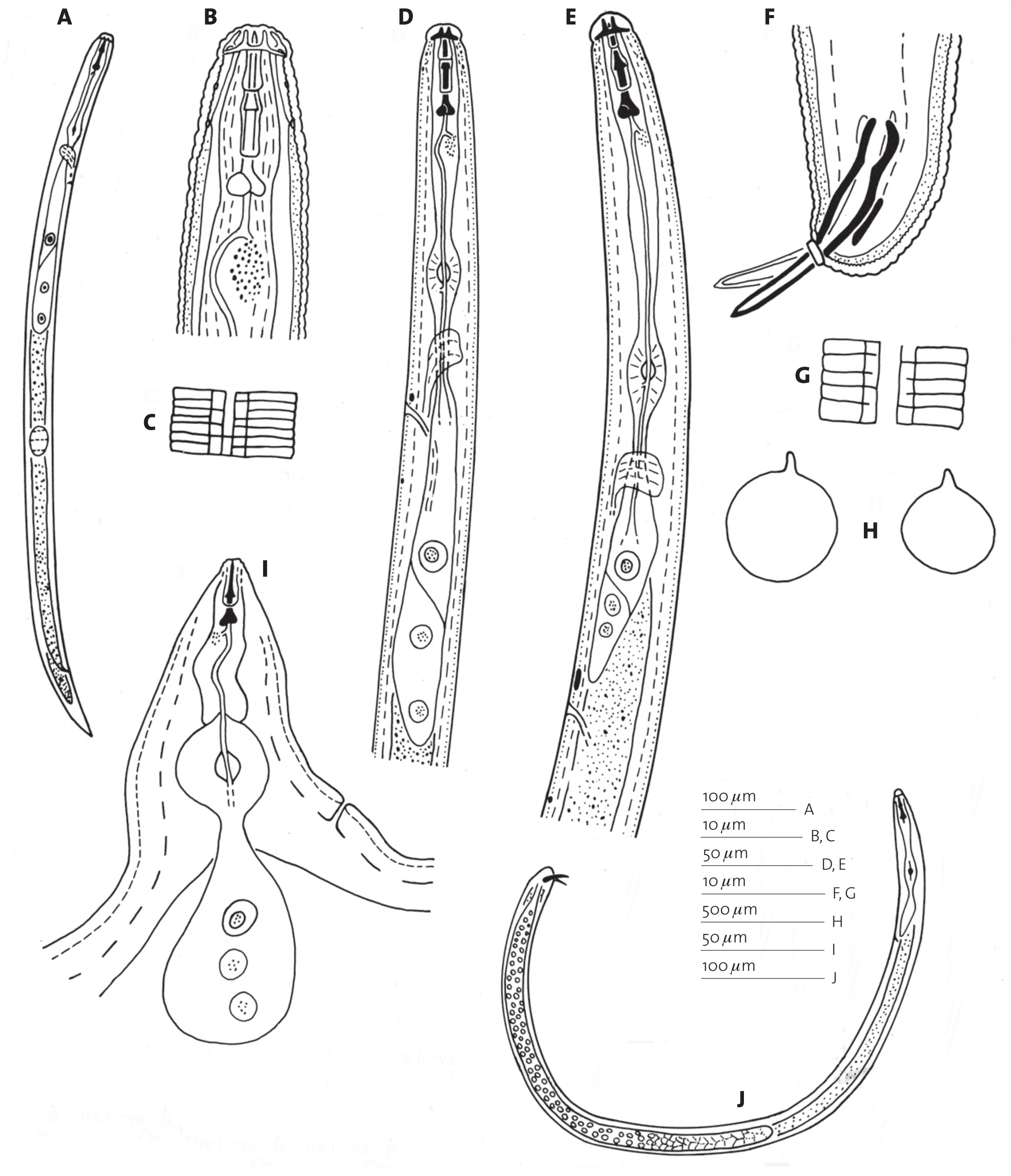

Fig. 10.16. *Globodera rostochiensis*. A-D: J2; A: Entire body; B: Lip region; C: Lateral field; D: Pharyngeal region; E-G: Male; E: Pharyngeal region; F: Tail; G: Lateral field; H: Entire cysts; I: Anterior region of female; J: Entire male. After Stone, (1973a).

Description: *Female* color changing from white to yellow to light golden as female matures to cyst stage. *Cyst* brown, ovate to spherical in shape with protruding neck, circumfenestrate (Fig. 10.15A, B), abullate. Fenestra circular, anus conspicuous at apex of a V-shaped subsurface cuticular mark. *J2* body tapering at both extremities but more at posterior end. Stylet well developed, with prominent rounded knobs as viewed laterally. Lateral fields with four lines extending for most of body length. Tail tapering to small, rounded terminus.

Remarks: *Globodera rostochiensis* is morphologically similar to *G. pallida* and *G. tabacum*. It differs from *G. pallida* by yellow or gold vs cream colored maturing females, higher number of ridges between the vulva and anus, larger mean for Granek's ratio, stylet knob shape, shorter average stylet length and rounded *vs* more pointed J2 tail terminus.

