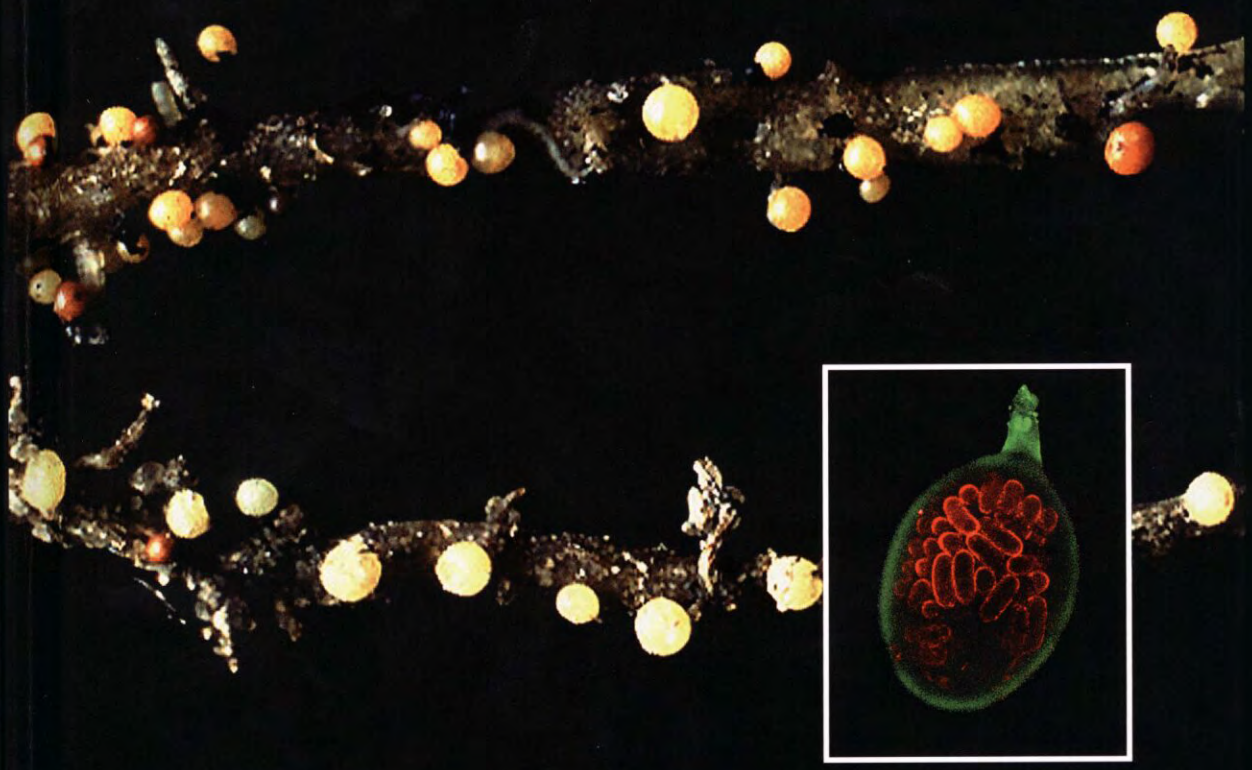
Nematology Monographs and Perspectives 8A

Systematics of Cyst Nematodes (Nematoda: Heteroderinae)

Sergei A. Subbotin, Manuel Mundo-Ocampo
and James G. Baldwin



Series Editors: David J. Hunt and Roland N. Perry

BRILL

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(Nematoda: Heteroderinae)

Sergei A. Subbotin, Manuel Mundo-Ocampo and James G. Baldwin

David J. Hunt and Roland N. Perry (Series Editors)

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Foreword . . .
Acknowledger

Introduction

1. Morpholog

Egg and em
Juvenile sta
Male adult
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2. Biology an

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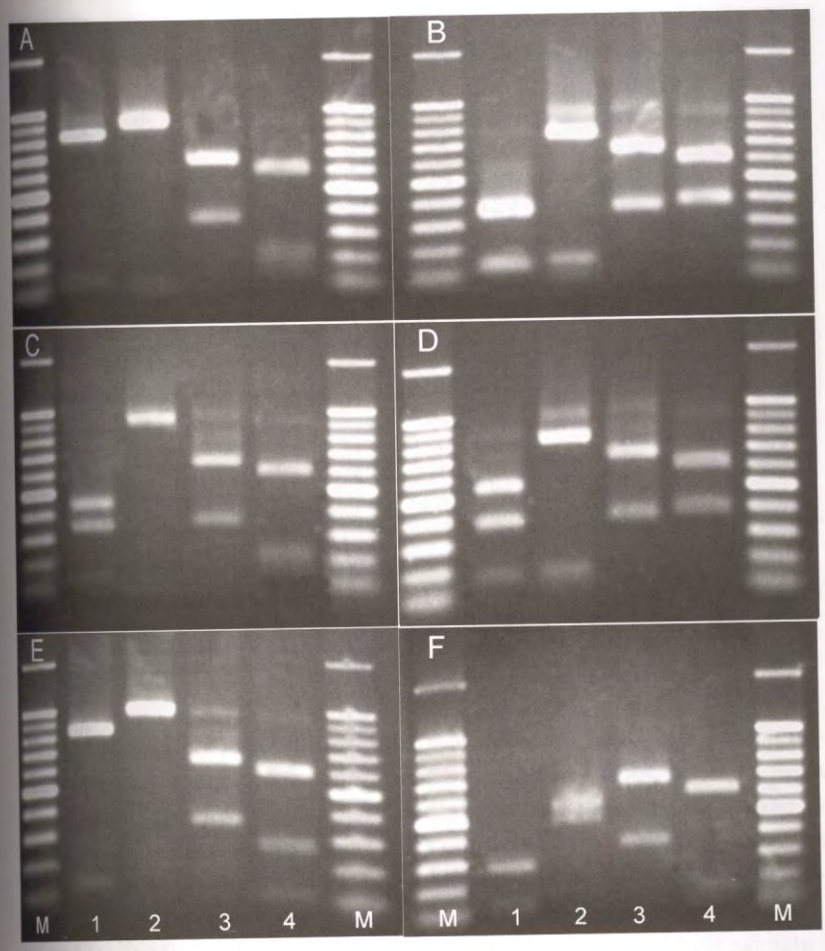


