15 Taxonomy, Identification and Principal Species

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15.1 Introduction

15.1.1 History

The first cyst-forming nematode was discovered on sugar beets by Schacht (1859) and was later described by Schmidt (1871), who established the first new genus of cyst nematode, *Heterodera*, and named this nematode as *Heterodera schachtii* in honour of Hermann Schacht. Presently, the genus *Heterodera* includes species with cyst bodies more or less lemon-shaped and ambifenestrate, bifenestrate vulval fenestration or without fenestration. To accommodate the potato cyst nematodes and related species having round cysts, Skarbilovich (1959) erected the subgenus *Globodera*, which later was elevated to generic status by Behrens (1975). Around the same time, Mulvey and Stone (1976) proposed recognizing *Globodera* at generic level. These authors also described the genus *Punctodera* for species having extensive anal fenestration. Krall and Krall (1978) erected the genus *Cactodera* for species with cysts that have a posterior protuberance and circumfenestrate vulva region. Sturhan (2002) established a new genus, *Betulodera*, to accommodate *Cactodera betulae* based on peculiarities of its host range and cyst morphology. Later three genera, *Dolichodera*, *Paradolichodera* and *Vittatidera*, were described with morphological peculiarities; each of these genera presently contains only single species. The introduction of cyst nematodes and detailed historical background of various cyst-forming nematode genera is given in several books and chapters (Franklin, 1951; Baldwin and Mundo-Ocampo, 1991; Ferris, 1998; Wouts and Baldwin, 1998; Siddiqi, 2000; Baldwin and Perry, 2004; Subbotin *et al.*, 2010; Subbotin and Franco, 2012; Turner and Subbotin, 2013).

The cyst-forming nematode species have been instrumental in stimulating growth of nematology worldwide. Presently this nematode group contains eight genera: *Heterodera* (85 species), *Globodera* (14 species), *Cactodera* (14 species), *Dolichodera* (1 species), *Paradolichodera* (1 species), *Betulodera* (1 species), *Punctodera* (4 species) and *Vittatidera* (1 species), with a total number of 121 valid species.

15.1.2 Major reference sources

Cyst nematode taxonomy and specific diagnostic variability were studied by a number of authors, with the earlier monographs and major reference sources discussed in great detail in several book chapters and articles by Mulvey (1959, 1972, 1973), Franklin (1972), Stone (1973a, b, 1975, 1979), Stone and Rowe (1976), Mulvey and Ebsary (1980), Stone and Hill (1982), Mulvey and Golden (1983), Vovlas (1985), Vovlas *et al.* (1985), Golden (1986), Baldwin and Mundo-Ocampo (1991), Wouts and Baldwin (1998), Zunke and Eisenback (1998), Siddiqi (2000), Sturhan (2002, 2010) and Sturhan *et al.* (2007). The importance of the cone mounts in morphology and identification of cyst nematode was first stressed by Oostenbrink and den Ouden (1954), followed by Cooper (1955), Mulvey (1957, 1960, 1972) and Green (1971). In the book chapter on morphology and identification of cyst nematodes, Golden (1986) gives detailed information on cyst wall pattern, cyst posterior, vulval cone and terminal region, together with other characters useful for identification of males and second-stage juveniles. Other major sources include the two-volume treatise written by Subbotin *et al.* (2010). The latest chapters by Subbotin and Franco (2012) and Turner and Subbotin (2013) contain a brief introduction to the cyst-forming nematode groups, together with information on life cycle and behaviour and general morphology of the subfamily Heteroderidae, including diagnosis and descriptions of the principal species.

15.2 Identification

Morphology is the essential basis for identification of cyst nematodes (Fig. 15.1). Molecular approaches in taxonomy are more widely used now and will be used in future to supplement and extend our morphological knowledge, and will no doubt provide new understanding of the identity and systematics of various populations and species. Accurate and rapid identification of cyst and other nematodes is crucial for the implementation of effective control measures. In addition, sound decisions regarding quarantine of imported and exported plant material and commodities also demand timely and accurate diagnostics. However, the identification of cyst nematodes to species level is fraught with difficulty. In cyst nematodes, conserved morphology, variable morphometrics, host effects, intraspecific variation and existence of cryptic species complicate the situation, and the ever-increasing number of described species, of which the diagnosis and relationships of many vary from less than ideal to doubtful (as is also the case with root-knot nematodes) causes problems.

Verification of mixed populations and/or detection of rare species requires identification techniques, including morphological (cone mounts of posterior end of cysts; male, female and second-stage juvenile (J2) labial region shape, and stylet morphology; length and shape of J2 tail) and biochemical or molecular methodologies. Detailed diagnostic characters differentiating various genera of cyst-forming nematode species have been given by authors such as Mulvey and Golden (1983), Golden (1986), Baldwin and Mundo-Ocampo (1991), Ferris (1998), Wouts and Baldwin (1998), Zunke and Eisenback (1998), Siddiqi (2000), Subbotin *et al.*

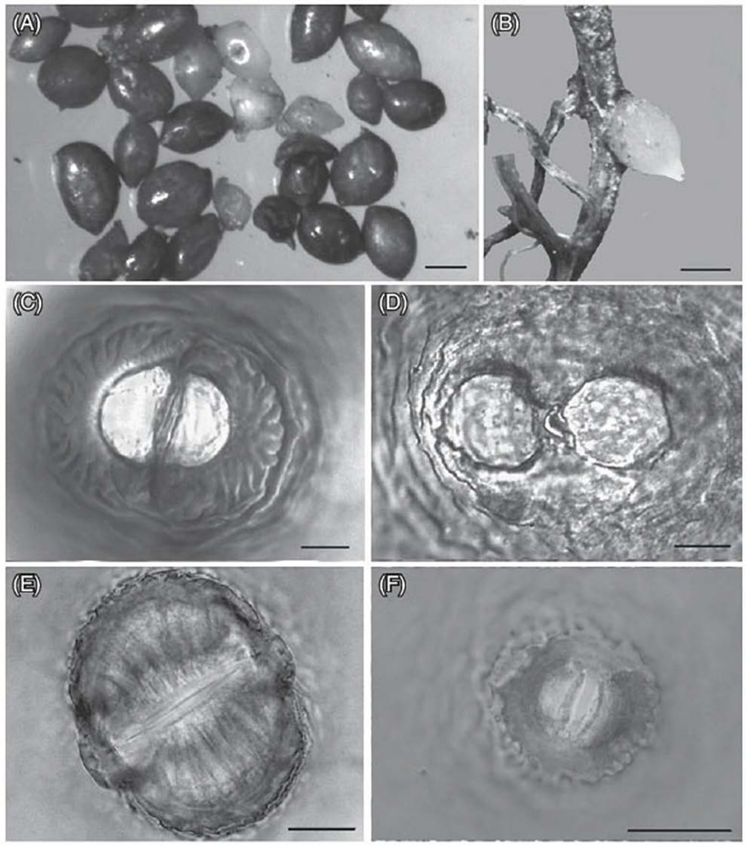

Fig. 15.1. (A) Cysts of *Heterodera cruciferae*. (B) White female of *H. cruciferae* on a root. Vulval plates. (C) *H. medicaginis* (ambifenestrate). (D) *Heterodera* sp. (bifenestrate). (E) *H. orientalis* (no fenestration). (F) *Cactodera rosae* (circumfenestrate). A–D, Courtesy of V.N. Chizhov; E, after Mundo-Ocampo *et al.*, 2008; F, courtesy of I. Cid del Prado Vera. Scale bars: A, B = 200 μm; C = 15 μm; D, E, F = 25 μm.

(2000, 2010), Handoo (2002), Skantar *et al.* (2007, 2011), Nakhla *et al.* (2010), Subbotin and Franco (2012) and Turner and Subbotin (2013). They are also detailed in in Chapter 14, this volume, under 'Conclusions and future prospects'.

15.2.1 General techniques

For morphological observation, J2 and males can be recovered from fresh infected roots or cysts/eggs incubated in Petri dishes with a small amount of water. They may also be recovered from soil by sieving and Baermann funnel techniques. Cysts and white females are dissected from infected roots after fixation overnight in 3% formaldehyde. Procedures for measuring and preparing specimens are given in Golden and Birchfield (1972). Females have the anterior and posterior ends cut with a sharp knife, cleaned with a dental root canal file and mounted permanently on a glass slide in a drop of lactophenol solution (see Chapter 14, this volume). Photomicrographs of cone mounts, J2 and males can be done with a digital camera attached to a dissecting microscope.

For more details on killing, fixing, processing nematodes to glycerin, preparing temporary and permanent slide mounts and preserving nematode structures in a life-like manner, the reader is referred to Whitehead (1968), Hooper (1970, 1986, 1990), Golden (1990) and Carta (1991).

15.2.2 Cone mounts

The character most frequently used for cyst species identification is the morphology of the cone, which is located in the posterior body region of the cyst. To investigate fully the posterior part of the cyst (perinea), it must first be mounted on a slide. Oostenbrink and den Ouden (1954) were the first to use cone top structures, including fenestra, bullae and underbridge, in the separation of cyst-forming nematodes. Cooper (1955) provided detailed information and later Mulvey (1957, 1960), Fenwick (1959) and Hesling (1965) added more information. The identification of *Heterodera* cysts by terminal and cone top structures was discussed by Mulvey (1972) in a detailed examination of 39 of the 53 species of *Heterodera* described at that time to provide adequate basis for identification of these species; brief information on making vulval cones of cyst nematodes is given by Riggs (1990). A protocol for preparation of the vulval cones of cyst nematodes plus figures showing techniques for preparing cyst cones for light microscope observation, are given in Appendix II of Subbotin *et al.* (2010). A more detailed account on cone mounts is covered in Chapter 14, this volume.

15.2.3 Root staining

Many methods have been developed for staining and clearing nematode-infected root tissues. Staining with acid fuchsin-lactophenol or lacto-glycerol are the most widely used methods. In addition, a method that utilizes chlorine bleach as a prestaining treatment has proved very reliable and is relatively simple to use (Byrd *et al.*, 1983). For more detail, see McBeth *et al.* (1941), Hooper (1986, 1990) and Hussey (1990).

15.2.4 Scanning electron microscopy

For scanning electron microscopy (SEM), living specimens are fixed in 3% glutaraldehyde solution buffered with 0.05 M phosphate (pH 6.8), dehydrated in a graded series of ethanol, critical point dried from liquid CO_2 and sputter-coated with a 20–30-nm layer of gold-palladium. For more detail, the papers by Eisenback (1991) and Charchar and Eisenback (2000), and Chapter 14, this volume, are recommended.

15.2.5 Diagnostic characters

The most important diagnostic features used for identification of cyst nematodes include: **Cyst**: Size, shape and colour, nature of cone (ambifenestrate, bifenestrate, circumfenestrate or no fenestration; Fig. 15.1C–F). Fenestra length and width, presence or absence of bullae, underbridge, vulval slit length, nature of cyst wall pattern, Granek's ratio (distance from the anus to the nearest edge of the fenestra divided by the length of the fenestra). **Female**: Shape of body, labial region, stylet length, shape of stylet cone, shaft and basal knobs, and excretory pore/stylet length ratios (EP/ST). **Male**: Size, height and shape of labial cap, the number of annulations, diameter of the labial region as compared to the first body annule, stylet length, form of stylet cone, shaft and basal knobs, distance of the dorsal gland orifice (DGO) from the stylet base and length and form of spicule and gubernaculum. **J2**: Body and stylet length, form of labial region and shape of stylet knobs, location of the hemizonid in relation to the excretory pore, distance of DGO from stylet base, number of incisures in the lateral field and shape and length of the tail and hyaline terminus. For more details about these and other differentiating characters, see Baldwin and Mundo-Ocampo (1991), Golden (1986), Mulvey and Golden (1983), Golden (1986), Subbotin *et al.* (2010), Turner and Subbotin (2013) and Chapters 14 and 16, this volume.

15.3 Systematic Position

Because of certain similarities in morphology and biology, root-knot nematodes and cyst-forming nematodes have often been thought to be closely related. As a consequence, in many old systematic schemes both groups were often placed in a single family or subfamily, the Heteroderidae or Heteroderinae, respectively, closely related to the hoplolaimids. However, a growing suspicion indicated that the two groups had evolved separately and had achieved their similarities via the process of convergent evolution. According to this view, the cyst and root-knot nematodes each justify their own family or subfamilies, but the root-knot nematodes are closer to the pratylenchids than to the hoplolaimids (Hunt and Handoo, 2009). Systematics of the family Heteroderidae and its genera are given in great detail by Subbotin *et al.* (2010) as well as the classification scheme followed after Siddiqi (2000) presented here in Table 15.1. The taxonomic history and problems of Heteroderinae are discussed by Hesling (1965), Krall and Krall (1978), Golden, (1986), Baldwin and Mundo-Ocampo (1991) and Siddiqi (2000).