Globodera pallida **Stone, 1973 – the pale potato cyst nematode**
(Fig. 10.17)

The pale cyst nematode, *G. pallida*, is considered to be a major pest of potato crops in cool temperate climates. It is reported from several counties in Europe, Asia, Africa and South America. In Central and North America, *G. pallida* has been reported in Panama, USA and Canada, but in the last two countries PCN have a rather restricted distribution with only small infested areas because of rigorous phytosanitary regulations and seed potato certification programs, compared to the widespread infestations found in European countries. Recently, mtDNA analysis has been used to study genetic relationships among Peruvian populations of *G. pallida*, thus identifying the origin of western European populations of this species (Picard *et al*., 2007; Plantard *et al*., 2008). Using mtDNA gene, cytochrome b (cytb) sequences and microsatellite loci, Plantard *et al*. (2008) showed that the *G. pallida* presently distributed in Europe derived from a single restricted area in the extreme south of Peru, located between the north shore of Lake Titicaca and Cusco. *Globodera pallida* develops one generation for a vegetation season. This species is adapted to cool temperatures and is able to hatch earlier in the year and develop at 2°C cooler than *G. rostochiensis* (Langeslag *et al*., 1982). The symptoms of attack by *G. pallida* are similar to those for *G. rostochiensis* and the damage threshold is 1-2 eggs/g soil. Hosts includes potato, *Solanum tuberosum* (Solanaceae, Solanales), Eggplant, *Solanum melongena* L., tomato, *S. lycopersicum*, many species of *Solanum*; black henbane, *Hyoscyamus niger*.

Measurements

Cysts: L = 420-748 μm; W = 400-685 μm; fenestral diam. = 17.5-25 μm; number of ridges between anus and fenestra = 7 – 17; Granek's ratio = 1.2-3.6.

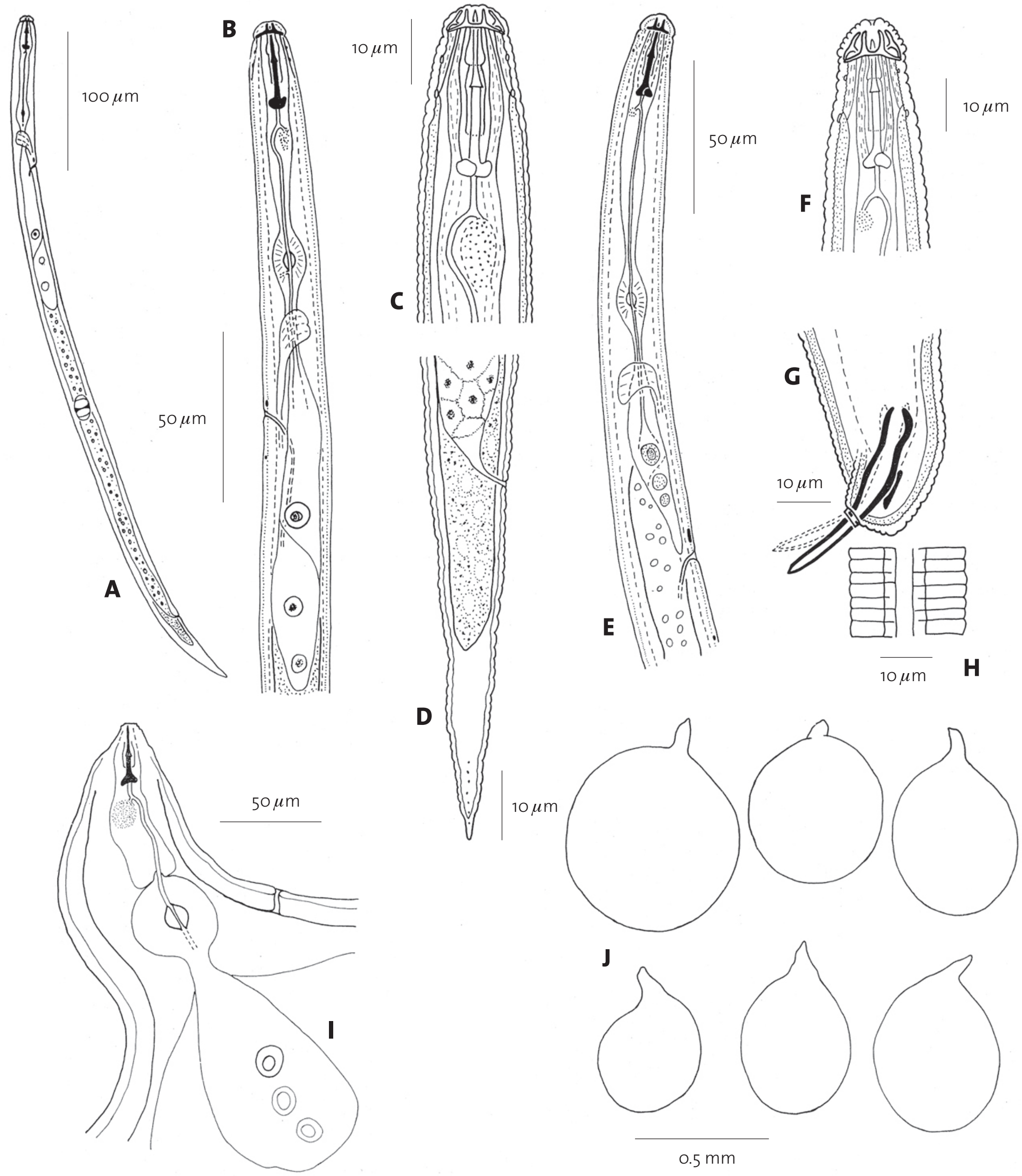

Fig. 10.17. *Globodera pallida*. A-D: J2. A: Entire body; B, C: Anterior region; D: Tail; E-H: Males. E, F: Anterior region; G: Tail; H: Lateral field; I: Anterior region of female; J: Cysts. After Stone (1973b).

J2: L = 380-533 μm; stylet = 22.5-25 μm; DGO = 2.7-5 μm; hyaline region = 20-31 μm; tail = 40-57 μm.