Fig. 39. *PCR-ITS-RFLP profiles for* Globodera *species. A:* G. rostochiensis*; B:* G. pallida*; C:* G. tabacum*; D:* G. mexicana *sp. n.; E:* G. artemisiae*; F:* G. zelandica*; M = 100 bp DNA ladder (Promega); 1 =* Bsh1236*I; 2 =* Hinf*I; 3 =* Pst*I; 4 =* Rsa*I. (Original.)*

SPECIES DESCRIPTIONS

1. *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959
(Figs 7E; 40, 41A, B; 42)

Common name: yellow or golden potato cyst nematode.

The golden cyst nematode associated with potato plants was first reported by Kühn (1881) and was considered to be *H. schachtii*, this be-

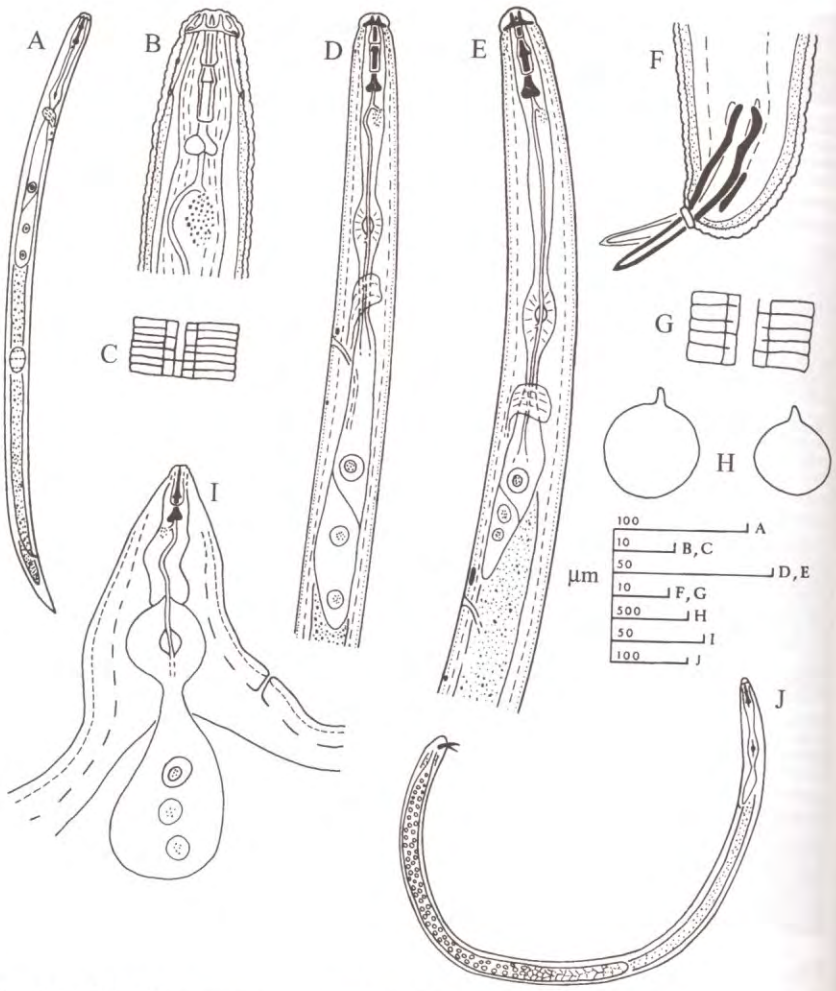


Fig. 40. Globodera rostochiensis. *A-D: J2. A: Entire body; B: Lip region; C: Lateral field; D: Pharyngeal region; E-G: Male. E: Pharyngeal region; F: Tail; G: Lateral field; H: Entire cysts; I: Anterior region of female; J: Entire male. After Stone (1973).*

ing the only known species of cyst nematode at that time. This was, however, an isolated observation and later, during the early 1900s, the potato cyst nematode became more widely known throughout Europe. Many concluded that at least one form of *H. schachtii* was specialised on solanaceous plants, causing "soil sickness of potatoes" (Spears, 1968).