The advances in molecular methodologies have facilitated a better understanding of the phylogeny of nematodes. We have proposed here a new improved classification for the family Heteroderidae on the basis of up-to-date information collected on the classification of cyst nematodes (Table 15.1) (Subbotin *et al.*, 2017). Regrouping of some genera under these subfamilies has been undertaken, wherever it was felt necessary and these genera have been shifted to their appropriate subfamiles. These additions seemed desirable and necessary to provide more useful groupings. The changes are incorporated to make the classification easier to handle. Classification of the family Heteroderidae by other authors, including our current proposed one, is given in Table 15.1. Cyst-forming nematodes are placed in two subfamilies: Heteroderinae, Filipjev & Schuurmans Stekhoven, 1941, and Punctoderinae, Krall & Krall, 1978.

15.4 Subfamily Heteroderinae Filipjev & Schuurman Stekhoven, 1941

Diagnosis: Heteroderidae: Filipjev & Schuurmans Stekhoven, 1941. **Mature female and cyst**: body more or less lemon-shaped, with a posterior cone. Female cuticle not annulated, changing colour after death. 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 eggsac also present. **Male**: developed through metamorphosis, labial region annulated, lateral field with four or three incisures, tail short, hemispherical, without bursa. **J2**: lateral field marked by three or four incisures, phasmids punctiform or lens-like. **Type and only genus**: *Heterodera* Schmidt, 1871

15.5 Genus *Heterodera* Schmidt, 1871

Diagnosis: (Heteroderinae) is the same as for the subfamily.

Genus: *Heterodera* Schmidt, 1871
= *Tylenchus* (*Heterodera* Schmidt, 1871)
= *Heterodera* (*Heterodera* Schmidt, 1871)
= *Heterobolbus* Railliet, 1896
= *Bidera* Krall & Krall, 1978
= *Ephippiodera* Shagalina & Krall, 1981
= *Afenestrata* Baldwin & Bell, 1985
= *Afrodera* Wouts, 1985
= *Brevicephalodera* Kaushal & Swarup, 1989

Molecular and morphological data support the division of most *Heterodera* species into several groups: *Afenestrata*, *Avenae*, *Bifenestra*, *Cardiolata*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii* (Table 15.2). Key features for study include the formation of the fenestra. These are classified as without fenestration (*Afenestrata* group), ambifenestrate (two openings divided by a narrow vulval bridge) or bifenestrate (two openings separated by a much wider vulval bridge) (Fig. 15.1C–D). The length of the vulval slit varies. In the *Avenae* group it is very short at 8–10 μm, whereas members of the *Schachtii* group have a much longer slit averaging 45 μm in length.

15.5.1 List of species and synonyms

Type species:

1. Heterodera schachtii A. Schmidt, 1871

= *Tylenchus schachtii* (A. Schmidt) Örley, 1880
= *Heterodera schachtii minor* O. Schmidt, 1930

Table 15.1. Classification of the family Heteroderidae Filipjev & Schuurman Stekhoven, 1941.

Krall and Krall (1978)	Wouts (1985)	Siddiqi (2000)	Handoo and Subbotin (this chapter)
Heteroderinae Filipjev & Schuurman Stekhoven, 1941	Heteroderinae Filipjev & Schuurman Stekhoven, 1941	Heteroderinae Filipjev & Schuurman Stekhoven, 1941	Heteroderinae Filipjev & Schuurman Stekhoven, 1941
Bidera Krall & Krall, 1978 *Cactodera* Krall & Krall, 1978 *Globodera* Skarbolovich, 1959 *Heterodera* Schmidt, 1871	*Afrodera* Wouts, 1985 *Bidera* Krall & Krall, 1978 *Heterodera* Schmidt, 1871 *Hylonema* Luc, Taylor & Cadet, 1978	*Afenestrata* Baldwin & Bell, 1985 *Cactodera* Krall & Krall, 1978 *Dolichodera* Mulvey & Ebsary, 1980 *Globodera* Skarbolovich, 1959 *Heterodera* Schmidt, 1871 *Punctodera* Mulvey & Stone, 1976	*Heterodera* Schmidt, 1871
Punctoderinae Krall & Krall, 1978	Punctoderinae Krall & Krall, 1978		Punctoderinae Krall & Krall, 1978
Punctodera Mulvey & Stone, 1976	*Cactodera* Krall & Krall, 1978 *Dolichodera* Mulvey & Ebsary, 1980 *Globodera* Skarbolovich, 1959 *Punctodera* Mulvey & Stone, 1976		*Betulodera* Sturhan, 2002 *Cactodera* Krall & Krall, 1978 *Dolichodera* Mulvey & Ebsary, 1980 *Globodera* Skarbolovich, 1959 *Paradolichodera* Sturhan, Wouts & Subbotin, 2007 *Punctodera* Mulvey & Stone, 1976 *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010
    Sarisoderinae Krall & Krall, 1978	Meloidoderinae Golden, 1971 Cryphoderinae Cooman, 1978 Ataloderinae Wouts, 1973 Verutinae Esser, 1981	Meloidoderinae Golden, 1971 Ataloderinae Wouts, 1973	Meloidoderinae Golden, 1971 Ataloderinae Wouts, 1973 Verutinae Esser, 1981
Sarisodera Wouts & Sher, 1971			

Table 15.2. Morphological characterizations of *Heterodera* groups.

Stage and Characters	Cyst					J2	
Group	Shape	Fenestration	Bullae	Underbridge	Vulval slit	Lateral field	Host
Afenestrata	Lemon or rounded	Absent	Absent	Absent or weak	Long	3 or 4	Monocotyledons
Avenae	Lemon	Bifenestrate	Well developed	Absent or present	Short	4	Monocotyledons
Bifenestra	Lemon	Bifenestrate	Absent	Absent	Short	3	Monocotyledons
Cardiolata	Lemon	Ambifenestrate	Absent	Present	Long	3	Monocotyledons
Cyperi	Lemon or rounded	Ambifenestrate	Absent or present	Absent or present	Long	3 or 4	Monocotyledons
Goettingiana	Lemon	Ambifenestrate	Absent or present	Weak	Long	4	Dicotyledons
Humuli	Lemon	Bifenestrate (ambifenestrate for *H. fici*)	Absent or present	Weak	Long	4	Dicotyledons
Sacchari	Lemon or rounded	Ambifenestrate	Finger-like	Strong	Long	3	Monocotyledons
Schachtii	Lemon	Ambifenestrate	Well developed	Strong	Long	4	Dicotyledons

Other species:

2. *Heterodera africana* (Luc, Germani & Netscher, 1973) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008

= *Sarisodera africana* Luc, Germani & Netscher, 1973
= *Afenestrata africana* (Luc, Germani & Netscher, 1973) Baldwin & Bell, 1985
= *Afrodera africana* (Luc, Germani & Netscher, 1973) Wouts, 1985

3. *H. agrostis* Kazachenko, 1993
4. *H. amygdali* Kirjanova & Ivanova, 1975
5. *H. arenaria* Cooper, 1955

= *Bidera arenaria* (Cooper, 1955) Krall & Krall, 1978

6. *H. aucklandica* Wouts & Sturhan, 1995
7. *H. australis* Subbotin, Rumpenhorst, Sturhan & Moens, 2002
8. *H. avenae* Wollenweber, 1924

= *Heterodera schachtii* var. *avenae* Wollenweber, 1924
= *Bidera avenae* (Wollenweber, 1924) Krall & Krall, 1978
= *Heterodera schachtii major* O. Schmidt, 1930
= *Heterodera major* O. Schmidt, 1930

9. *H. axonopi* (Souza, 1996) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008

= *Afenestrata axonopi* Souza, 1996

10. *Heterodera bamboosi* (Kaushal & Swarup, 1988) Wouts & Baldwin, 1998

= *Brevicephalodera bamboosi* Kaushal & Swarup, 1988
= *Afenestrata bamboosi* (Kaushal & Swarup, 1989) Siddiqi, 2000

11. *H. bergeniae* Maqbool & Shahina, 1988
12. *H. betae* Wouts, Rumpenhorst & Sturhan, 2001
13. *H. bifenestra* Cooper, 1955

= *Bidera bifenestra* (Cooper, 1955) Krall & Krall, 1978
= *Heterodera longicaudata* Seidel, 1972
= *Bidera longicaudata* (Seidel, 1972) Krall & Krall, 1978

14. *H. cajani* Koshy, 1967

= *Heterodera vigni* Edward & Misra, 1968

15. *H. canadensis* Mulvey, 1979
16. *H. cardiolata* Kirjanova & Ivanova, 1969

= *Heterodera cynodontis* Shahina & Maqbool, 1989

17. *H. carotae* Jones, 1950a
18. *H. ciceri* Vovlas, Greco & Di Vito, 1985
19. *H. circeae* Subbotin & Sturhan, 2004
20. *H. cruciferae* Franklin, 1945
21. *H. cyperi* Golden, Rau & Cobb, 1962
22. *H. daverti* Wouts & Sturhan, 1978
23. *H. delvii* Jairajpuri, Khan, Setty & Govindu, 1979
24. *H. elachista* Ohshima, 1974
25. *H. fengi* Wang, Zhuo, Ye, Zhang, Peng & Liao, 2013
26. *H. fici* Kirjanova, 1954
27. *H. filipjevi* (Madzhidov, 1981) Stelter, 1984

= *Bidera filipjevi* Madzhidov, 1981

28. *H. galeopsidis* Goffart, 1936

= *Heterodera schachtii galeopsidis* Goffart, 1936

29. *H. gambiensis* Merny & Netscher, 1976
30. *H. glycines* Ichinohe, 1952
31. *H. glycyrrhizae* Narbaev, 1987
32. *H. goettingiana* Liebscher, 1892
33. *H. goldeni* Handoo & Ibrahim, 2002
34. *H. graminis* Stynes, 1971
35. *H. graminophila* Golden & Birchfield, 1972
36. *H. guangdongensis* Zhuo, Wang, Zhang & Liao, 2014
37. *H. hainanensis* Zhuo, Wang, Ye, Peng & Liao, 2013
38. *H. hordecalis* Andersson, 1975

= *Bidera hordecalis* (Andersson, 1975) Krall & Krall, 1978

39. *H. humuli* Filipjev, 1934
40. *H. johanseni* (Sharma, Kaushal, Singh, Pande, Pokharel & Upreti, 2001) Sturhan, 2002

= *Cactodera johanseni* Sharma, Kaushal, Singh, Pande, Pokharel & Upreti, 2001

41. *H. kirjanovae* Narbaev, 1988
42. *H. koreana* (Vovlas, Lamberti & Choo, 1992) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008

= *Afenestrata koreana* Vovlas, Lamberti & Choo, 1992

43. *H. latipons* Franklin, 1969

= *Bidera latipons* (Franklin, 1969) Krall & Krall, 1978
= *Ephippiodera latipons* (Franklin, 1969) Shagalina & Krall, 1981

44. *H. lespedezae* Golden & Cobb, 1963
45. *H. leuceilyma* Di Edwardo & Perry, 1964
46. *H. litoralis* Wouts & Sturhan, 1996
47. *H. longicolla* Golden & Dickerson, 1973
48. *H. mani* Mathews, 1971

= *Bidera mani* (Mathews, 1971) Krall & Krall, 1978

49. *H. medicaginis* Kirjanova in Kirjanova & Krall, 1971
50. *H. mediterranea* Vovlas, Inserra & Stone, 1981
51. *H. ment hae* Kirjanova & Narbaev,1977
52. *H. mothi* Khan & Husain, 1965
53. *H. orientalis* (Kazachenko, 1989) Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008

= *Afenestrata orientalis* Kazachenko, 1989

54. *H. oryzae* Luc & Brizuela, 1961
55. *H. oryzicola* Rao & Jayaprakas, 1978
56. *H. pakistanensis* Maqbool & Shahina, 1986
57. *H. persica* Tanha Maafi, Sturhan, Subbotin & Moens, 2006
58. *H. phragmitidis* Kazachenko, 1986
59. *H. plantaginis* Narbaev & Sidikov, 1987
60. *H. pratensis* Gäbler, Sturhan, Subbotin & Rumpenhorst, 2000
61. *H. raskii* Basnet & Jayaprakash, 1984
62. *H. ripae* Subbotin, Sturhan, Rumpenhorst & Moens, 2003

= *Heterodera riparia* Subbotin, Sturhan, Waeyenberge & Moens, 1997 [= junior homonym] *nec* Kazachenko, 1993

63. *H. riparia* (Kazachenko, 1993) Subbotin, Sturhan, Rumpenhorst & Moens, 2003

= *Bidera riparia* Kazachenko, 1993

64. *H. rosii* Duggan & Brennan, 1966
65. *H. sacchari* Luc & Merny, 1963
66. *H. saccharophila* Mundo-Ocampo, Troccoli, Subbotin, Del Cid, Baldwin & Inserra, 2008