Description: *Female* white in color, some populations passing, after 4-6 weeks, through a cream stage, turning glossy brown when dead. *Cyst* vulval region intact or fenestrated with single circumfenestrate (Fig. 10.15B, D) opening occupying all or part of vulval basin, abullate. *J2* lateral field with four incisures but with three anteriorly and posteriorly, occasionally completely areolated. Stylet well developed, basal knobs with distinct anterior projection as viewed laterally. Tail tapering uniformly with a finely rounded point, hyaline region forming about half of tail region.

Remarks: *Globodera pallida* is most closely related to *G. rostochiensis* and *G. tabacum*. It differs from *G. rostochiensis* by cream colored females *vs* yellow or gold, smaller number of ridges between the vulva and anus, smaller mean for Granek's ratio, stylet knob shape, longer stylet length, tail terminus and presence of refractive bodies on hyaline part of tail (usually 4-7 refractive bodies vs absence) in J2.

Globodera tabacum (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959 – the tobacco cyst nematode

Globodera tabacum is considered as a serious and important pest of shade and broadleaf tobacco. It recorded from several countries in Europe, Asia, Africa, South and North America. *Globodera tabacum* is a polytypic species containing the following subspecies: *G. tabacum tabacum* (Lownsbery & Lownsbery, 1954); *G. tabacum virginiae* (Miller & Gray, 1968) and *G. tabacum solanacearum* (Miller & Gray, 1972). All three subspecies develop on tobacco and horsenettle, but otherwise differ in host preference. *Globodera tabacum* parasitizes *Nicotiana tabacum* L., Horsenettle, *Solanum carolinense* L., tomato and species of the genera: *Nicotiana*, *Solanum*, as well as *Atropa belladona* L., *Hyoscyamus niger* L., *Nicandra physalodes* (L.) Gaertn., *Capsicum annuum* L. Two or more generations usually occur. Infected tobacco plants have small root systems and above-ground symptoms are similar to those associated with severe root-knot and lesion nematode infestations. Nematode infection is often associated with increased damage from bacterial wilt, black shank. Farmers in Virginia, USA, have recorded complete crop failures, but losses generally average 15%. A high density of nematode populations early in the growing season can reduce flue-cured tobacco yield by 25-50%, although tobacco may escape significant losses from moderate populations, especially under favorable growing conditions.

Measurements

Cysts: L = 337-937 µm; W = 232-812 µm; L/W ratio = 0.9-1.5; fenestral diam. = 13-36 µm; number of ridges between anus and fenestra = 5-15; Granek's ratio = 1.4- 4.2.

J2: L = 458-621 µm; stylet = 20-27 µm; DGO = 4.3-9 µm; hyaline region = 17-35 µm; tail = 34-64 µm.

Description: *Female* body ovate to spherical with elongate neck, white, becoming yellow. *Cyst* light shiny brown, circumfenestrate, abullate. *J2* with well developed rounded basal knobs. Terminus of tail finely rounded.

Remarks: *Globodera tabacum* differs from *G. rostochiensis* by J2 with longer mean values of body length, mean stylet and by cysts with smaller mean number of cuticular ridges. It differs from *G. mexicana* sp. n. by J2 with longer mean body length and from *G. pallida* by cysts with a smaller mean number of cuticular ridges and by J2 with longer means of body length.

10.10.3. Genus *Betulodera* Sturhan, 2002
(Fig. 10.18)

Diagnosis (after Sturhan, 2002): *Cyst*: lemon-shaped, pear-shaped or spheroid with insignificant, obtuse, vulval cone. Cyst wall thick, with irregular network-like pattern, D-layer absent (no punctations in inner, deeper layers of cyst wall), subcrystalline layer heavily developed. Vulva terminal, surrounded by circumfenestration, vulval slit short (<10 µm), underbridge absent, denticles occasionally present, anus without fenestration. *Male*: body twisted, no cloacal tube, spicules with bifid distal tips, phasmid openings punctiform. *J2*: lateral field with three incisures, phasmid openings punctiform, without lens-like structure, labial region with three or four labial annuli and labial disc fused with submedial lips.

Type and only species: ***Betulodera betulae* (Hirschmann & Riggs, 1969) Sturhan, 2002.**

10.10.4. Genus *Cactodera* Krall & Krall, 1978

Diagnosis (after Sturhan, 2002): *Mature female* and *cyst* lemon-shaped to spherical, with posterior protuberance. Vulva terminal, vulval slit <30 µm, fenestra circumfenestrate (Fig. 10.4C). Anus without fenestration. Bullae and underbridge absent, vulval denticles usually present. Cuticle with D-layer. *J2* have lateral field