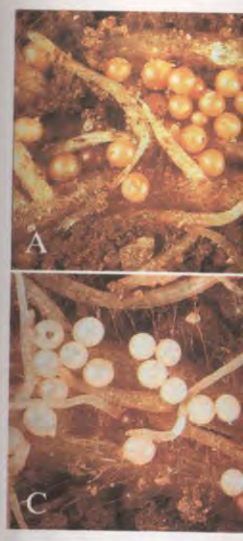
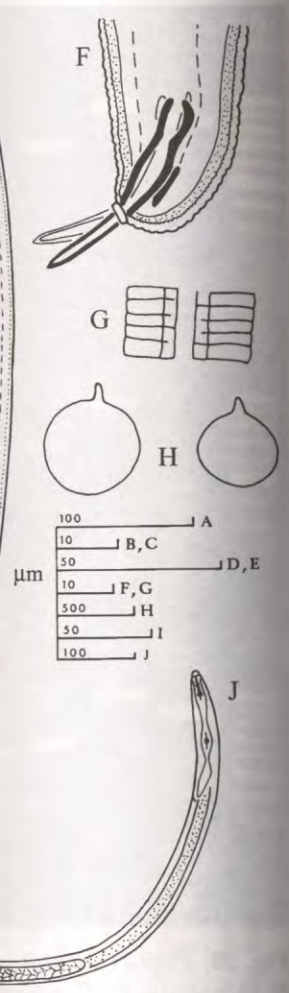


Fig. 41. *Potato cyst ne*
A, C: Females and cys
del Prado, Mexico; B
C = 500 µm; B, D =

Wollenweber (1923)
matodes parasitising
two separate species
on potato. However,
nematode as a subsp
lished a more compl
rostochiensis (Frankl
the neotype and gav
and descriptions of c
vided and reviewed b
1941; Franklin, 1951
Behrens, 1972; Øydv
Baldwin & Mundo-C
Andersson, 2004; Ma
al., 2006; Bacic *et al.*
from many countries
nematode pest of pota



re body; B: Lip region; C: Pharyngeal region; F: Tail; of female; J: Entire male.

at that time. This was, ing the early 1900s, the own throughout Europe. *achtii* was specialised on otatoes" (Spears, 1968).

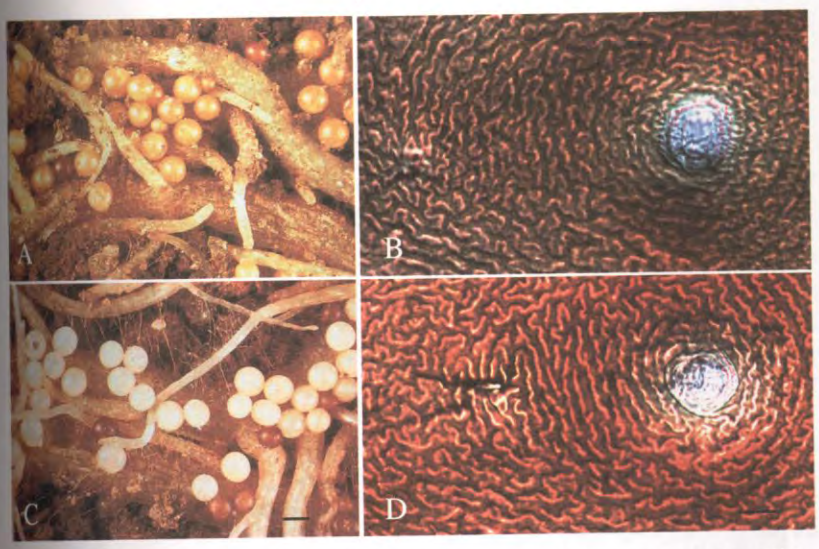


Fig. 41. *Potato cyst nematodes. A, B:* Globodera rostochiensis*; C, D:* G. pallida. *A, C: Females and cysts on roots; B, D: Vulval plates. (A, C courtesy of I. Cid del Prado, Mexico; B, D courtesy of V.N. Chizhov, Russia.) (Scale bars: A, C = 500 µm; B, D = 10 µm.)*

Wollenweber (1923) recognised morphological differences between nematodes parasitising potato and sugar beet and proposed to distinguish two separate species, *H. schachtii* on sugar beet and *H. rostochiensis* on potato. However, some researchers continued to consider potato cyst nematode as a subspecies of *H. schachtii* until 1940 when Franklin published a more complete morphological description and diagnosis of *H. rostochiensis* (Franklin, 1951). Golden and Ellington (1972) designated the neotype and gave a redescription of this species. Morphometrics and descriptions of different stages of *G. rostochiensis* have been provided and reviewed by many authors (Filipjev & Schuurmans Stekhoven, 1941; Franklin, 1951; Trudgill *et al.*, 1970; Webley, 1970; Green, 1971; Behrens, 1972; Øydvin, 1973; Stone, 1973a, b; Evans & Franco, 1977; Baldwin & Mundo-Ocampo, 1991; Gonzales *et al.*, 1992; Manduric & Andersson, 2004; Manduric *et al.*, 2004; Sirca & Urek, 2004; Knoetze *et al.*, 2006; Bacic *et al.*, 2008). Today, *G. rostochiensis* has been reported from many countries and is probably the most economically important nematode pest of potato.