= *Afenestrata sacchari* Kaushal & Swarup, 1988

67. *H. salixophila* Kirjanova, 1969
68. *H. scutellariae* Subbotin & Sturhan, 2004
69. *H. sinensis* Chen & Zheng, 1994
70. *H. skohensis* Kaushal, Sharma & Singh, 2000
71. *H. sojae* Kang, Eun, Ha, Kim, Park & Choi, 2016
72. *H. sonchophila* Kirjanova, Krall & Krall, 1976
73. *H. sorghi* Jain, Sethi, Swarup & Srivastava, 1982
74. *H. spinicauda* Wouts, Shoemaker, Sturhan & Burrows, 1995
75. *H. spiraeae* Kazachenko, 1993
76. *H. sturhani* Subbotin, 2015
77. *H. swarupi* Sharma, Siddiqi, Rahaman, Ali & Ansari, 1998
78. *H. trifolii* Goffart, 1932

= *Heterodera schachtii* var. *trifolii* Goffart, 1932
= *Heterodera scleranthii* Kaktina, 1957
= *Heterodera rumicis* Poghossian, 1961
= *Heterodera paratrifolii* Kirjanova, 1963

79. *H. turangae* Narbaev, 1988
80. *H. turcomanica* Kirjanova & Shagalina, 1965

= *Bidera turcomanica* (Kirjanova & Shagalina, 1965) Krall & Krall, 1978
= *Ephippiodera turcomanica* (Kirjanova & Shagalina, 1965) Shagalina & Krall, 1981

81. *H. urticae* Cooper, 1955
82. *H. ustinovi* Kirjanova, 1969

= *Bidera ustinovi* (Kirjanova, 1969) Krall & Krall, 1978
= *Heterodera iri* Mathews, 1971

83. *H. uzbekistanica* Narbaev, 1980
84. *H. vallicola* Eroshenko, Subbotin & Kazachenko, 2001
85. *H. zeae* Koshy, Swarup & Sethi, 1971

Species inquirendae:
H. aquatica Kirjanova, 1971
H. chaubattia Gupta & Edward, 1973
H. graduni Kirjanova *in* Kirjanova & Krall, 1971
H. limonii Cooper, 1955
H. methwoldensis Cooper, 1955
H. oxiana Kirjanova, 1962
H. polygoni Cooper, 1955
H. tajikistanica Kirjanova & Ivanova, 1966

Nomen nudum:
H. indocyperi Husain & Khan, 1964

15.5.2 Principal species

15.5.2.1 *European cereal cyst nematode,* H. avenae

Presently, several *Heterodera* species are included under the common name 'cereal cyst nematodes' (CCN) comprising European cereal cyst nematode (ECCN) *H. avenae*; Filipjev cereal cyst nematode *H. filipjevi*; Australian cereal cyst nematode *H. australis*; Sturhan or Chinese cereal cyst nematode *H. sturhani*; and Mediterranean cereal cyst nematode *H. latipons*.

ECCN was first recorded by Kühn (1874) as a parasite of cereals in Germany and was later found in other countries. This nematode is now reported in most wheat-growing regions of Europe, Asia, North Africa and North America. ECCN 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 and Cook, 1993), with annual yield losses reaching £3 million (Nicol and Rivoal, 2008). At least US$3.4 million is estimated to be lost annually in wheat production in the states of Idaho, Oregon and Washington, USA, because of the cereal cyst nematodes *H. avenae* and *H. filipjevi*. The yield losses it causes on wheat range from 15–20% in Pakistan, and 40–92% on wheat and 17–77% on barley in Saudi Arabia. Hosts of *H. avenae* include species of cereals and grasses from the following genera: *Agropyron*, *Agrostis*, *Alopecurus*, *Anisantha*, *Arrhenatherum*, *Avena*, *Brachypodium*, *Bromus*, *Dactylis*, *Echinochloa*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Phalaris*, *Phleum*, *Poa*, *Polypogon*, *Secale*, *Setaria*, *Sorghum*, *Trisetum*, *Triticum*, *Vulpia*, *Zerna* and *Zea* (Williams and Siddiqi, 1972). In several regions with cereals *H. avenae* occurs in a mixture with *H. filipevi*. *Heterodera avenae* develops only one generation per year, with J2 hatch from the eggs determined largely by temperature (Rivoal and Cook, 1993).

Description: **Cyst**: 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. **Male**: L = 1020–1590 μm; stylet = 27–33 μm; spicules = 33–38 μm; gubernaculum = 10–13 μm. **J2**: L = 505–598 μm; stylet = 24–27.5 μm; tail hyaline region = 34–50 μm; tail = 52–79 μm. **Cyst**: lemon-shaped, with prominent neck and vulval cone (Fig. 15.2). Subcrystalline layer conspicuous, sloughing off with formation of dark brown cyst. Bifenestrate, bullae prominent, crowded beneath vulval cone (Figs 15.3A and 15.6A). **J2**: stylet well developed, with large, anteriorly flattened to concave basal knobs (Fig. 15.4A). Lateral field with four incisures. Tail with a sharply pointed terminus (Fig. 15.5A).

ECCN belongs to the *Avenae* group and is a member of the *H. avenae* species complex. It differs from closely related species (*H. australis*, *H. aucklandica*, *H. riparia*, *H. sturhani*, *H. pratensis* and *H. arenaria*) by morphometrical characters of the J2 and cysts and sequences of the internal transcribed spacer (ITS)-rRNA and mitochondrial cytochrome oxidase I (COI mtDNA) genes. Molecular analysis revealed that the world populations of the ECCN can be divided into two types: (i) European and North American populations of *H. avenae* – type A; (ii) Asian and African populations of *H. avenae* – type B.

15.5.2.2 Yellow beet cyst nematode, *H. betae*

The yellow beet cyst nematode (YBCN) was first observed by Maas *et al.* (1976) during examination of samples collected from the southern sugar beet regions of The Netherlands and it has been considered as a biotype of the clover cyst nematode, *H. trifolii*. After comparative morphological and molecular analysis of several populations of the YBCN with closely related species, Wouts *et al.* (2001) described it as a new species, *H. betae*. Further studies showed that this species was preferentially distributed in the warm environments of southern Europe. YBCN has been reported in several European countries and Morocco. Recently, *H. betae* was found in natural conditions on *B. vulgaris* ssp. *maritima* along the Atlantic and North Sea coastlines from northern France to Spain and Portugal (Gracianne *et al.*, 2014). *Heterodera betae* is considered another important cyst nematode after *H. schachtii*, causing considerable damage to sugar beet. YBCN induces yield losses mainly on sandy soils. In several locations this species co-occurs with *H. schachtii*. *Heterodera betae* reproduces on Brassicaceae (*Brassica* spp., *Sinapis alba*), Amaranthaceae (*Betae vulgare*, *Spinacia oleracea*), Popygonaceae (*Rumex* spp.), Caryophyllaceae (*Stellaria media*), Fabaceae (*Trifolium* spp.) and Solanaceae (*Solanum esculentum*) (Ambrogioni *et al.*, 2004). Cabbage and broad beans are also good hosts for *H. betae*. YBCN may complete three or four generations during the vegetation period. The species can multiply on sugar beet varieties with resistance genes to *H. schachtii*. YBCN has 35 or 36 chromosomes and reproduces by mitotic parthenogenesis, indicating that this species is

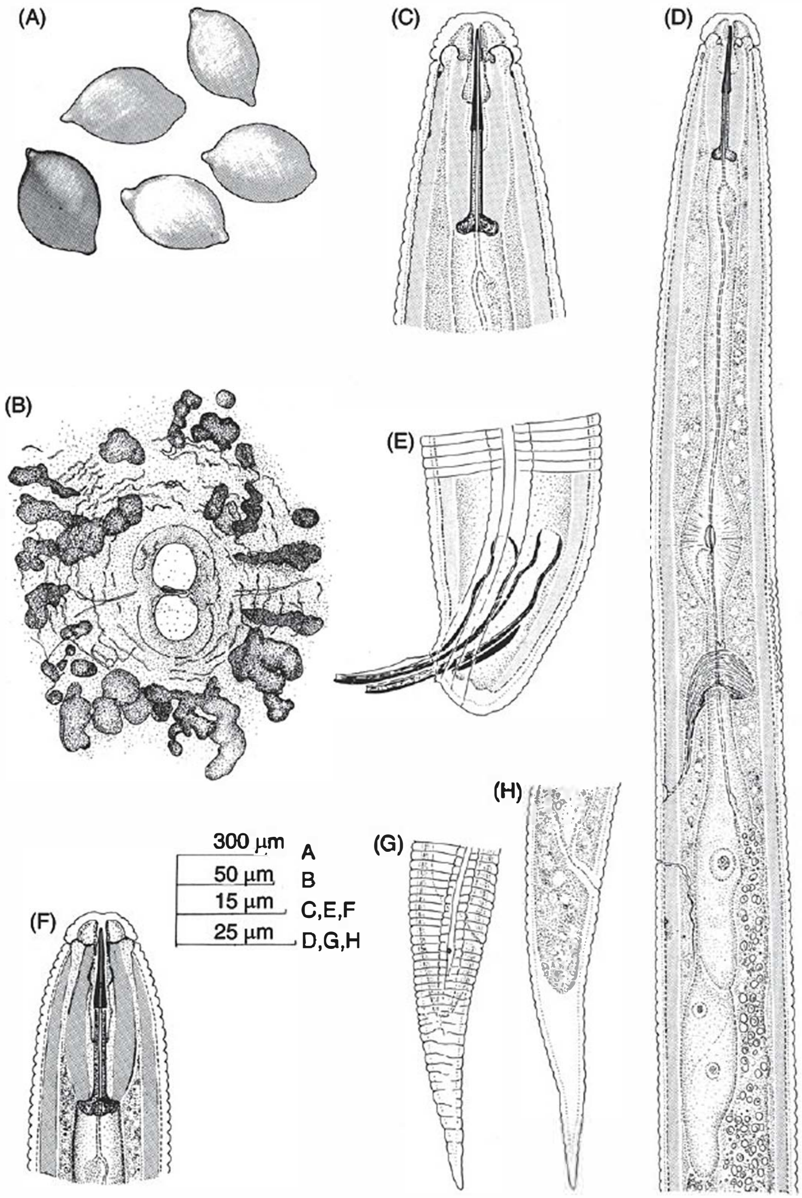

Fig. 15.2. *Heterodera avenae*. (A) Cysts. (B) Vulval cone. (C) Anterior region of male. (D) Pharyngeal region of male. (E) Tail of male. (F) Anterior region of J2. (G, H) Tail of second-stage juvenile. (After Williams and Siddiqi, 1972.)

potentially tetraploid (Steele and Whitehand, 1984).

Description: **Cyst**: L = 475–1160 μm; W = 168–702 μm; **L/W** ratio = 1.4–2.5; fenestral length = 30–73 μm; vulval slit = 48–67 μm. **Male**: not found. **J2**: L = 525–672 μm; stylet = 25–33 μm; tail hyaline region = 32–50 μm; tail = 64–84 μm. **Female**: passes through a distinct yellow phase. **Cyst**: lemon-shaped, often asymmetrical. Vulval cone ambifenestrate (Fig. 15.3B), in young cysts surrounded by eggsac. Vulval bridge narrow. Underbridge distinct,

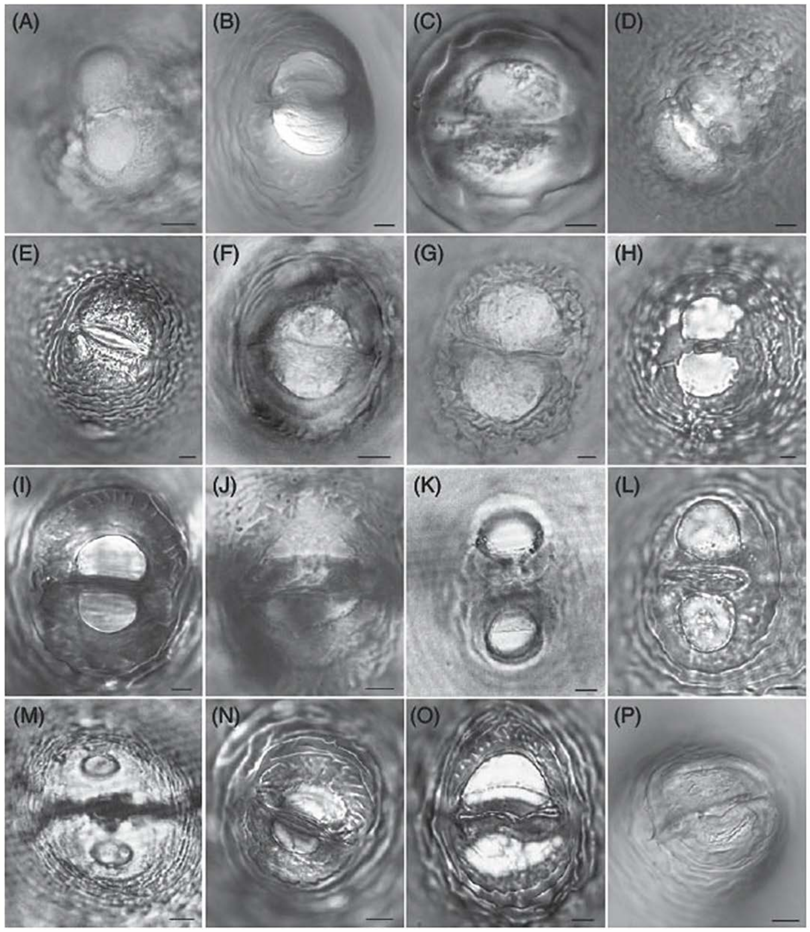

Fig. 15.3. Vulval plates. (A) *Heterodera avenae*. (B) *H. betae*. (C) *H. cajani*. (D) *H. carotae*. (E) *H. cruciferae*. (F) *H. elachista*. (G) *H. fici*. (H) *H. filipjevi*. (I) *H. glycines*. (J) *H. goettingiana*. (K) *H. hordecalis*. (L) *H. humuli*. (M) *H. latipons*. (N) *H. schachtii*. (O) *H. trifolii*. (P) *H. zeae*. B, C, D, P, after Subbotin *et al*. (2010) with modifications; K, after Andersson (1975); M, after Franklin (1969); E, H, I, L, N, O, courtesy of V.N. Chizhov. Scale bars = 10 μm.

long, heavily pigmented, with bifurcate ends. Heavily pigmented bullae present, mainly anterior to underbridge, globular. **J2:** slightly curved ventrally. Stylet robust, knobs heavy, deeply concave anteriorly, convex posteriorly. Lateral field with four incisures. Tail gradually tapering to slender rounded terminus.