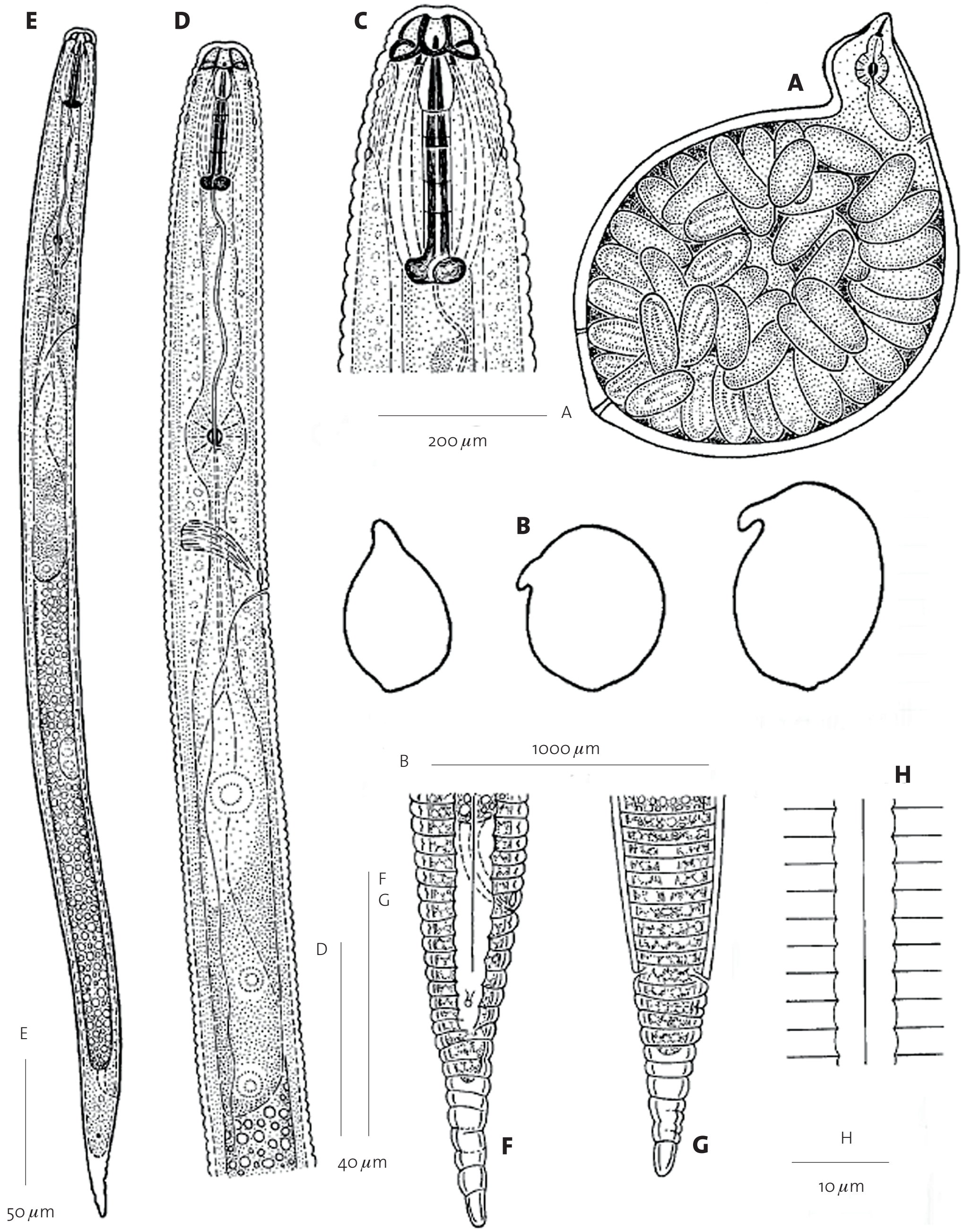

Fig. 10.18. *Betulodera betulae*. Females and cysts. A: Female filled with eggs (lateral); B: Shape and size ranges of cysts. J2. C: Labial region (lateral); E: Full-length outline (lateral); D: Pharyngeal region (lateral); F: Tails (lateral); G: Tails (ventral); H: Lateral field. After Hirschmann & Riggs (1969).

with four incisures, phasmid openings punctiform. Labial disc and six labial sectors present.

Type species: ***Cactodera cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978**

Total species number: 13

***Cactodera cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978 – the cactus cyst nematode**

A *cyst* nematode infecting cacti, *Discocactus akkermannii* (Lindl) Barthlott and *Cereus speciosus* K. Schumacher, both of which were expressing declining symptoms, was first recorded and described from Maartensdijk, near Utrecht, The Netherlands. The cactus cyst nematode is distributed worldwide, mainly on plants of the family Cactaceae grown in glasshouses as ornamentals. The dispersal of *C. cacti* from native regions in the Americas is beyond doubt associated with the international trade of infested ornamental cactus plants around the world. The cactus cyst nematode has been associated with, or infecting, plants belonging to three families: Cactaceae (Caryophyllales); Umbelliferae (order Apiales): and Euphorbiaceae (order Malpighiales) (Kirjanova & Krall, 1971; Mulk, 1977; Esser, 1992). Infected plants may exhibit various symptoms including branched roots and increased numbers of rootlets. Plants become reddish brown to yellow in color, wilted, stunted, with reduced flower production and shortening of the flowering period. With high infection, the host may die.

Measurements

Cysts: L = 328-780 μm; W = 240-598 μm; L/W ratio = 1.1-2.0; fenestral diam. = 16-48 μm.

J2: L = 344-584 μm; stylet = 21-26 μm; hyaline region = 12-21 μm; tail = 34-60 μm.

Description: *Female* Body lemon-shaped to almost spherical, pearly white, yellow, or golden, maturing to light brown. *Cyst* usually lemon-shaped, but may be rounded with protruding neck and vulva, light or medium brown, sometimes reddish brown. Vulval denticles generally present, visible beneath fenestral surface. Cone tops abullate, circumfenestrate. *J2* body vermiform, tapering anteriorly and posteriorly. Tail tapering, with hyaline region often shorter than stylet. Eggshells heavily punctuate as seen under optical microscope with oil immersion.

Remarks: *Cactodera cacti* resembles *C. weissi*, *C. acnidae*, *C. milleri* and *C. galinsogae*. It differs from *C. weissi* and *C. acnidae* in having eggshells heavily punctate *vs* shells without visible markings, J2 with larger stylet. It differs from J2 *C. galinsogae* by a longer tail and from *C. milleri* by cysts with a larger fenestral diam.