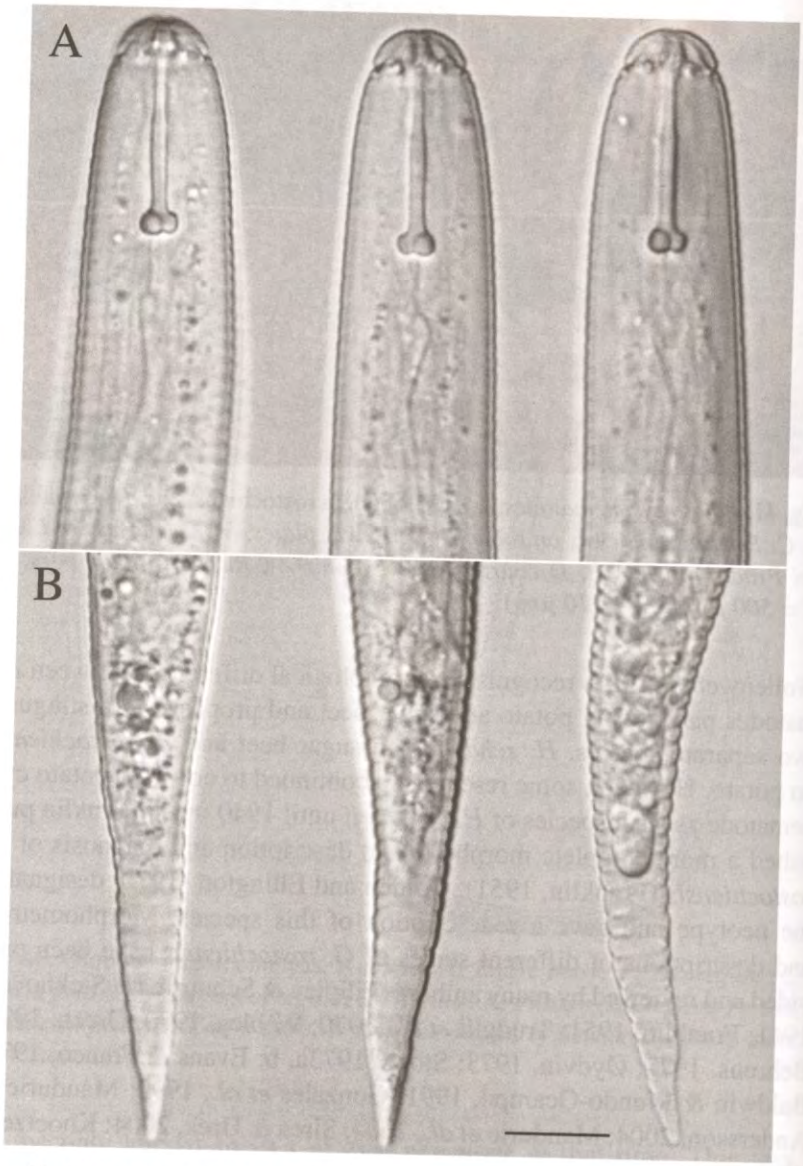


Fig. 42. Globodera rostochiensis. *J2. A: Anterior region; B: Tail. (Original.) (Scale bar = 10 μm.)*

MEASUREMENTS

After Golden and Elling

– *Neotype female*:
1.4; stylet = 23.8 μm; D
130 μm; vulval slit = 1

– *Female* (n = 50)
W = 0.34 (0.27-0.43) m
24) μm; DGO = 6.2 (5.
(105-175) μm; vulval sl
fenestral width = 18-2
(39-80) μm.

– *Cyst*: see Table 1

– *Male* (n = 50): L
a = 27 (22-36); b = 5
(25-27) μm; DGO = 6
gubernaculum = 12 (10-

– *J2*: see Table 16.

– *Egg* (n = 50): L =
ratio = 2.3 (2.0-2.6).

After Stone (1973b).

– *Female* (n = 25):
labial region diam. =
valve = 73.2 ± 14.6 μm
diam. = 22.4 ± 2.8 μm
basin = 60.0 ± 10.1 μ
axis = 21.6 ± 3.5.

– *Male* (n = 50): L =
0.6 μm; labial region hei
DGO = 5.3 ± 0.9 μm;
7.4 μm, excretory pore
body diam. at anus level
gubernaculum = 10.3 ±

Onions (1953) reporte
stylet = 33 μm.

region; B: Tail. (Original.)

MEASUREMENTS

After Golden and Ellington (1972).

– *Neotype female*: Length = 470 μm; W = 320 μm; L/W ratio = 1.4; stylet = 23.8 μm; DGO = 6.4 μm; anterior end to excretory pore = 130 μm; vulval slit = 10 μm; vulva-anal distance = 41 μm.

– *Female* (n = 50): L (including neck) = 0.52 (0.42-0.64) mm; W = 0.34 (0.27-0.43) mm; L/W ratio = 1.5 (1.2-2.0); stylet = 23 (22-24) μm; DGO = 6.2 (5.8-7.0) μm; anterior end to excretory pore = 131 (105-175) μm; vulval slit = 9 (6-11) μm; fenestral length = 15-30 μm; fenestral width = 18-20 μm; distance from anus to vulva edge = 47 (39-80) μm.

– *Cyst*: see Table 16.

– *Male* (n = 50): L = 1.08 (0.89-1.27) mm; W = 39 (31-46) μm; a = 27 (22-36); b = 5.9 (4.9-7.3); c = 267 (161-664); stylet = 26 (25-27) μm; DGO = 6.4 (5.3-7.0) μm; spicules = 35 (32-39) μm; gubernaculum = 12 (10-14) μm; tail = 4.4 (1.7-6.7) μm.

– *J2*: see Table 16.

– *Egg* (n = 50): L = 105 (95-115) μm; W = 45 (42-48) μm; L/W ratio = 2.3 (2.0-2.6).

After Stone (1973b).