Heterodera betae belongs to the *Schachtii* group. It differs from other related species owing to its longer body length, stylet and tail of J2. This species can be differentiated from other species by polymerase chain reaction-internal transcribed spacers-restriction fragment length polymorphism (PCR–ITS–RFLP).

15.5.2.3 Pigeonpea cyst nematode, H. cajani

The pigeonpea cyst nematode (PPCN) was described from roots of pigeon pea, *Cajanus cajan*, and later found parasitizing cowpea, *Vigna sinensis*, in India. The list of host plants includes several dozen plant species of the families Fabaceae (*Vigna* spp. and others) and Pedaliaceae (*Sesamum indicum*). *Heterodera cajani* is now present in all major pigeon pea-growing regions of India and Pakistan and is considered as the most important nematode pathogen of this crop. More than nine generations may be developed during

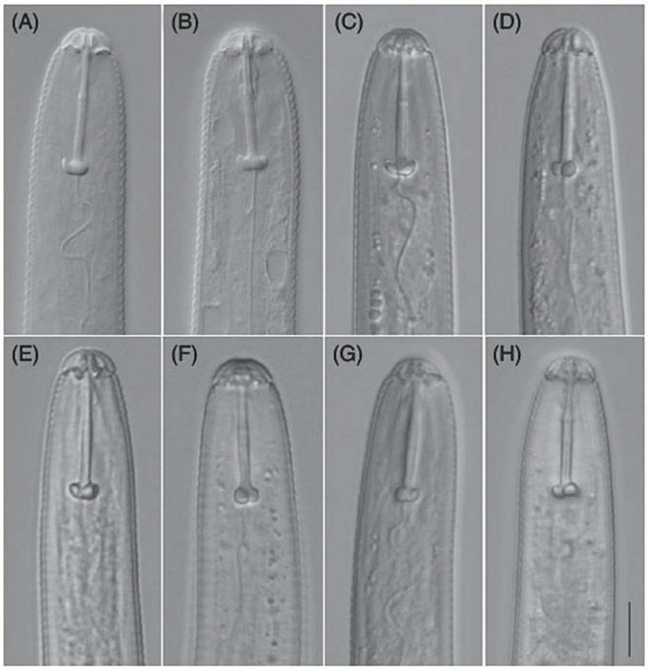

Fig. 15.4. Heads of second-stage juveniles. (A) *Heterodera avenae*. (B) *H. filipjevi*. (C) *H. schachtii*. (D) *H. cruciferae*. (E) *H. humuli*. (F) *Globodera rostochiensis*. (G) *Betulodera* sp. (H) *Punctodera punctata*. A, B, after Subbotin *et al.* (2003). Scale bar, A–H = 10 μm.

a vegetation season. Three races of PPCN are distinguished by their ability to multiply on cluster bean (*Cyamopsis tetragonolobus*) and sunn hemp (*Crotalaria juncea*) (Walia and Bajaj, 1986, 1988; Siddiqi and Mahmood, 1993). Walia and Bajaj (2000) found these races could be distinguished by vulval cone structure and male morphology.

Description: **Cyst**: L = 390–690 μm; W = 175–510 μm; L/W ratio = 1.0–2.5; fenestral length = 31–69 μm; vulval slit = 31–60 μm. **Male**: L = 780–1280 μm; stylet = 27–30 μm; spicules = 31–41 μm; gubernaculum = 8–12 μm. **J2**: L = 324–515 μm; stylet = 20–31 μm; tail hyaline region = 17–40 μm; tail = 32–64 μm. **Cyst**: light to dark brown, typically lemon-shaped with protruding neck and vulva. Subcrystalline layer present on young cysts. Ambifenestrate with strong underbridge (Fig. 15.3C). Bullae prominent dark brown, located beneath underbridge. **J2**: body tapering anteriorly and posteriorly but more so posteriorly. Stylet strong, well developed with anteriorly directed or rounded knobs. Lateral field with four incisures. Tail with bluntly rounded and narrow terminus.

Heterodera cajani belongs to the *Schachtii* group. It differs from several species of this group by a smaller cyst size. The ITS-rRNA gene sequence clearly distinguishes this species from all other species of the *Schachtii* group.

15.5.2.4 Carrot cyst nematode, *H. carotae*

Heterodera carotae occurs throughout the carrot-growing areas of Europe and is also found in the USA, Canada and South Africa. The species has a rather narrow list of host plants. It was found to parasitize only several subspecies of *Daucus carota* and several wild Apiaceae, such as *Torilis* spp. Up to four generations may occur in favourable 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 significant crop losses.

Description: **Cyst**: 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. **Male**: L = 1090–1220 μm; stylet = 31–38 μm;

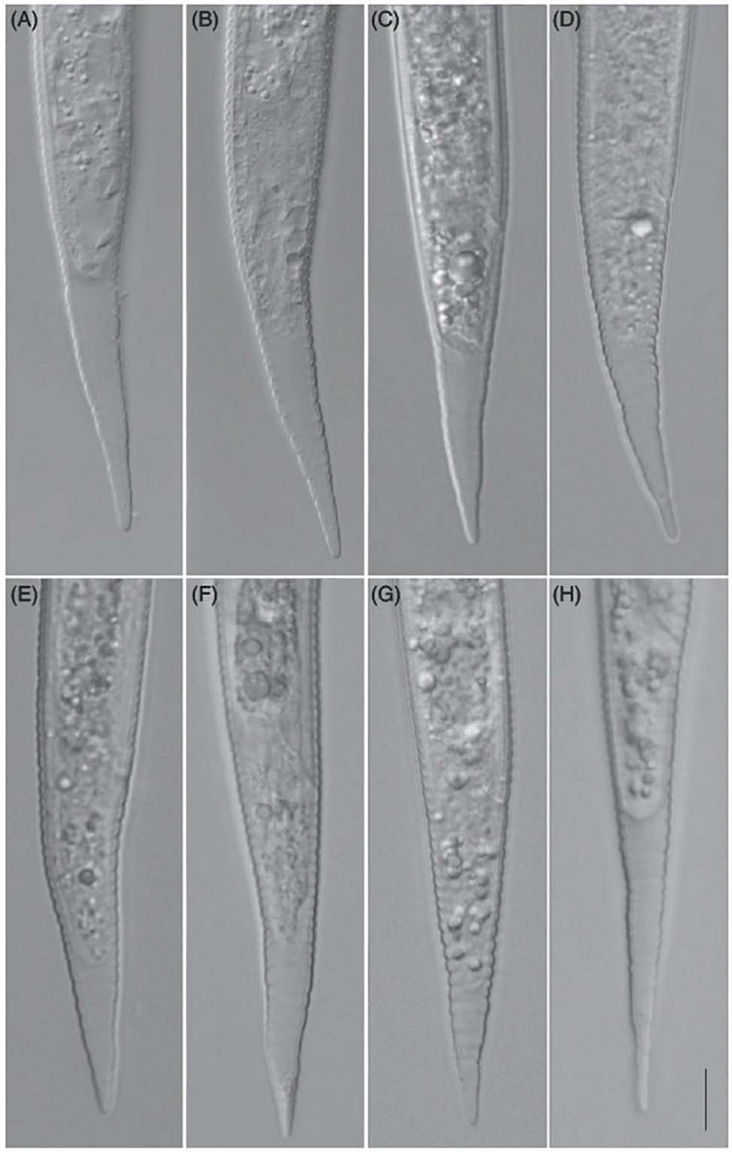

Fig. 15.5. Tails of second-stage juveniles. (A) *Heterodera avenae*. (B) *H. filipjevi*. (C) *H. schachtii*. (D) *H. cruciferae*. (E) *H. humuli*. (F) *Globodera rostochiensis*. (G) *Betulodera* sp. (H) *Punctodera punctata*. A, B, after Subbotin *et al.* (2003). Scale bar A–H = 10 μm.

spicules = 31–36 μm; gubernaculum = 10–13 μm. **J2**: L = 375–452 μm; stylet = 22–25 μm; tail hyaline region = 20–31.8 μm; tail = 43.5–59 μm. **Cysts**: small, lemon-shaped without bullae and slender underbridge. Fenestration ambifenestrate (Fig. 15.3D). **J2**: labial region slightly offset with four indistinct annuli. Stylet knobs with concave anterior faces. Lateral field with four incisures. Tail with a pointed terminus.

Heterodera carotae belongs to the *Goettingiana* group. It most closely resembles *H. cruciferae*, but molecular and morphological differentiation of these species is problematic.

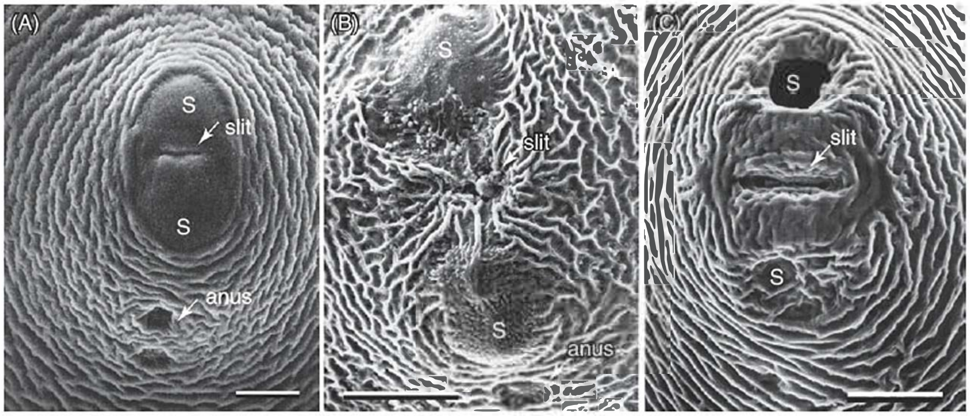

Fig. 15.6. SEM micrographs of the vulval area. (A) *Heterodera avenae*. (B) *H. latipons*. (C) *H. hordecalis*. S – semifenestrae. Scale bars = 20 μm. After Greco *et al.* (2002).

15.5.2.5 Cabbage cyst nematode, H. cruciferae

The cabbage cyst nematode is known in several cabbage growing areas in Europe, Asia, Australia and North America, where it is considered to be a major pest of cabbage crops. Plants infected by this nematode have short and bushy roots, due to secondary root production, resulting in patches of plants showing severe decline and stunting. This species is often associated with *H. schachtii*. *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 may occur in Europe. The cabbage cyst nematode has a narrower host range than *H. schachtii*, but apparently infects all species of *Brassica*.

Description: Cyst: 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. **Male**: L = 718–1343 μm; stylet = 20–28 μm; spicules = 16–38 μm; gubernaculum = 7–11 μm. **J2**: L = 333–504 μm; stylet = 20–25 μm; tail hyaline region = 16–30 μm; tail = 26–58 μm. **Cyst**: broad, almost spherical to lemon-shaped, ambifenestrate (Fig. 15.3E), without bullae, with very low semifenestral arches separated by a narrow vulval bridge, semifenestrae unobstructed in mature cysts. **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 (Fig. 15.4D). Lateral field with four incisures. Tail tapering uniformly to a fine rounded terminus (Fig. 15.5D).

Heterodera cruciferae belongs to the *Goettingiana* group, and is closely related to and morphologically similar to *H. carotae*.

15.5.2.6 Japanese cyst nematode, H. elachista

This nematode species was described from Japan on upland rice fields and later in Iran, China and Italy. In addition to *Oryza sativa*, host plants include *Zea mays* and *Carex* spp. This nematode causes 7–19% rice yield loss. Greater yield losses were observed when the roots were invaded by the nematodes before tillering. Nematodes had the most severe impact during the later stages of plant growth. Corn plants infected by the nematode were stunted with a marked proliferation of short lateral roots, resulting in plant decline and infested patches in the field.