10.10.5. Genus *Dolichodera* Mulvey & Ebsary, 1980
(Fig. 10.19)

Diagnosis (after Siddiqi, 2000): *Female* body elongate-oval, without terminal protuberance, white, swollen part 400-500 μm long, 140-270 μm wide, 2-2.8 times as long as wide, neck moderately long. Cuticle not annulated but with fine irregular striae. Vulval area terminal or just subterminal, circumfenestrate, fenestra *ca* 20 μm in diam., bullae present, perineal tubercles absent. Anus pore-like, lacking a fenestra, located 10-13 μm dorsal to vulval fenestral margin. Cyst light brown, elongate oval. Several large bullae present. Perineal tubercles absent. Vulva circumfenestrate, underbridge absent. Anus lacking fenestra. Male not found. *J2* with long tail (95-120 μm). Lateral field with three incisures, inner one faint. Labial region hemispherical, offset, with two annuli. Tail tip narrowly rounded. Phasmid openings lacking a lens-like ampulla, located about one anal body diam. posterior to anus.

Type and only species: ***Dolichodera fluvialis* Mulvey & Ebsary, 1980**.

10.10.6. Genus *Paradolichodera* Sturhan,Wouts & Subbotin, 2007
(Fig. 10.20)

Diagnosis (after Sturhan *et al*., 2007): *Mature female* and *cyst* elongate-ovoid, with rounded posterior end. Cuticle transparent, with faint transverse striations on anterior part of body and posteriorly mostly with faint irregular ridges superimposed on distinct punctations. Cuticle turning yellowish to light brown on death, covered by a subcrystalline-like film. Eggs retained in body, egg-sac not observed. Labial disc squarish. Stylet well developed. Vulva terminal or subterminal, vulval slit short, circumfenestrate. Anus lacking fenestration. *Male* body not twisted, lateral field with four incisures. Cloacal tube present, spicules rounded at tip. Phasmids lacking. *J2* long, extremely slender for family, lateral fields indistinct. Stylet short (<20 μm). Dorsal gland orifice located more than half stylet length posterior to stylet base, pharyngeal glands long, filling body cavity. Tail long, slender, phasmid openings punctiform.

Type and only species: ***Paradolichodera tenuissima* Sturhan, Wouts & Subbotin, 2007**

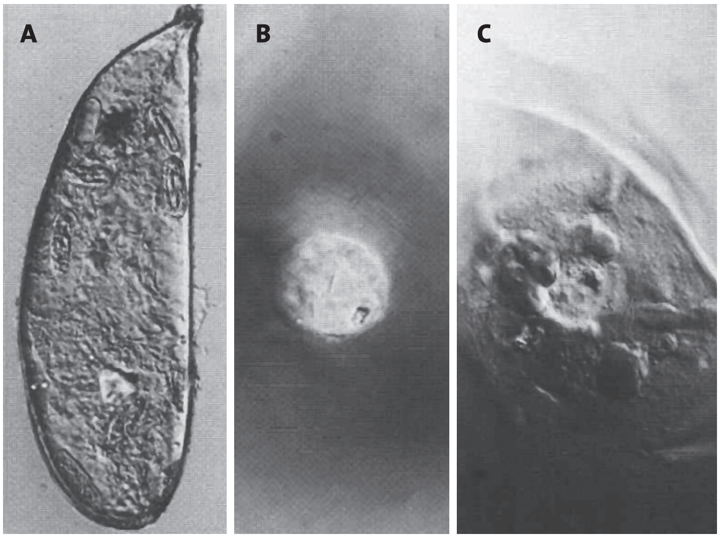

Fig. 10.19. *Dolichodera fluvialis*. Cyst. A: Whole cyst with slightly subterminal vulva fenestra; B: Fenestral area which has deteriorated to form a round aperture; C: Bullae in fenestral area. After Mulvey & Ebsary (1980).