– *Female* (n = 25): Stylet = 22.9 $\pm$ 1.2 μm; DGO = 5.7 $\pm$ 0.9 μm; labial region diam. = 5.2 $\pm$ 0.7 μm; anterior end to: median bulb valve = 73.2 $\pm$ 14.6 μm, excretory pore = 145.3 $\pm$ 17.4 μm; fenestral diam. = 22.4 $\pm$ 2.8 μm; vulval slit = 9.7 $\pm$ 1.9 μm; anus to vulval basin = 60.0 $\pm$ 10.1 μm; number of cuticular ridges on anal vulval axis = 21.6 $\pm$ 3.5.

– *Male* (n = 50): L = 1197 $\pm$ 100 μm; labial region diam. = 11.8 $\pm$ 0.6 μm; labial region height = 6.8 $\pm$ 0.3 μm; stylet = 25.8 $\pm$ 0.9 μm; DGO = 5.3 $\pm$ 0.9 μm; anterior end to: median bulb valve = 98.5 $\pm$ 7.4 μm, excretory pore = 172.3 $\pm$ 12.1 μm; tail = 5.4 $\pm$ 1.1 μm; body diam. at anus level = 13.5 $\pm$ 0.4 μm; spicules = 35.5 $\pm$ 2.8 μm; gubernaculum = 10.3 $\pm$ 1.5 μm.

Onions (1953) reported giant J2 of *G. rostochiensis*: L = 783-817 μm; stylet = 33 μm.

Table 16. *Morphometrics of cysts and J2 of* Globodera rostochiensis *(all measurements in μm).*

Population Characters	Germany, Golden & Ellington (1972)	Germany, Rostock, Stone (1973b)	UK, Feltwell, Evans & Franco (1977)	Russia, Kazachenko (1993)	Pakistan, Shahina & Maqbool (1995)	Slovenia, Libelice, Sirca & Urek (2004)	Serbia, Bata, Bacic *et al.* (2008)
Cyst (n)	50	25	–	25	25	10	10
Length (excl. neck)	680 (450-990)	445 ± 50	–	589 (467-725)	533 (480-665)	–	–
Width	540 (250-810)	382 ± 61	–	559 (430-713)	502 (387-654)	–	–
Length/width	1.27 (1.0-1.8)	–	–	1.04 (0.9-1.3)	–	–	–
Fenestral diam.	15.0 (8-20)	18.8 ± 2.2	20.7	14.1 (10.4-19.5)	17.2 (16-19.5)	17.9 ± 1.7 (14.6-19.4)	18.4 ± 2.1 (14.6-20.7)
Number of ridges		–	17.2	–	–	18.3 ± 1.6 (16-22)	20-24
Anus-fenestra distance	68 (29-116)	66.5 ± 10.3	70.2	50.7 (33.8-78)	61 (53.5-72.3)	64.8 ± 4.7 (58.1-73.3)	55.9 ± 8.3 (44.9-72.9)
Granek's ratio	4.5 (2.0-7.0)	3.6 ± 0.8	3.4	3.7 (2.1-5.4)	–	3.6 ± 0.4 (3.0-4.3)	3.0 ± 0.8 (2.3-4.6)
J2 (n)	50	50	–	25	20	30	10
Length	430 (370-470)	468 ± 20	457.3	392 (366-406)	465 (439-502)	434.4 ± 16.7 (402.7-468.0)	426.1 ± 32.7
a	19 (16-23)	–	–	21.3 (18.6-25.4)	23 (19-29.5)	21.4 ± 1.1 (18.9-23.0)	–
b	2.3 (2.2-2.5)	–	–	3 (2.7-3.4)	–	–	–
c	8 (7-9)	–	–	9 (8.1-11)	12.5 (10.0-15.5)	9.0 ± 0.6 (7.8-11.0)	–

Table 16. *(Continued).*

Population Characters	Germany, Golden & Ellington (1972)	Germany, Rostock, Stone (1973b)	UK, Feltwell, Evans & Franco (1977)	Russia, Kazachenko (1993)	Pakistan, Shahina & Maqbool (1995)	Slovenia, Libelice, Sirca & Urek (2004)	Serbia, Bata, Bacic *et al.* (2008)
Stylet	22 (21-23)	21.8 ± 0.7	21.0	19.9 (19.5-20.8)	22.5 (20.5-23.5)	20.7 ± 0.6	21.2 ± 0.7

Table 16. *(Continued).*

Population / Characters	Germany, Golden & Ellington (1972)	Germany, Rostock, Stone (1973b)	UK, Feltwell, Evans & Franco (1977)	Russia, Kazachenko (1993)	Pakistan, Shahina & Maqbool (1995)	Slovenia, Libelice, Sirca & Urek (2004)	Serbia, Bata, Bacic *et al.* (2008)
Stylet	22 (21-23)	21.8 ± 0.7	21.0	19.9 (19.5-20.8)	22.5 (20.5-23.5)	20.7 ± 0.6 (18.9-22.4)	21.2 ± 0.7 (19.8-21.7)
Labial region height	–	4.6 ± 0.6	–	–	–	–	–
Labial region diam.	–	9.9 ± 0.4	–	–	–	–	–
DGO	5.5 (5.0-6.7)	2.6 ± 0.6	–	5.0 (3.9-5.2)	4.5-5.5	3.8 ± 0.8 (2.4-5.0)	–
Anterior end to excretory pore	–	100.5 ± 2.4	95.5	84.7 (75.6-91)	80-90	–	–
Anterior end to median bulb valve	68 (64-76)	69.2 ± 1.9	64.5	61 (54.6-66.3)	–	65.0 ± 6.9 (55.3-77.6)	–
Body diam. at mid-body	23 (19-26)	–	–	–	–	20.1 ± 0.6 (18.9-22.4)	–
Body diam. at anus level	–	11.4 ± 0.6	–	10.9 (10.4-11.7)	–	12.7 ± 0.7 (11.3-14.4)	–
Hyaline region	24 (18-30)	26.5 ± 1.8	–	19.9 (19.5-20.8)	25.0 (22.2-28.4)	25.1 ± 1.6 (22.5-29.9)	22.4 ± 2.0 (19.4-24.3)
Tail	51 (44-57)	43.9 ± 11.6	46.4	43.5 (36.4-46.8)	48.5 (41.0-51.4)	48.5 ± 2.7 (42.6-53.8)	44.9 ± 4.8 (36.5-53.5)