Description: Cyst: L = 278–586 μm; W = 207–540 μm; L/W ratio = 1.1–1.9; fenestral length = 23–50 μm; vulval slit = 26–55 μm. **Male**: L = 820–940 μm; stylet = 20–21 μm; spicules = 26–29 μm; gubernaculum = 9–13 μm. **J2**: L = 330–535 μm; stylet = 16–25 μm; tail hyaline region = 25–50 μm; tail = 44–87 μm. **Cyst**: light to dark brown, spherical to lemon-shaped. Subcrystalline layer present. Ambifenestrate (Fig. 15.3F), vulval cone with semifenestrae almost as wide as long. Narrow vulval bridge and weak underbridge, and prominent dark brown bullae. **Male**: very rare. **J2**: body slightly curved ventrally. Lip region hemispherical with three annuli. Stylet well developed with rounded or anteriorly concave knobs. Lateral field with three incisures. Tail long, narrowly tapering to a very fine, rounded terminus.

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* as it lacks finger-shaped projections on the slender underbridge of the cysts and from *H. oryzae* as it has smaller cysts and a shorter body of the J2.

15.5.2.7 Fig cyst nematode, H. fici

The fig cyst nematode, *H. fici*, was first described from roots of the plant *Ficus elastica*, which was imported from Harbin, China, to Russia. Subsequent surveys revealed that this nematode is widely distributed in natural conditions in the Mediterranean and other countries on roots of *F. carica* and ornamental species of the genus *Ficus*. The nematode can be considered a potential threat in fig nurseries. Plants infected by this nematode showed symptoms of retarded growth and yellowing of leaves.

Description: **Cyst**: L = 340–697 µm; W = 272–560 µm; L/W = 1.0–1.7; fenestral length = 42–74 µm; vulval slit = 35–56 µm. **Male**: L = 760–1002 µm; stylet = 26–32 µm; spicules = 27–32 µm; gubernaculum = 6–9 µm. **J2**: L = 320–470 µm; stylet = 20–25 µm; tail hyaline region = 18–33 µm; tail = 40–61 µm. **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. 15.3G). Bullae dome shaped, small, scattered around underbridge plane. Underbridge weakly developed, with furcate ends. **J2**: stylet well developed, basal knobs rounded, directed slightly anteriorly. Lateral field with four incisures. Tail terminus rounded.

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*) as it has ambifenestrate rather than bifenestrate cysts and a longer vulval slit.

15.5.2.8 Filipjev cereal cyst nematode, H. filipjevi

This species was described by Madzhidov (1991) from Tajikistan and later identified from many European and Asian countries and the USA. Presently, *H. filipjevi* is considered to be an important worldwide pest of cereals. The list of plant hosts of this species includes more than 20 species of cereals and grasses. One generation develops during the growing season. At least two pathotypes of this species are recognized.

Description: **Cyst**: L = 455–936 µm; W = 306–792 µm; L/W = 1.0–1.9; fenestral length = 38–66 µm; vulval slit = 6–12 µm. **Male**: L = 1160–1400 µm; stylet = 27–29 µm; spicules = 28–34 µm. **J2**: L = 431–614 µm; stylet = 22–30 µm; hyaline region = 28–41 µm; tail = 49–64 µm. **Cyst**: lemon-shaped with prominent vulval cone. Subcrystalline layer present. Colour varying from light to dark brown. Bifenestrate with massive underbridge (Fig. 15.3H). Bullae large and numerous. **J2**: labial region offset with two faint annuli. Stylet with anteriorly projecting knobs (Fig. 15.4B). Lateral field with four incisures. Tail conical (Fig. 15.5B).

Heterodera filipjevi belongs to the *Avenae* group and the *H. avenae* complex. It differs from most species owing to the presence of an underbridge. The ITS-rRNA and COI gene sequences clearly distinguishes this species from other species of the *Avenae* group.

15.5.2.9 Soybean cyst nematode, H. glycines

Soybean cyst nematode is a major pest of soybean in Asia, North and South America and is found in most countries of the world where soybean is produced. In Japan, the yield loss was estimated to be 10–75% (Ichinohe, 1988), in China 10–20% and in the USA annual losses in north central states attributable to *H. glycines* parasitism were estimated to be worth over $200 million (Doupnik, 1993). In six north central states (Illinois, Indiana, Iowa, Minnesota, Missouri and Ohio) surveyed for the presence of *H. glycines*, 47–83% of soybean hectarage was found to be infested (Workneh *et al*., 1999). In central China *H. glycines* was also recognized as a pest of tobacco (Shi and Zheng, 2013).

A cyst nematode parasitizing soybean plants and causing 'yellow dwarf' symptoms was recorded from Japan, in 1915. Ichinohe (1952) was the first to make careful morphological comparisons with other *Heterodera* species and to give a specific name to and brief description of this nematode. *Heterodera glycines* has a broad host range, especially Fabaceae, but also on other families. More than 66 weed species of nine families are suitable hosts. Riggs (1992) provided a list of non-fabaceous hosts comprising species from 22 families (e.g. Boraginaceae, Capparaceae, Caryophyllaceae, Chenopodiaceae, Brassicaceae, Lamiaceae, Fabaceae, Scrophulariaceae and 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.

Heterodera glycines disrupts 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 (see Chapter 12, this volume). Sudden death syndrome is a soil-borne disease of soybean caused by the fungus *Fusarium solani* in association with *H. glycines*. However, soybean cyst nematode can reduce soybean yields without causing above-ground symptoms (Wang *et al.*, 2003).

Description: Cyst: 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. **Male**: L = 911–1400 μm; stylet = 24–27 μm; spicules = 28–45 μm; gubernaculum = 8–13 μm. **J2**: L = 345–504 μm; stylet = 21–25 μm; tail hyaline region = 18–36 μm; tail = 35–59 μm. **Cyst**: mainly lemon-shaped, sometimes round with a protruding neck and cone. Ambifenestrate, bullae prominent, located at or anterior to underbridge, extending into vulval cone from interior of body wall cuticle (Fig. 15.3I). 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 with anteriorly protruding knobs. Tail tapering uniformly to a finely rounded terminus.

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* in the shape of the stylet knobs of J2 (slightly convex *vs* moderately or strongly concave), shorter average J2 stylet length and longer average fenestral length. The ITS-rRNA and *COI* gene sequences distinguish this species from other species of the *Schachtii* group.

15.5.2.10 *Pea cyst nematode,* H. goettingiana

Liebscher described the pea cyst nematode in 1892 and also reported symptoms of infection and yield loss of pea (*Pisum sativum*) and vetch (*Vicia sativa*) caused by this nematode in fields of the Agricultural Institute at Göttingen, Germany. Presently, *H. goettingiana* has been found in many countries of Europe, North Africa, Asia and the USA. Infested 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. Hosts of *H. goettingiana* also include several species of the genera belonging to the Fabaceae family: *Cicer*, *Glycine*, *Lathyrus*, *Lens*, *Lupinus*, *Medicago*, *Pisum*, *Trifolium* and *Vicia*. The number of generations produced depends on environmental conditions. One or two generations occur during the growing season in the UK, and three generations may develop in southern Italy.

Description: Cyst: 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. **Male**: L = 1270 μm; stylet = 27 μm; spicules = 27 μm; gubernaculum = 12 μm. **J2**: L = 408–519 μm; stylet = 23–26 μm; tail hyaline region = 27–38 μm; tail = 54–74 μm. **Cyst**: lemon-shaped with light to dark brown cyst wall. Vulval cone ambifenestrate (Fig. 15.3J). 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 very weak. **J2**: body curved ventrally after fixation. Labial region hemispherical, with two to five 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.

Heterodera goettingiana belongs to the *Goettingiana* group. It differs from several other representatives of the *Goettingiana* group (*H. cruciferae*, *H. carotae*, *H. circeae*, *H. scutellariae* and others) owing to longer average J2 body, longer tail and longer hyaline region. The ITS-rRNA gene sequence distinguishes this species from other species of the *Goettingiana* group.

15.5.2.11 *Barley cyst nematode,* H. hordecalis

The barley cyst nematode was found and described from a barley field in Halland province, Sweden in 1975. Presently, *H. hordecalis* is reported from several central and north European and Asian countries. The nematode also parasitizes rye, winter cereals and many grasses from the genera *Ammophila*, *Leymus*, *Bromus*, *Calamagrostis*, *Dactylis*, *Festuca*, *Lolium* and *Phleum*.

Description: **Cyst**: L = 330–950 µm; W = 255–680 µm; L/W ratio = 1.0–1.5; fenestral length = 47– 80 µm. **Male**: L = 805–1390 µm; stylet = 24–29 µm; spicules = 34–42 µm; gubernaculum = 9–13 µm. **J2**: L = 410–550 µm; stylet = 21–26 µm; tail hyaline region = 29–46 µm; tail = 44–60 µm. **Cyst**: body ovoid, with distinct neck and vulval cone. Bifenestrate, semifenestrae widely separated (Figs 15.3K and 15.6C). In centre of vulval bridge, a rigid, often dumb-bell like structure, surrounding anterior end of vagina. 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 absent. **J2**: labial region slightly offset with three or four annuli. Lateral field with four incisures. Tail tip finely rounded.

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 ITS-rRNA and *COI* gene sequences clearly differentiate this species from all others.

15.5.2.12 *Hop cyst nematode,* H. humuli

Heterodera humuli was described by Filipjev (1934) from hop plants based on morphological data provided by several researchers. Presently, the hop cyst nematode is reported from the hop-growing areas in European and Asian countries, South Africa, Australia, New Zealand, the USA and Canada. In an experimental study (Hafez *et al.*, 1999), 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. The growth of nematode-infested hop cultivars was significantly reduced when the plant pathogenic fungus *Verticillium albo-atrum* was also present. Hosts include *Humulus lupulus*, *Urtica dioica* and *U. urens*. Only one generation is produced per year.

Description: **Cyst**: 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. **Male**: L = 670–1000 µm; stylet = 20–28 µm; spicules = 29–33 µm; gubernaculum = 7–8 µm. **J2**: L = 336–468 µm; stylet = 21–25 µm; tail hyaline region = 22–30 µm; tail = 42–53 µm. **Cyst**: lemon-shaped, occasionally nearly spherical. Abullate, thin walled, light coloured, darkening with age. Bifenestrate, vulval bridge broad, semifenestrae circular or subcircular, often obscured by thin membrane with a fine fingerprint-like pattern (Fig. 15.3K). Underbridge slender, weak, with furcate ends. **J2**: labial region rounded, offset with two to four annuli (Fig. 15.4E). Lateral field with four incisures, not areolated. Tail tapering, terminus often constricted and irregularly shaped (Fig. 15.5E).

Heterodera humuli belongs to the *Humuli* group and is similar to *H. ripae* and *H. vallicola*. It differs from *H. ripae* as it has a longer J2 tail and hyaline region and from *H. vallicola* as it has a more slender cyst and longer fenestral length. The ITS-rRNA gene sequence distinguishes this species from other species of the *Humuli* group.

15.5.2.13 *Mediterranean cereal cyst nematode,* H. latipons

This species was collected from roots of stunted wheat plants in Israel and described by Franklin (1969). Presently, *H. latipons* has been recorded in many countries of the Mediterranean basin, North Africa, the Near East and Japan. Hosts include *Hordeum vulgare*, *Avena sativa*, *Secale cereale*, *Phalaris minor*, *P. paradoxa* and *Elytrigia repens*. 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 (Scholz, 2001). Moreover, damage is more severe in fields infested concomitantly by *H. latipons* and the fungus *Cochliobolus sativus*. In all areas studied, *H. latipons* completed only one life cycle during the growing season.

Description: **Cyst**: L = 300–700 μm; W = 310–560 μm; L/W ratio = 0.6–1.7; fenestral length = 45–76 μm; vulval slit = 6–11 μm. **Male**: L = 960–1406 μm; stylet = 22–29 μm; spicules = 32–36 μm; gubernaculum = 8 μm. **J2**: L = 401–598 μm; stylet = 22–26 μm; tail hyaline region = 20–36 μm; tail = 42–72 μm. **Cyst**: dark to mid brown covered with white subcrystalline layer. Bifenestrate, semifenestrae separated by a distance greater than fenestral width, vulval slit short (Figs 15.3M and Fig. 15.6B). Strong underbridge with pronounced thickening in middle and with ends splayed. Bullae usually absent, sometimes present at underbridge level. **J2**: labial region with three annuli. Stylet with well developed, anteriorly concave knobs. Tail with finely rounded terminus.

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. The ITS-rRNA and *COI* gene sequences clearly differentiate this species from all others.