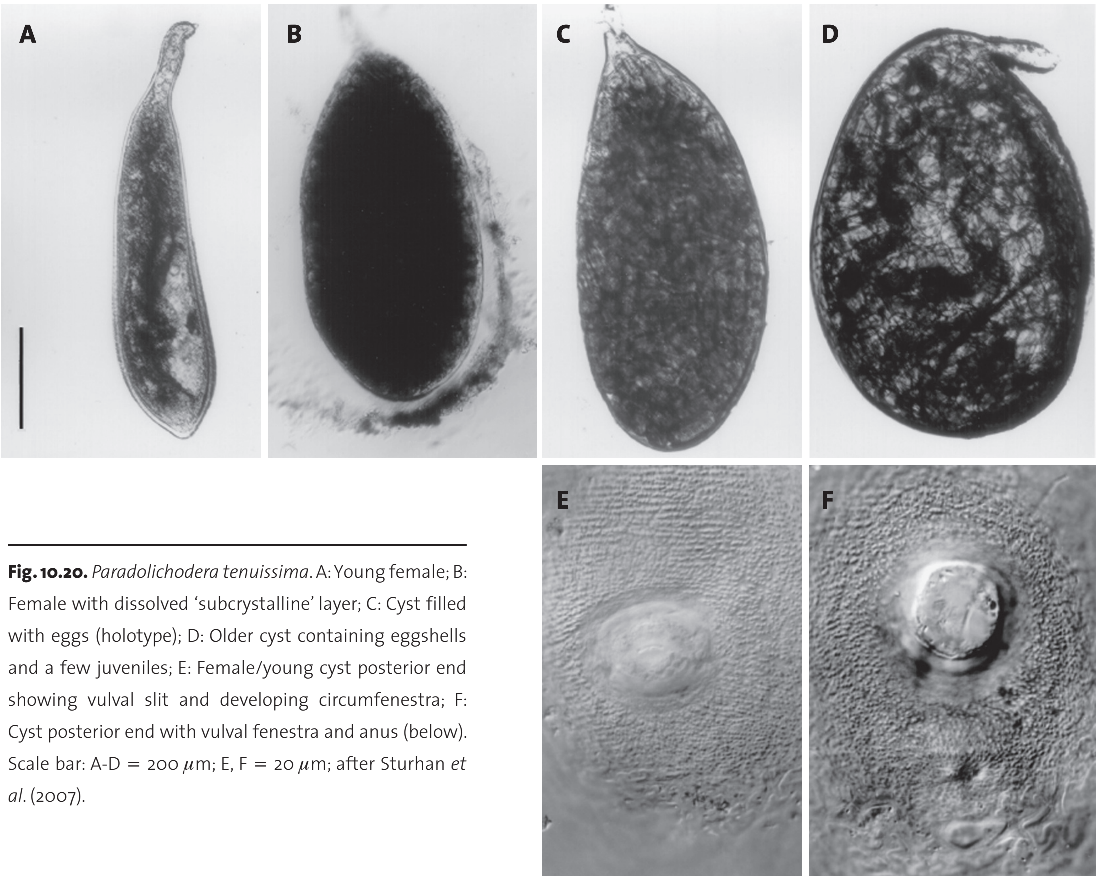

Fig. 10.20. *Paradolichodera tenuissima*. A: Young female; B: Female with dissolved 'subcrystalline' layer; C: Cyst filled with eggs (holotype); D: Older cyst containing eggshells and a few juveniles; E: Female/young cyst posterior end showing vulval slit and developing circumfenestra; F: Cyst posterior end with vulval fenestra and anus (below). Scale bar: A-D = 200 μm; E, F = 20 μm; after Sturhan *et al.* (2007).

10.10.7. Genus *Punctodera* Mulvey & Stone, 1976

Diagnosis (after Siddiqi, 2000): *Mature female* and *cyst*: Spherical, pear-shaped or ovoid, with short projecting neck and heavy subcrystalline layer. Cuticle reticulate, subcuticle with punctations. D-layer present. Terminal region not cone-shaped; cyst light to dark brown. Vulval slit extremely short ($<5\ \mu$m), anus at a short distance from vulval fenestra. Circumfenestrate (Fig. 10.4D), fenestra surrounding vulva 16-40 μm (*ca* 20 μm in type species) in diam., anus offset toward ventral margin of fenestra, an anal fenestra of similar shape and size to vulval fenestra present. Underbridge and perineal papilla-like tubercles absent. Bullae present or absent. Egg retained in body, no egg-sac. *Male*: Vermiform, less than 1.5 mm long. DGO 2.6-4.6 μm. Spicules 31-33 μm long, distally pointed. Tail less than 0.5 anal body diam. long, cloacal lips not forming a tube. *J2*: Body 0.35-0.49 mm, stylet 24-26 μm and tail conical, 63-78 μm long, hyaline region in type species 38-41 μm long. Lateral field with four incisures. Pharyngeal glands filling body diam. Phasmid openings punctiform, without a lens-like structure. Parasites of monocotyledonous plants.

Type species: ***Punctodera punctata* (Thorne, 1928) Mulvey & Stone, 1976**

Total number of species: 4

***Punctodera chalcoensis* Stone,**
Sosa Moss & Mulvey, 1976 – the Mexican corn cyst nematode
(Fig. 10.21)

The Mexican corn cyst nematode was first observed in the late 1950s in maize fields in Huamantla, Tlaxcala, Mexico, and was originally identified as a Mexican race of *Heterodera punctata* (Baldwin & Mundo-Ocampo, 1991). Further detailed morphological and morphometric observations revealed differences between these populations and *P. punctata* and *P. matadorensis* and led to the description of a new species, *P. chalcoensis*, by Stone *et al*. (1976). It is highly damaging to maize in Mexico, causing severe yellowing, stunting and even death of young seedlings (Baldwin & Mundo-Ocampo, 1991). Heavily attacked plants have stunted root systems with many short laterals, giving a bottle-brush effect, and the aerial parts of the plant appear unthrifty. Pathogenicity studies of *P. chalcoensis* on maize in the glasshouse showed an inverse correlation between root and top weight of mature plants and the number of nematode cysts in the inoculum.

Measurements

Cysts: L = 441 μm; W = 416 μm; L/W ratio = 1.1; fenestral diam. = 18.1 -20 μm.