DESCRIPTION

After Stone (1973b).

Female

Pearly white, subspherical to ovate, with elongate, protruding neck. Colour changing from white to yellow to light golden as female matures to cyst stage. Cuticle thick, with superficial, rugose, lace-like pattern, D-layer present, punctations resolved near or beneath surface. Labial region slightly offset, bearing two annuli. Labial framework weakly developed. Stylet fairly strong, straight to slightly curved, with well developed rounded basal knobs, sloping posteriorly. Median bulb large, nearly spherical, with well developed valve. Pharyngeal glands often obscured but appearing clustered. Excretory pore conspicuous, always at or near base of neck. Vulva terminal, slit of medium length. Vulval area circumfenestrate. No anal fenestration, but anus and vulva both lying in a 'vulval basin', anal area not encircled by cuticular rings. Often beneath vulva, generally in a cluster, are vulval bodies of highly variable size and shape, large superficial tubercles clumped near vulva. Vulva ellipsoid in shape, anus shorter than vulva. All eggs retained in body, no egg mass.

Cyst

Brown, ovate to spherical in shape with protruding neck, circumfenestrate, abullate, without distinct 'vulval bodies' commonly seen in white females. Fenestra circular, anus conspicuous at apex of a V-shaped subsurface cuticular mark. Cyst wall pattern basically as in female but often more prominent, especially near mid-body, tending to form wavy lines going around body. Subcrystalline layer absent. Punctations generally present but variable in intensity and arrangement. Each cyst containing 200-1000 eggs.

Male

Body vermiform, slightly tapering at both anterior and posterior regions. Cuticle with prominent annulation. Labial region slightly offset, hemispherical, with six annuli. Labial framework heavily sclerotised. Stylet strong, with prominent knobs. Anterior and posterior cephalids present. Lateral fields with four equally spaced lines. Median bulb ellipsoidal. Excretory pore *ca* two annuli posterior to often distinct hemizonid. One testis. Spicules slightly arcuate, tips rounded, not notched. Tail short, variable in length and shape.

J2

Body tapering at both ext
annulation well defined. L
most of body length, outer
Labial region slightly offs
at base than anteriorly, pre
flattened, appearance. Labia
developed, with prominent
and posterior cephalids p
ellipsoidal. Isthmus and p
Excretory pore almost adj
Genital primordium slightl
commonly resolved. Tail tap
generally difficult to see, wh

TYPE HOST AND LOCALITY

Roots of potato, *Solanum*
Rostock, Germany.

OTHER HOSTS

Tomato, *Solanum lycoper*
agronomic crops other than
Other hosts include many *Sc*
L., *Nicotiana acuminata* Ho
G. Don, *Salpiglossis* spp.,
Schlecht (Mai & Lownsber
& Engel, 1973; Stone, 197
Stelter, 1987; CAB Internatio
(Table 17). *Chenopodium*
Polygonum nepalense Meisn
species (Inagaki, 1984).

DISTRIBUTION

The centre of origin of pot
in South America from wh
potatoes, probably around 1
were spread with seed potat
covers temperate zones dow

J2

Body tapering at both extremities but more at posterior end. Cuticular annulation well defined. Lateral fields with four lines extending for most of body length, outer two lines crenate but without areolation. Labial region slightly offset, bearing 4-6 annuli, considerably wider at base than anteriorly, presenting a rounded, though rather anteriorly flattened, appearance. Labial framework heavily sclerotised. Stylet well developed, with prominent rounded knobs as viewed laterally. Anterior and posterior cephalids present. Valve of median bulb prominent, ellipsoidal. Isthmus and pharyngeal glands typical for the genus. Excretory pore almost adjacent yet slightly posterior to hemizonid. Genital primordium slightly posterior to mid-body, with four cells commonly resolved. Tail tapering to small, rounded terminus. Phasmids generally difficult to see, when visible, located about halfway along tail.

Type host and locality

Roots of potato, *Solanum tuberosum* (Solanaceae, Solanales) from Rostock, Germany.

Other hosts

Tomato, *Solanum lycopersicum* L. and eggplant, *S. melongena* L., are agronomic crops other than potato that are attacked by *G. rostochiensis.* Other hosts include many *Solanum* spp., *Datura* spp. *Hyoscyamus niger* L., *Nicotiana acuminata* Hook., *Physalis* spp. *Physochlaina orientalis* G. Don, *Salpiglossis* spp., *Capsicum annuum* L., *Saracha jaltomata* Schlecht (Mai & Lownsbery, 1948; Mai, 1952; Ellenby, 1954; Stelter & Engel, 1973; Stone, 1973b; Roberts & Stone, 1981; Brodie, 1984; Stelter, 1987; CAB International, 2005a; Sullivan *et al.*, 2007 and others) (Table 17). *Chenopodium album* var. *centrorubrum* Makino (CAC), *Polygonum nepalense* Meisn. and *P. convolvus* L. are poor hosts for this species (Inagaki, 1984).