15.5.2.14 Sugar beet cyst nematode, H. schachtii

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. *Heterodera schachtii* is found in all major sugar beet production areas of the world and is widespread in Europe, the USA and Canada, and considered as one of the most serious pests of sugar beet. Annual yield loss in EU countries based upon world market sugar prices was estimated in 1999 to be up to US$90 million (Müller, 1999). *Heterodera schachtii* parasitizes more than 200 different plants, mainly from the families Amaranthaceae (including many species of *Beta* and *Chenopodium*) and Brassicaceae (*Brassica oleracea*, *B. napus*, *B. rapa*, *Rhaphanus sativus* and many others including a diversity of common weeds). Some plants from Polygonaceae, Scrophulariaceae, Caryophyllaceae and Solanaceae are susceptible to nematode infection. In some climates, three to five generations may complete development on sugar beet in one season.

Description: **Cyst**: L = 480–960 μm; W = 396–696 μm; L/W ratio = 0.9–2.0; fenestral length = 28–51 μm; vulval slit = 33–54 μm. **Male**: L = 1038–1638 μm; stylet = 27–30 μm; spicules = 27–39 μm; gubernaculum = 10–11 μm. **J2**: L = 400–512 μm; stylet = 23–28 μm; tail hyaline region = 17–33 μm; tail = 40–56 μm. **Cyst**: colour light to dark brown. Ambifenestrate (Fig. 15.3N), 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 the vulval bridge. **J2**: labial region offset, with four indistinct annuli (Fig. 15.4C). Stylet moderately heavy with prominent, forwardly directed knobs. Tail acutely conical with rounded tip (Fig. 15.5C).

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. The PCR–ITS–RFLP, ITS–rRNAand *COI* sequences distinguish this species from other species in the *Schachtii* group.

15.5.2.15 Clover cyst nematode, H. trifolii

The clover cyst nematode is considered to be a pest of diverse agricultural crops and several pasture plants, especially parasitizing white clover, *Trifolium repens*. *Heterodera trifolii* is a cosmopolitan species and reported from all continents. Some authors propose considering species *H. trifolii* as a conglomerate of independently evolved mitotic parthenogenetic populations, comprising polyploidy and aneuploid forms with 3n = 24–28 and 4n = 34–35 chromosomes, and host races with more or less extended host ranges. Host plants include many species of Fabaceae and other families. Several generations may occur during a vegetative period. This nematode may cause a reduction in yield, nitrogen fixation and persistence of clover plants in pastures. Several studies have shown yield losses and growth suppression of white clover due to *H. trifolii* infection.

Description: **Cyst**: L = 360–1020 μm; W = 195–680 μm; L/W = 1.2–2.7; fenestral length = 40–80 μm; vulval slit = 39–66 μm. **Male**: not found. **J2**: L = 461–678 μm; stylet = 23–31 μm; tail hyaline region = 27–45 μm; tail = 49–78 μm. **Cyst**: brown to dark brown, ambifenestrate with strong underbridge and elongated bullae (Fig. 15.3O). Long vulval slit and strongly pigmented underbridge with bifurcate ends. **J2**: labial region offset, with three or four annuli. Lateral field with four incisures. Stylet robust, anterior surfaces of knobs concave. Tail conoid, tapering uniformly to a finely rounded terminus.

Heterodera trifolii is a member of the *H. trifolii* species complex including *H. betae*, *H. daverti*, *H. lespedezae*, *H. medicaginis* and *H. galeopsidis*, and belongs to the *Schachtii* group. The clover cyst nematode can be found to parasitize clover in a mixture with *H. daverti*; the latter species has males. Molecular characterization of this species complex is given by Subbotin *et al.* (2010) and more recently by Vovlas *et al.* (2015).

15.5.2.16 Maize cyst nematode, H. zeae

This species was described in India, where it is considered to be the most important nematode problem in maize, causing yield loss to the maize crop of 21–29% (Srivastava and Chawla, 2005). It is presently also found in Egypt, Pakistan, Afganistan, Thailand, Portugal and Greece. *Heterodera zeae* was first detected in the western hemisphere in North America, in Kent County, Maryland in 1981 (Sardanelli *et al.*, 1981). The maize cyst nematode *H. zeae* can cause serious losses in maize yields, especially under conditions meeting its high optimum temperature requirements (Krusberg *et al.*, 1997). This species was found mainly on *Zea mays*; however, other cultivated or wild Poaceae species are also considered as suitable host plants including sorghum, rice, wheat, barley, foxtail millet, barnyard millet, finger millet, little millet, oat, rye, sugarcane and khus-khus grass. *Heterodera zeae* may complete six to seven generations during a maize-growing season. Laboratory experiments showed that the host races of *H. zeae* can be differentiated on the basis of their ability to reproduce on maize and vetiver (*Vetiveria zizanioides*) (Bajaj and Gupta, 1994).

Description: Cyst: 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. **Male**: L = 641–994 μm; stylet = 24–25 μm; spicules = 25–32 μm; gubernaculum = 8–11 μm. **J2**: L = 350–484 μm; stylet = 19–25 μm; tail hyaline region = 17–30 μm; tail = 32–50 μm. **Cyst**: light brown, basically lemon-shaped, cuticle thin walled, ambifenestrate. Semifenestra separated by fairly wide vulval bridge (Fig. 15.3P), fenestral length and width variable, basin wide but generally poorly defined. Bullae prominent, immediately beneath to underbridge and characteristically arranged as four finger-like bullae in a distinct formation. Underbridge simple, short, and thin, found in all but a few of cysts examined, lacking forking at ends. **J2**: labial region slightly offset, rounded, with low profile, with three to five 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.

Heterodera zeae differs from other species by the location of the bullae at two levels, that is, four, finger-like bullae located immediately beneath a short, thin, underbridge and with many randomly located bullae further below. The ITS-rRNA sequence distinguishes this species from other species.

15.6 Subfamily Punctoderinae Krall & Krall, 1978

Diagnosis: Heteroderidae Filipjev & Schuurmans Stekhoven, 1941.

Mature female and cyst: body spherical or with vulva cone. Perineal area subterminal or terminal. Female cuticle not annulated, changing colour after death. Vulval lips absent, vulval slit short. Vulval fenestration circumfenestrate. Cuticle thick, D- layer present or absent. Bullae absent or present. Underbridge absent. Subcrystalline layer

present or absent. **Male**: developed through metamorphosis, labial region annulated, lateral field with four incisures, tail short, hemispherical, without bursa. **Second-stage juvenile**: with lateral field marked by four incisures, phasmids punctiform without lens-like structure.
Type genus: Genus *Globodera* Skarbilovich, 1959
Other genera:
Punctodera Mulvey & Stone, 1976
Cactodera Krall & Krall, 1978
Dolichodera Mulvey & Ebsary, 1980
Betulodera Sturhan, 2002
Paradolichodera Sturhan, Wouts & Subbotin, 2007
Vittatidera Bernard, Handoo, Powers, Donald & Heinz, 2010

15.7 Genus *Globodera* Skarbilovich, 1959

Diagnosis: (Punctoderinae) (after Baldwin and 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 μm. No anal fenestra. Vaginal remnants, underbridge and bullae rarely present. Cuticle with distinct D-layer. All eggs retained in body, eggsac absent. **Male**: lateral field with four lines, spicules >30 μm, distally pointed. **J2**: with four incisures in lateral field. Tail conical, pointed, phasmids punctiform. **Egg**: surface smooth.

15.7.1 List of species and synonyms

Type species:

1. *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959

= *Heterodera schachtii rostochiensis* Wollenweber, 1923
= *Heterodera rostochiensis* Wollenweber, 1923
= *Heterodera* (*Globodera*) *rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959
= *Heterodera schachtii solani* Zimmermann, 1927
= *Heterodera solani* Zimmermann, 1927
= *Heterodera pseudorostochiensis* Kirjanova, 1963
= *Globodera pseudorostochiensis* (Kirjanova, 1963) Mulvey & Stone, 1976
= *Globodera arenaria* Chizhov, Udalova & Nasonova, 2008

Other species:

2. *G. agulhasensis* Knoetze, Swart, Wentzel & Tiedt, 2017
3. *G. artemisiae* (Eroshenko & Kazachenko, 1972) Behrens, 1975

= *Heterodera artemisiae* Eroshenko & Kazachenko, 1972
= *Globodera hypolysi* Ogawa, Ohshima & Ichinohe, 1983

4. *G. bravoae* Franco, Cid del Prado & Lamothe-Argumedo, 2000
5. *G. capensis* Knoetze, Swart & Tiedt, 2013
6. *G. ellingtonae* Handoo, Carta, Skantar, & Chitwood, 2012
7. *G. leptonepia* (Cobb & Taylor, 1953) Skarbilovich, 1959

= *Heterodera leptonepia* Cobb & Taylor, 1953
= *Heterodera* (*Globodera*) *leptonepia* (Cobb & Taylor, 1953) Skarbilovich, 1959

8. *G. mali* (Kirjanova & Borisenko, 1975) Behrens, 1975

= *Heterodera mali* Kirjanova & Borisenko, 1975
= *Globodera mali* (Kirjanova & Borisenko, 1975) Mulvey & Stone, 1976

9. *G. mexicana* Subbotin, Mundo-Ocampo & Baldwin, 2010

= *Heterodera mexicana* Campos-Vela, 1967 (= *nomen nudum*)

10. *G. millefolii* (Kirjanova & Krall, 1965) Behrens, 1975

= *Heterodera millefolii* Kirjanova & Krall, 1965
= *Heterodera* (*Globodera*) *millefolii* (Kirjanova & Krall, 1965) Mulvey, 1973
= *Globodera achilleae* (Golden & Klindie, 1973) Behrens, 1975
= *Heterodera achilleae* Golden & Klindie, 1973

11. *G. pallida* Stone, 1973

= *Heterodera pallida* Stone, 1973
= *Heterodera (Globodera) pallida* Stone, 1973

12. *G. sandveldensis Knoetze, Swart, Wentzel & Tiedt, 2017*

13. *G. tabacum tabacum* (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959

= *Heterodera tabacum* Lownsbery & Lownsbery, 1954

= *Globodera tabacum* (Lownsbery & Lownsbery, 1954) Behrens, 1975
= *G. tabacum solanacearum* (Miller & Gray, 1972) Behrens, 1975
= *Heterodera solanacearum* Miller & Gray, 1972
= *Heterodera tabacum solanacearum* (Miller & Gray, 1972) Stone, 1983
= *Globodera solanacearum* (Miller & Gray, 1972) Behrens, 1975
= *G. tabacum virginiae* (Miller & Gray, 1968) Stone, 1973
= *Heterodera virginiae* Miller & Gray, 1968
= *Heterodera tabacum virginiae* (Miller & Gray, 1968) Stone, 1983
= *Globodera virginiae* (Miller & Gray, 1968) Behrens, 1975

14. *G. zelandica* Wouts, 1984

15.7.2 Principal species

15.7.2.1 Golden potato cyst nematode, G. rostochiensis

Presently, the potato cyst nematodes (PCN) include three species, two species of them, *G. rostochiensis* and *G. pallida*, are reported from many countries and are considered to be economically important pests of potato. The recently described *G. ellingtonae* was found parasitizing potatoes in the USA and some regions of South America (Handoo *et al.*, 2012; Zasada *et al.*, 2013, 2015; Lax *et al.*, 2014).

The golden potato cyst nematode (GPCN), *G. rostochiensis*, is a serious pest of potatoes around the world and is a target of strict regulatory actions in many countries. The GPCN was first found associated with potato plant, *Solanum tuberosum*, from Rostock, Germany in 1881 and was considered to be *H. schachtii*, this being the only known species of cyst nematode at that time. During the early 1900s, GPCN became more widely known throughout Europe and was formally described in 1923. Presently, GPCN are reported in most potato-producing regions of Europe, Africa, Asia, North, Central and South America, and Oceania. Hosts of GPCN include potato (*S. tuberosum*), tomato (*S. lycopersicum*) and eggplant (*S. melongena*). Other hosts include many *Solanum* spp., *Datura* spp., *Hyoscyamus niger*, *Nicotiana acuminata*, *Physalis* spp., *Physochlaina orientalis*, *Salpiglossis* spp., *Capsicum annuum* and *Saracha jaltomata*.

In temperate regions, *G. rostochiensis* usually completes only one generation, although a second generation may be initiated but not completed. 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 or more years (see Chapter 3, this volume). The main routes of spread are infested seed potatoes and movement of soil through farm machinery. Heavily infected plants become yellow and stunted. Infected plants have reduced root systems, which are abnormally branched and brownish in colour. 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 8 and 64 eggs (g soil)$^{-1}$, yield losses of about 20 and 70%, respectively, can be expected. The damaging effect of PCN is not only determined by nematode density, but also by such factors as cultivar, crop husbandry and environmental conditions.

Description: **Cyst**: L = 450–990 μm; W = 250–810 μm; L/W ratio = 0.9–1.8; fenestral diameter = 14–21 μm; number of ridges between anus and fenestra = 16–24; Granek's ratio = 2.0–7.0. **Male**: L = 860–1406 μm; stylet = 26–27 μm; spicules = 32–39 μm; gubernaculum = 10–14 μm. **J2**: L = 366–502 μm; stylet = 19–23 μm; tail hyaline region = 18–30 μm; tail = 37–57 μm. **Female**: colour 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, abullate (Fig. 15.8). Fenestra circular (Fig. 15.7A), 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 (Fig. 15.4F). Lateral fields with four lines extending for most of body length. Tail tapering to small, rounded terminus (Fig. 15.5F).