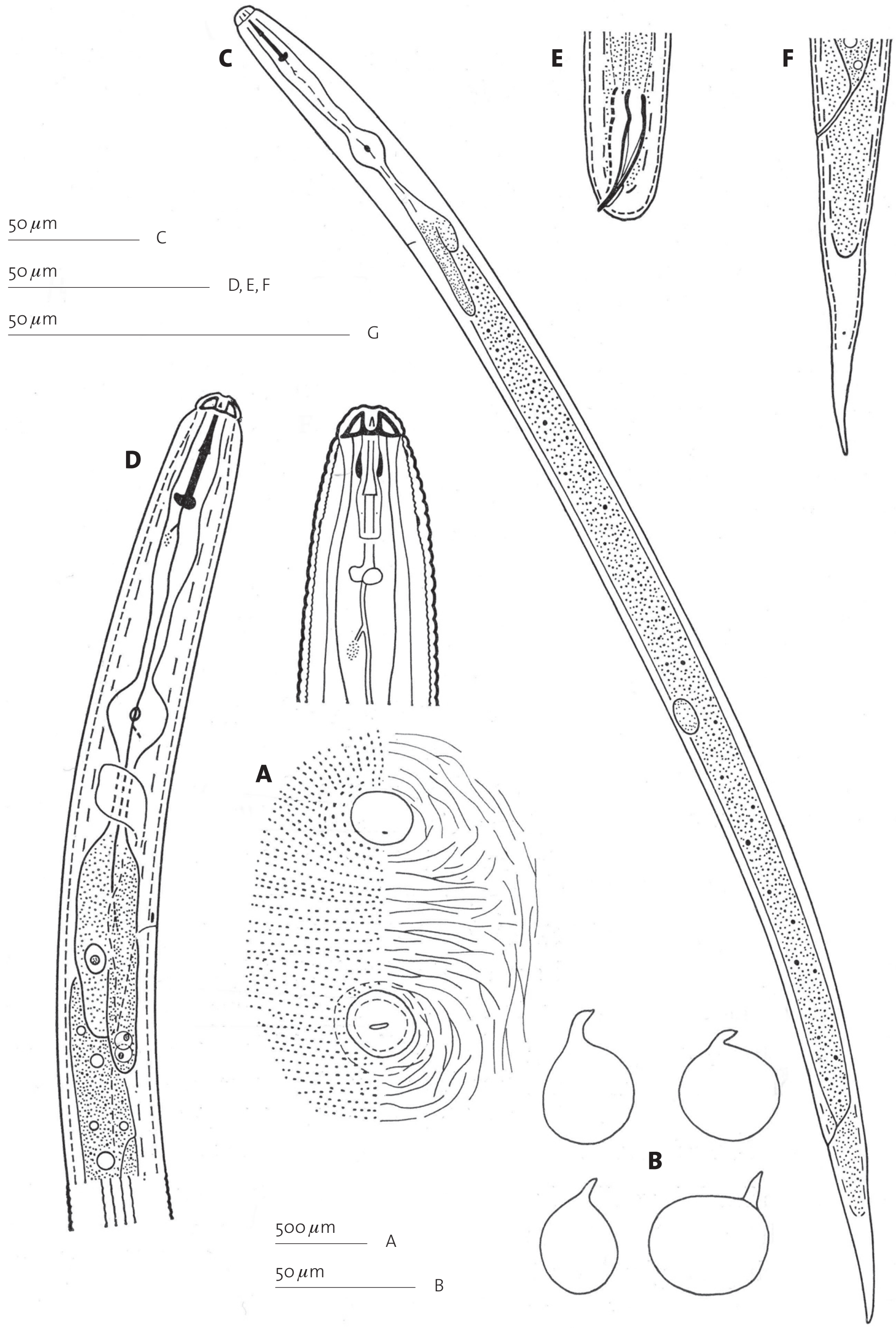

Fig. 10.21. *Punctodera chalcoensis*. A: Perineum of female; B: Females; C: J2; D: Pharyngeal region of J2; F: Anterior region of J2; E: Male tail; F: Tail of J2. After Stone *et al*. (1976).

J2: L = 522 μm; stylet = 24.4 μm; hyaline region = 38.6 μm; tail = 66.2 μm.

Description: *Cyst* spherical or subspherical with protruding neck, pale to dark brown, darkening with age. New cysts often retaining subcrystalline layer. Old cysts losing thin walls of vulval and anal fenestrae, younger cysts showing incomplete fenestration. Some specimens with small scattered bullae in perineal region or just anterior to vulval fenestra, but bullae may be lacking from many cysts. *J2* vermiform body adopting a slight ventral curve on heat relaxation. Well developed stylet with massive basal knobs, knobs rounded posteriorly, flat to shallowly concave anteriorly.

Remarks: *Punctodera chalcoensis* most resembles *P. matadorensis* and *P. stonei*. It differs from *P. matadorensis* by the shape of the J2 stylet knobs, which are anteriorly flat to slightly concave in *P. chalcoensis vs* strongly concave. They also differ in the distribution of bullae in the cyst perineum, these being small scattered or absent in *P. chalcoensis vs* massive and always present in *P. matadorensis*. *Punctodera chalcoensis* differs from *P. stonei* by the shorter hyaline region of J2 and by its distinctive host range.

10.10.8. Genus *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010

Diagnosis (after Bernard *et al*, 2010 with modifications): *Cyst*: orange-brown to brown, lemon-shaped with short necks and vulval cone. vulval aperture circular to rhomboid, circumfenestrate, with irregular denticle-like protuberances around the periphery of orifice. Bullae, vulval bridge and vulval underbridge absent. *Male*: variable length, stylet knobs rounded. *J2* having conoid tail with narrowly rounded tip, phasmid apertures pore-like. Stylet length less than 18 μm. Stylet knobs rounded. Lateral field with four incisures. Eggshell smooth.