Distribution

The centre of origin of potato cyst nematodes is the Andes Mountains in South America from where they were introduced to Europe with potatoes, probably around 1850 (Evans *et al.*, 1975). From here they were spread with seed potatoes to other areas. The present distribution covers temperate zones down to sea level and in the tropics at higher

Table 17. *Differential host range of* Globodera *parasitising solanaceous plants (after Campos-Vela, 1967; Stelter, 1987 and Mugniéry and Phillips, 2007 with modification).*

	Solanum tuberosum (potato)	*Solanum lycopersicum* (tomato)	*Nicotiana tabacum* (tobacco)	*Solanum nigrum* (nightshade)	*Solanum dulcamara* (bittersweet)
G. rostochiensis	+	+	−	−	+
G. pallida	+	+	−	−	+
G. tabacum	−	+	+	+	+
G. mexicana **sp. n.**	−	+	−/+	+	+

altitudes. In these areas, the distribution is linked with that of the potato crop.

Europe: Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, UK, Ukraine, Yugoslavia (former); *Asia*: Armenia, Georgia, India, Indonesia, Iran, Japan, Lebanon, Oman, Pakistan, Philippines, Sri Lanka, Tajikistan, Turkey; *Africa*: Algeria, Egypt, Libya, Morocco, Sierra Leone, South Africa, Tunisia; *North and Central America*: Canada, Costa Rica, Mexico, Panama, USA (New York; eradicated in Delaware); *South America*: Argentina (eradicated), Bolivia, Brazil, Chile, Colombia, Ecuador, Guatemala, Peru, Venezuela; *Oceania*: Australia, New Zealand (EPPO Quarantine Pest).

Note

Globodera arenaria, recently described by Chizhov *et al.* (2008), is synonymised here with *G. rostochiensis*, because it does not differ morphologically and molecularly from the golden potato cyst nematode (Subbotin, unpubl.).

Relationships

Globodera rostochiensis is morphologically similar to *G. pallida* and *G. tabacum*. It differs from *G. pallida* by yellow or gold *vs* cream

coloured maturing females, higher number of ridges between the vulva and anus (16-31 *vs* 8-20), larger mean for Granek's ratio (3.0-4.5 *vs* 2.1-2.5), stylet knob shape (rounded *vs* anteriorly projected), shorter average stylet length (20-22 *vs* 23-24 μm) and rounded *vs* more pointed J2 tail terminus. It differs from *G. tabacum* by shorter mean J2 body length (392-468 *vs* 476-556 μm), shorter mean stylet (20-22 *vs* 21-25 μm) and in the cyst by higher mean number of cuticular ridges (17-20 *vs* 7-9) (Table 14).

PCR-RAPD (Thiéry *et al.*, 1997a) and ITS-rRNA analysis (Thiéry & Mugniéry, 1996; Subbotin *et al.*, 2000b) have revealed that *G. rostochiensis* is more closely related to *G. tabacum* than to other species (Fig. 27).

Biology

In temperate regions, *G. rostochiensis* usually completes only one generation (Fig. 2) (Morris, 1971), although a second generation may be initiated but not completed (Jones, 1950; Evans, 1969). In subtropical regions two generations might occur (Philis, 1980; Greco *et al.*, 1988). 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.

Pathogenicity

Heavily infected plants become yellow and stunted. Infected plants have reduced root systems which are abnormally branched and are 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 eight and 64 eggs/g of soil, yield losses of about 20 and 70%, respectively, can be expected (Greco, 1988). Seinhorst (1965, 1982), Brown (1969) and Elston *et al.* (1991) proposed a model that described the relationships of potato cyst nematode population densities before planting with potato yield and post-harvest nematode populations. The damaging effect of potato cyst nematodes is not only determined by nematode density, but also by such factors as cultivar, crop husbandry and environmental conditions (Mulder *et al.*, 1997). Potato cyst nematodes are responsible for annual potato tuber losses of up to 9% in Europe (Evans & Stone, 1977).

BIOCHEMICAL AND MOLECULAR CHARACTERISATION

Application of the IEF of proteins and PCR-ITS-RFLP for differentiation of *G. rostochiensis* from other *Globodera* species has been tested by several authors (Tables 9, 12). The enzymes *Alu*I generated a unique profile for *G. rostochiensis* after restriction of the PCR-ITS product. RFLP profile and approximate sizes of restriction fragments generated by diagnostic enzymes for this species are given in Figure 39 and Table 15, respectively. Species specific PCR primers for identification of this species are given in Table 13. IEF profiling is a reliable tool to distinguish the two species of potato cyst nematodes from each other (Fig. 33).

2. *Globodera artemisiae* (Eroshenko & Kazachenko, 1972) Behrens, 1975

(Figs 43, 44)

Common name: wormwood cyst nematode.