Globodera rostochiensis is morphologically similar to *G. pallida*, *G. ellingtonae*, *G. mexicana* and *G. tabacum*. It differs from *G. pallida* as it has yellow or gold *vs* cream coloured maturing females, a higher number of ridges between the vulva and anus, a larger mean for Granek's

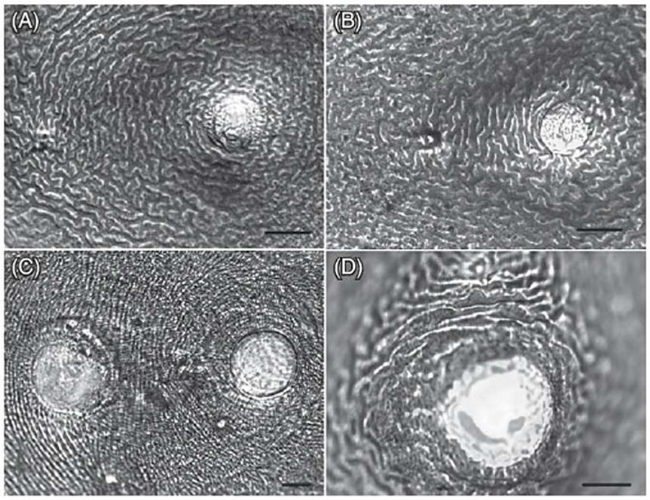

Fig. 15.7. Vulval plates. (A) *Globodera rostochiensis*. (B) *G. pallida*. (C) *Punctodera punctata*. (D) *Cactodera cacti*. Courtesy of V.N. Chizhov. Scale bars = 15 μm.

ratio, a different stylet knob shape, shorter average stylet length and bluntly rounded *vs* more pointed J2 tail terminus. The ITS-rRNA and *COI* gene sequences clearly distinguish GPCN from all other PCN and *Globodera* species.

15.7.2.2 *Ellington potato cyst nematode, G. ellingtonae*

Ellington potato cyst nematode (EPCN) was recently recognized as a new species from Oregon and Idaho, USA. Glasshouse experiments have demonstrated that this nematode can reproduce on potatoes (Handoo *et al*., 2012) and biological information, including hatch in potato root diffuste, was provided by Zasada *et al.* (2013, 2015), who confirmed that this nematode reproduces well on various cultivars of potato and tomato. In 2014, EPCN was also identified on roots of Andean potatoes collected from the Salta region of Northern Argentina (Lax *et al*., 2014). Isolates of *Globodera* sp. from Chile also showed a high degree of molecular similarity with populations of *G. ellingtonae* from Argentina and the USA, suggesting that this nematode also occurs in Chile (see also Chapter 7, this volume).

Description: **Cyst**: L = 370–860 μm; W = 320–890 μm; L/W ratio = 0.9–1.4; fenestral diameter = 12–42.5 μm; number of ridges between anus and fenestra = 8–25; Granek's ratio = 0.9–5.9. **Male**: L = 717–1368 μm; stylet = 21–27 μm; spicules = 30–44 μm; gubernaculum = 8–15 μm. **J2**: L = 365–526 μm; stylet = 19–24 μm; hyaline region = 19–33 μm; tail = 39–56 μm. **Female**: body white becoming yellow to pale brown as eggs mature, ovate to rounded or subspherical in shape with elongate, protruding neck. **Cysts**: light brown to brown in colour, generally spherical to occasionally oval with a protruding neck. Vulval region fenestrated with a single circumfenestrate opening occupying all or part of vulval basin. Cyst wall pattern ridge-like to irregular, wavy to whorled with heavy punctations. **J2**: lip region with three or four complete or incomplete cephalic annules. Stylet short, robust; basal knobs rounded posteriorly; the anterior surface of three shapes: rounded, forward projection anteriorly or flattened, the latter being the most frequent. Tail tapering uniformly but abruptly narrowing with a pronounced to slight constriction near the posterior third of the hyaline portion, ending with a peg-like, finely rounded to pointed terminus.

EPCN differs morphologically from the related species, *G. pallida*, *G. rostochiensis*, *G. tabacum* and *G. mexicana* in the distinctive J2 tail that uniformly tapers but abruptly narrows with a pronounced to slight constriction near the posterior third of the hyaline portion, ending with a peg-like, finely rounded to pointed terminus. Detailed differentiating characters between other PCN and *Globodera* species are given in Handoo *et al.* (2012) and Lax *et al.* (2014). The ITS-rRNA and *COI* gene sequences clearly distinguish EPCN from all other PCN and *Globodera* species.

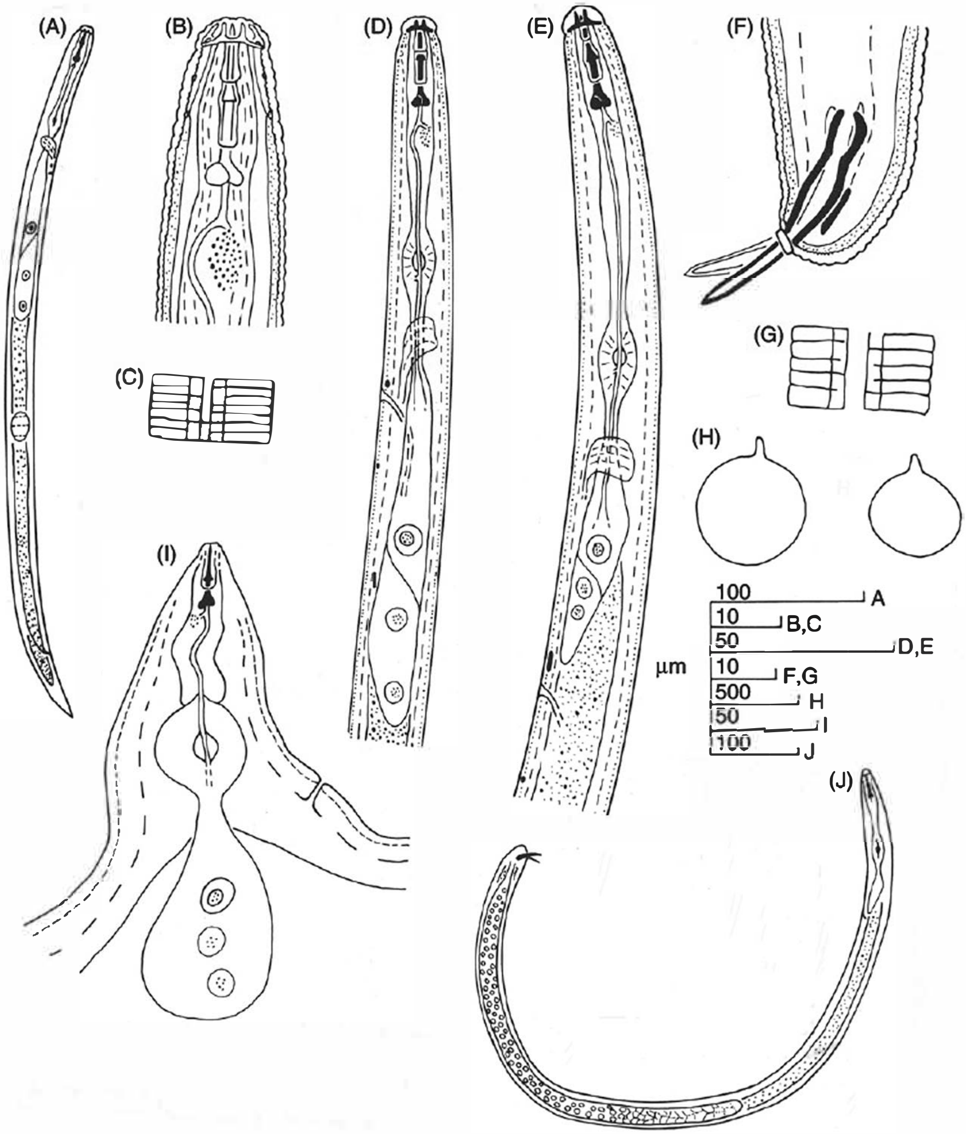

Fig. 15.8. *Globodera rostochiensis*. (A–D) J2. (A) Entire body. (B) Lip region. (C) Lateral field. (D) Pharyngeal region. (E–G) Male. (E) Pharyngeal region. (F) Tail. (G) Lateral field. (H) Entire cysts. (I) Anterior region of female. (J) Entire male. After Stone (1973a).

15.7.2.3 Pale potato cyst nematode, G. pallida

The pale potato cyst nematode (PPCN) is considered to be a major pest of potato crops in cool temperate climates. PPCN is reported from several counties in Europe, Asia, Africa and South America. *Globodera pallida* has not been recorded in Australia, but is found in New Zealand. In Central and North America *G. pallida* has been reported in Panama, the USA and Canada, but in the last two countries *Globodera* species on potato have a rather restricted distribution, with only small infested areas, because of rigorous phytosanitary regulations and seed potato certification programmes. 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 the 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 temperatures 2°C cooler than those required by *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)$^{-1}$. Hosts include potato (*S. tuberosum*), eggplant (*S. melongena*), tomato (*S. lycopersicum*), many other species of *Solanum* and black henbane (*Hyoscyamus niger*).

Description: **Cyst**: L = 420–748 µm; W = 400–685 µm; fenestral diameter = 17.5–25 µm; number of ridges between anus and fenestra = 7–17; Granek's ratio = 1.2–3.6. **Male**: L = 1198 µm; stylet = 27 µm; spicules = 36 µm; gubernaculum = 11 µm. **J2**: L = 380–533 µm; stylet = 22.5–25 µm; tail hyaline region = 20–31 µm; tail = 40–57 µm. **Female**: white in colour, 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 opening occupying all or part of vulval basin, abullate (Fig. 15.7B). **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 is about half of tail region.

Globodera pallida is most closely related to *G. mexicana*, *G. rostochiensis*, *G. tabacum* and *G. ellingtonae*. It differs from *G. rostochiensis* as it has cream coloured females *vs* yellow or gold, smaller number of ridges between the vulva and anus, a smaller mean for Granek's ratio, a different stylet knob shape, longer stylet length, tail terminus and presence of refractive bodies on the hyaline part of tail (usually four to seven refractive bodies *vs* absence) in J2. The ITS-rRNA and *COI* gene sequences clearly distinguish PPCN from all other PCN and *Globodera* species.

15.7.2.4 Tobacco cyst nematode, *G. tabacum*

Tobacco cyst nematode (TCN) is considered as a serious and important pest of shade and broadleaf tobacco. It is recorded from several countries in Europe, Asia, Africa, South and North America. *Globodera tabacum* was considered to be 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 horse nettle (*Solanum carolinense*), but otherwise differ in host preference. *Globodera tabacum* parasitizes *Nicotiana tabacum*, *S. carolinense*, tomato and other species of the genera *Nicotiana* and *Solanum*, as well as *Atropa belladona*, *Hyoscyamus niger*, *Nicandra physalodes* and *Capsicum annuum*. TCN may have four to five generations on tobacco under field conditions in the USA and Italy. Infected tobacco plants have small root systems and stunting of the aerial parts. TCN infection is often associated with increased damage from bacterial wilt and 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 favourable growing conditions.

Description: **Cyst**: L = 337–937 µm; W = 232–812 µm; L/W ratio = 0.9–1.5; fenestral diameter = 13–36 µm; number of ridges between anus and fenestra = 5–15; Granek's ratio = 1.0–4.2. **Male**: L = 710–1450 µm; stylet = 24–29 µm; spicules = 26–35 µm; gubernaculum = 9–12 µm. **J2**: L = 410–621 µm; stylet = 20–27 µm; tail hyaline region = 17–35 µm; tail = 33–64 µm. **Female**: body ovate to spherical with elongate neck, white, becoming yellow. **Cyst**: light shiny brown, circumfenestrate, abullate. **J2**: with well developed rounded basal knobs. Labial region offset by a slight constriction, marked by four striae. Terminus of tail finely rounded.

Globodera tabacum differs from *G. rostochiensis* as it has J2 with longer mean values of body length, mean stylet and cysts with a smaller mean number of cuticular ridges. It differs from *G. mexicana* as it has J2 with longer mean body length and from *G. pallida* owing to cysts with a smaller mean number of cuticular ridges and J2 with longer mean body length. The ITS-rRNA gene sequence clearly distinguishes TCN from all other *Globodera* species.

15.8 Genus *Punctodera* Mulvey & Stone, 1976

Diagnosis: (Punctoderinae) (after Siddiqi, 2000). **Mature females and cysts**: 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 μm), anus at a short distance from vulval fenestra. Circumfenestrate, fenestra surrounding vulva 16–40 μm in diameter, 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 eggsac. Parasites of monocotyledonous plants.