Type and only species: ***Vittatidera zeaphila* Bernard, Handoo, Powers, Donald & Heinz, 2010.**

***Vittatidera zeaphila* Bernard, Handoo, Powers, Donald & Heinz, 2010**
(Fig. 10.22)

In 2006, the Missouri Extension Nematology Laboratory discovered a new species of corn cyst nematode from a sample collected in a corn field of northwestern Tennessee, Obion County, USA. This nematode was considered as a representative of a new genus of the subfamily Heteroderinae. It resembles representatives of the genera *Cactodera* and *Betulodera* in having lemon shaped cysts and circumfenestrate vulval area. Similar cyst specimens had previously been collected in 1978 from

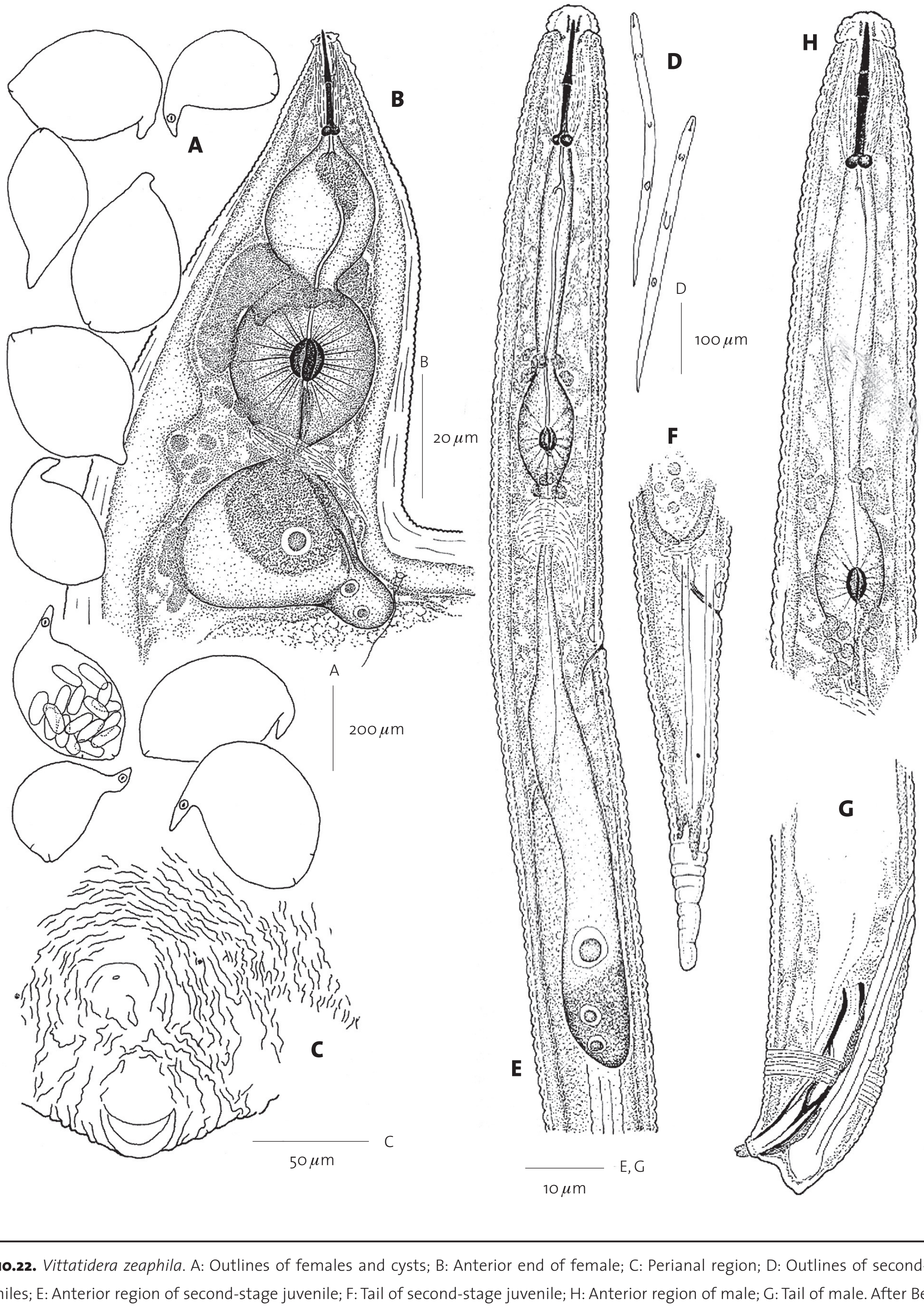

Fig. 10.22. *Vittatidera zeaphila*. A: Outlines of females and cysts; B: Anterior end of female; C: Perianal region; D: Outlines of second-stage juveniles; E: Anterior region of second-stage juvenile; F: Tail of second-stage juvenile; H: Anterior region of male; G: Tail of male. After Bernard *et al*. (2010) with modification.

Lauderdale County, Tennessee, on goosegrass, *Eleusine indica*. *Vittatidera zeaphila* is presently found only in the USA. (Bernard *et al*., 2010).

Measurements

Cysts: L = 470 (385-608) μm; W = 287 (179-391) μm; L/W = 1.7 (1.4-2.2); fenestral diam. = 36 (28-48) μm; anus - fenestral distance = 63 (52-74) μm.

J2: L = 365 (346-400) μm; a = 24.0 (22.3-26.9); b = 2.5 (2.4-2.7); stylet = 16.7 (15.7-17.5) μm; hyaline region = 15.3 (12.4-17.8) μm; tail = 41.5 (33.0-48.0) μm.

Description: *Cyst* lemon-shaped, vulval cone not protuberant, large egg masses persistent. In mature cysts vulval aperture circumfenestrate, circular to rhomboidal, membranous lips persistent on mature cysts, underbridge and bullae absent. *J2* stylet small with rounded knobs. Lip region rounded, slightly offset, with three annuli. Lateral field with four incisures. Tail elongate-conoid, tip narrowly rounded.

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