15.8.1 List of species and synonyms

Type species:

1. *Punctodera punctata* (Thorne, 1928) Mulvey & Stone, 1976

= *Heterodera punctata* Thorne, 1928
= *Heterodera* (*Globodera*) *punctata* (Thorne, 1928) Skarbilovich, 1959
Other species

2. *P. chalcoensis* Stone, Sosa Moss & Mulvey, 1976
3. *P. matadorensis* Mulvey & Stone, 1976
4. *P. stonei* Brzeski, 1998

15.8.2 Principal species

15.8.2.1 Grass cyst nematode, P. punctata

This species was described by Thorne in 1928 based on specimens from heavily infected wheat roots from a field in the Humboldt area, Saskatchewan, Canada. Subsequently, *P. punctata* was also reported as a common species infecting grasses from Europe, the USA and Canada. However, all attempts to infect wheat or other cereals with these nematodes failed to give any positive results. It has been suggested *P. punctata* might represent a complex of several closely related species. Several grasses are good hosts of this nematode. Only a single generation occurs each year.

Description: **Female and cyst**: L = 330–901 μm; W =170–720 μm; L/W ratio = 1.2–2.0; vulval fenestral diameter = 16–33 μm; anal fenestral diameter = 11–42 μm. **Male**: L = 910–1270 μm; stylet = 23–33 μm; spicules = 28–36 μm; gubernaculum = 8–10 μm. **J2**: L = 438–680 μm; stylet = 23–32 μm; DGO = 3.5–6.5 μm; tail hyaline region = 37–64 μm; tail = 63–93 μm. **Female and cyst**: ovoid, pear- or flask-shaped without vulval cone, white. Anal fenestra and vulval fenestra present (Fig. 15.7C). Vulva slit bordered by thickened ridges, set in a subcircular translucent area of cuticle. Newly formed cysts with conspicuous subcrystalline layer. **J2**: with well developed projecting anteriorly basal knobs (Fig. 15.4H). Conspicuous hyaline region at least twice as long as stylet, distal third of tail tapering, ending in a rounded point (Fig. 15.5H).

Punctodera punctata differs from other *Punctodera* species owing to the pear-shaped cysts and the absence of bullae.

15.9 Genus *Cactodera* Krall & Krall, 1978

Diagnosis: (Punctoderinae) (after Sturhan, 2002 with modification).
Mature females and cysts: lemon-shaped to spherical, with posterior protuberance. Vulva terminal, vulval slit <30 μm, fenestra circumfenestrate. Anus without fenestration. Bullae and underbridge absent, vulval denticles usually present. Cuticle with D-layer. The eggs are usually retained within the cyst body. Eggsac present or absent. Eggshell surface smooth or punctate. **J2**: have a lateral field with four incisures, phasmid openings punctiform.

15.9.1 List of species and synonyms

Type species:

1. *Cactodera cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978

= *Heterodera cacti* Filipjev & Schuurmans Stekhoven, 1941

Other species:

2. *C. acnidae* (Schuster & Brezina, 1979) Wouts, 1985

= *Heterodera acnidae* Schuster & Brezina, 1979

3. *C. amaranthi* (Stoyanov, 1972) Krall & Krall, 1978

= *Heterodera amaranthi* Stoyanov, 1972

4. *C. eremica* Baldwin & Bell, 1985
5. *C. estonica* (Kirjanova & Krall, 1963) Krall & Krall, 1978

= *Heterodera estonica* Kirjanova & Krall, 1963

6. *C. evansi* Cid del Prado & Rowe, 2000
7. *C. galinsogae* Tovar Soto, Cid del Prado, Nicol, Evans, Sandoval Islas & Martinez Garza, 2003
8. *C. milleri* Graney & Bird, 1990
9. *C. radicale* Chizhov, Udalova & Nasonova, 2008
10. *C. rosae* Cid del Prado & Miranda, 2008
11. *C. salina* Baldwin, Mundo-Ocampo & McClure, 1997
12. *C. thornei* (Golden & Raski, 1977) Mulvey & Golden, 1983

= *Heterodera thornei* Golden & Raski, 1977

13. *C. torreyanae* Cid del Prado Vera & Subbotin, 2014
14. *C. weissi* (Steiner, 1949) Krall & Krall, 1978

= *Heterodera weissi* Steiner, 1949

15.9.2 Principal species

15.9.2.1 *Cactus cyst nematode,* C. cacti

Cyst nematode-infecting cacti, *Discocactus akkermannii* and *Cereus speciosus*, both of which were expressing growth-declining symptoms, were 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 Mexico is associated with the international trade of infested ornamental cactus plants around the world. The cactus cyst nematode damages certain cacti grown as food-crops in Mexico and various ornamental cacti. It has been associated with succulent plants belonging to three families: Cactaceae: *Cereus*, *Cleistocactus*, *Coryphantha*, *Discocactus*, *Echinocactus*, *Echinopsis*, *Echinocereus*, *Epiphyllum*, *Gymnocalycium*, *Hatiora*, *Heliocereus*, *Hylocereus*, *Leuchtenbergia*, *Mammillaria*, *Melocactus*, *Notocactus*, *Nopalea*, *Notocactus*, *Opuntia*, *Oreocereus*, *Rebutia*, *Rhipsalis*, *Schlumbergera*, *Selenicereus* and *Thelocactus*; Umbelliferae: *Apium*; and Euphorbiaceae: *Euphorbia*. Infected plants may exhibit various symptoms including branched roots and increased numbers of rootlets. Plants become reddish-brown to yellow in colour, wilted and stunted, with reduced flower production and shortening of the flowering period. The life cycle takes around 30 days at 22°C.

Description: **Cyst**: L = 328–780 μm; W = 240–598 μm; L/W ratio = 1.1–2.0; fenestral diameter = 16–48 μm. **Male**: L = 910–1113 μm; stylet = 22–29 μm; spicules = 30–37 μm; gubernaculum = 10–15 μm. **J2**: L = 344–584 μm; stylet = 21–26 μm; tail hyaline region = 12–23 μm; tail = 34–60 μm. **Female**: body lemon-shaped to almost spherical, pearly white, yellow or golden. **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 (Fig. 15.7D). **Males**: have been reported as rare. **J2**: vermiform with hyaline region often shorter than stylet. Eggshells heavily punctuate.

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, and J2 with larger stylet. *Cactodera cacti* can be clearly differentiated from other species by sequences of the ITS-rRNA gene.

15.10 Genus *Dolichodera* Mulvey & Ebsary, 1980

Diagnosis: (Punctoderinae) (after Siddiqi, 2000, with modifications). **Females and cysts**: body elongate to oval, without terminal protuberance (Fig. 15.9A), white, swollen part 400–500 µm long, 140–270 µm wide, 2.0–2.8 times as long as wide, neck moderately long. Cuticle not annulated but with fine irregular striae. Vulval area terminal or just subterminal, circumfenestrate, fenestra approx. 20 µm in diameter, bullae present, perineal tubercles absent (Fig. 15.9B). Anus pore-like, lacking a fenestra, located 10–13 µm dorsal to vulval fenestral margin. Cyst with several large bullae. Perineal tubercles absent. Vulva circumfenestrate, underbridge absent. **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 diameter posterior to anus.

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

15.11 Genus *Betulodera* Sturhan, 2002

Diagnosis: (Punctoderinae) (after Sturhan, 2002). **Cysts**: 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

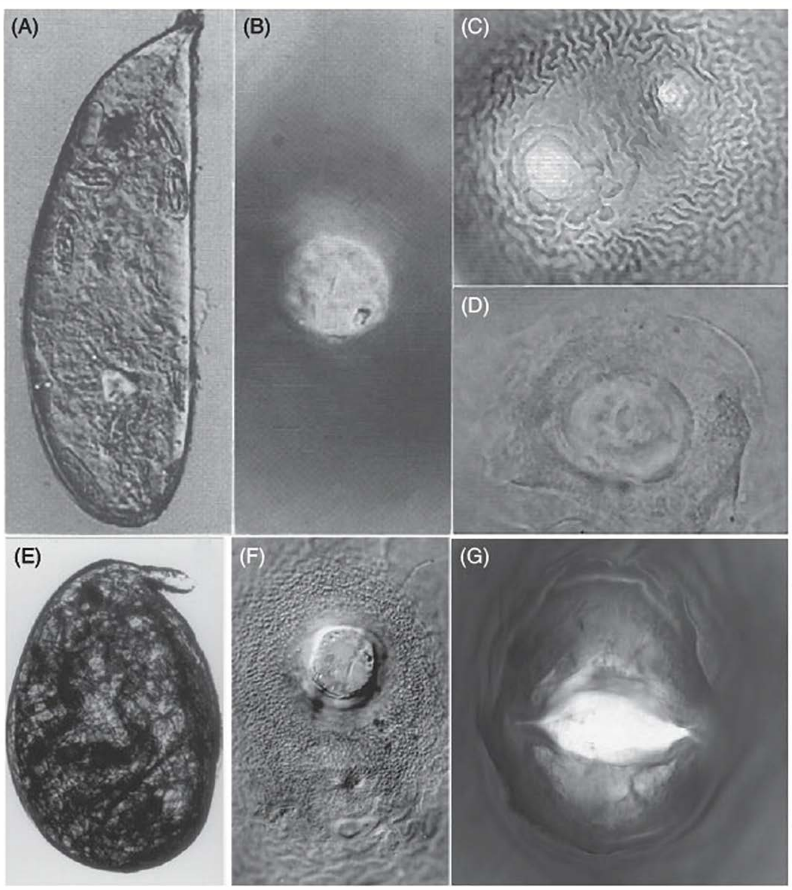

Fig. 15.9. *Dolichodera fluvialis*. (A) Cyst. (B) Fenestral area. *Betulodera betulae*. (C) Surface view of cyst cone top with single fenestra (large opening) and anus (small opening). (D) Fenestral area of white female during early stage of fenestration. *Paradolichodera tenuissima*. (E) Cyst. (F) Cyst posterior end with vulval fenestra and anus (below). *Vittatidera zeaphila*. (G) Fenestral area. A, B, After Mulvey and Ebsary (1980); C, D, Hirschmann and Riggs (1969); E, F, after Sturhan *et al.* (2007); G, Bernard *et al.* (2010).

circumfenestration, vulval slit short (<10 µm), underbridge absent, denticles occasionally present, anus without fenestration (Fig. 15.9C, D). **Male**: body twisted, no cloacal tube, spicules with bifid distal tips, phasmid openings punctiform. **J2**: has 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 (Figs 15.4G and 15.5G).

Type and only species:

Betulodera betulae (Hirschmann & Riggs, 1969) Sturhan, 2002

= *Heterodera betulae* Hirschmann & Riggs, 1969

= *Cactodera betulae* (Hirschmann & Riggs, 1969) Krall & Krall, 1978

15.12 Genus *Paradolichodera* Sturhan, Wouts & Subbotin, 2007

Diagnosis: (Punctoderinae) (after Sturhan *et al.*, 2007).

Female and cyst: elongate to ovoid, with rounded posterior end (Fig. 15.9E). 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, eggsac not observed. Labial disc squarish. Vulva terminal or subterminal, vulval slit short, circumfenestrate (Fig. 15.9F). Anus lacking fenestration. **Male**: body not twisted, lateral field with four incisures. 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

15.13 Genus *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010

Diagnosis: (Punctoderinae) (after Bernard *et al.*, 2010). **Cysts**: orange-brown to brown, lemon-shaped with short necks and vulval cone. Vulval cone slightly protuberant, membranous vulval lips persistent; vulval aperture circular to rhomboid, circumfenestrate, with irregular denticle-like protuberances around the periphery of orifice (Fig. 15.9G). Bullae, vulval bridge, vulval underbridge and internal denticles absent. Anus subterminal. **Male**: variable length, stylet knobs rounded. **J2**: with stylet less than 18 µm, conoid tail with narrowly rounded tip, phasmid apertures pore-like. Lateral field with four incisures. **Eggshell**: smooth.

Type and only species:

Vittatidera zeaphila Bernard, Handoo, Powers, Donald & Heinz, 2010

15.14 Conclusions and Future Prospects

For diagnostics of cyst nematodes, morphology is complementry to the use of molecular markers Accordingly, for any future proposals of new species in cyst nematode genera a blend of both morphological (including SEM), morphometric and molecular data is essential and desirable, with some of the genera rapidly approaching close to 121 valid species. The future prospects in cyst nematode taxonomy and diagnostics are dependent on molecular-based methodologies that will discriminate not only at the species level but also at the level of host races and pathotypes, thereby opening up opportunities for more focused management strategies. These techniques will offer rapid diagnostics and help resolve the present problems associated with relatively morphologically conserved organisms. Once these techniques are widely employed no doubt a number of the current nominal species will be shown to be junior synonyms, whilst others will be shown to be species complexes, possibly of sibling species. Molecular characterization will also enhance our understanding of the phylogeny of the genus and its relationship with other plant-parasitic nematodes.

15.15 Acknowledgements

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15.16 References

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