The wormwood cyst nematode was isolated from roots of *Artemisia rubripes* Nakai in the Primorsky Territory of the Russian Far East and was described by Eroshenko and Kazachenko (1972) as *H. artemisiae*. In 1976, similar cyst nematodes were found in roots of *A. princeps* Pamp. in the Shimabara Peninsula, Nagasaki Prefecture, Japan, and described by Ogawa *et al.* (1983) as *G. hypolysi*. Ogawa *et al.* (1983) reported that there were differences between *G. hypolysi* and *G. artemisiae* with respect to the distance from the anus to the fenestra in the cysts and the female and in body length and shape of stylet knobs of the J2. However, comparison of morphometrics of different stages of different populations provided by Chen *et al.* (1994), Brzeski (1998), Choi and Kim (2001), Manduric and Andersson (2004) and Dobosz *et al.* (2006) did not support this claim. Comparison of the ITS-rRNA gene sequences for both species did not reveal any significant differences and thus this data supported the synonym of *G. hypolysi* with *G. artemisiae* (Subbotin *et al.*, unpubl.).

MEASUREMENTS

After Eroshenko and Kazachenko (1972).

– *Paratype females* (n = 25): L = 562 (504-650) μm; W = 345 (275-388) μm; neck = 105 (10-110) μm; stylet = 23.2 (22.4-23.8) μm;

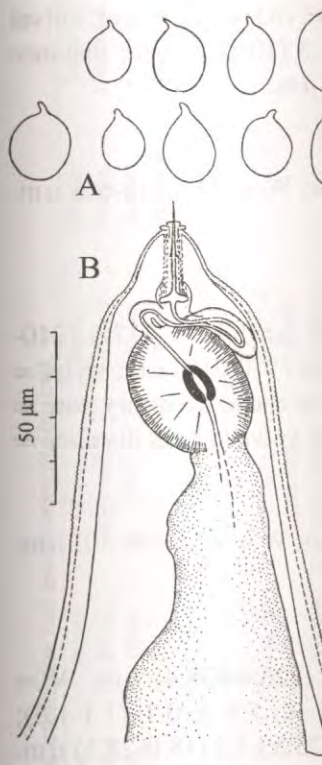


Fig. 43. Globodera artemisi
Anterior region of J2; D: Tai
G: Tail of male. After Eroshe

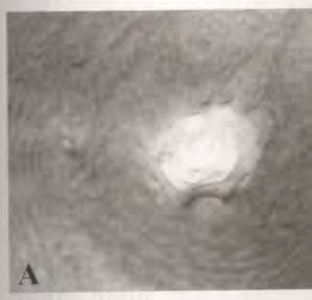


Fig. 44. Globodera artemisi
10 µm.)

DISTRIBUTION

Europe: Bosnia and Herzegovina (Golden & Klindić, 1973), Estonia (Krall, 1977), Germany (Sturhan, 1980, 1984), Latvia (Krall, 1977), Poland (Radziwinowicz, 1977), Slovakia (Sturhan & Liskova, 2004), Slovenia (Sirca & Urek, 2004), UK (Sykes & Webley, 1979).

OTHER HOSTS

Experimental testing revealed several hosts from the Asteraceae: *Achillea*, *Tripleurospermum*, *Matricaria*, *Pyrethrum*, *Leucanthemum*, *Tanacetum*, *Anthemis* and *Chrysanthemum* (Krall, 1977). Brzeski (1998) reported development of this species on *Anthemis cotula* L. and *Matricaria chamomilla* L.

RELATIONSHIPS

Globodera millefolii is similar to *G. artemisiae* from which it is distinguished by longer J2 stylet (mean = 25 *vs* 21-24 μm), longer gubernaculum (11.2-12.9 *vs* 6.0-9.9 μm) and different host ranges (Table 14).

BIOCHEMICAL AND MOLECULAR CHARACTERISATION

The PCR-ITS-rRNA profile for *G. millefolii* is given in Table 15. Enzymes *Alu*I and *Bsh*1236I generated unique profiles after digestion of PCR-ITS-rRNA product.

8. *Globodera pallida* Stone, 1973
(Figs 7F; 12A; 41C, D; 54, 55)

Common name: white or pale potato cyst nematode.

Guile (1966, 1970) was the first to note that females of pathotype A of the golden cyst nematode became golden yellow shortly after they ruptured the root cortex, whereas those of pathotype E remained white until they died and became brown cysts. Webley (1970) found that the J2 of pathotype A differed in measurements from those of pathotypes B and E and that the shape of the stylet knobs also differed among pathotypes. Jones *et al.* (1970) suggested that females with a cream or white colour represented an undescribed species and Trudgill

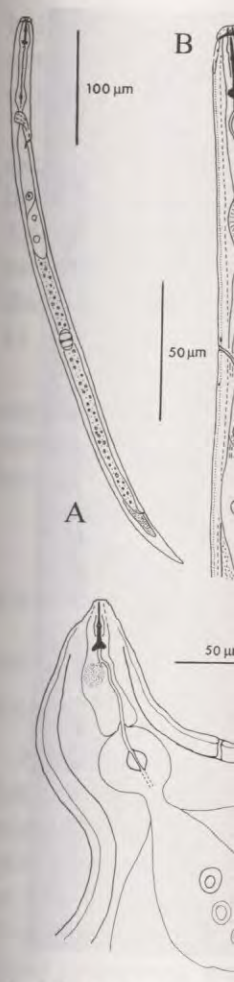


Fig. 54. Globodera pa
D: Tail; E-H: Males. E,
region of female; J: Cy

and Carpenter (197
electrophoresis in p
species as *Heteroder*
subgenus *Globodera.*
were also given by T
Ross (1972), Behren
and Ellis (1974), Ev

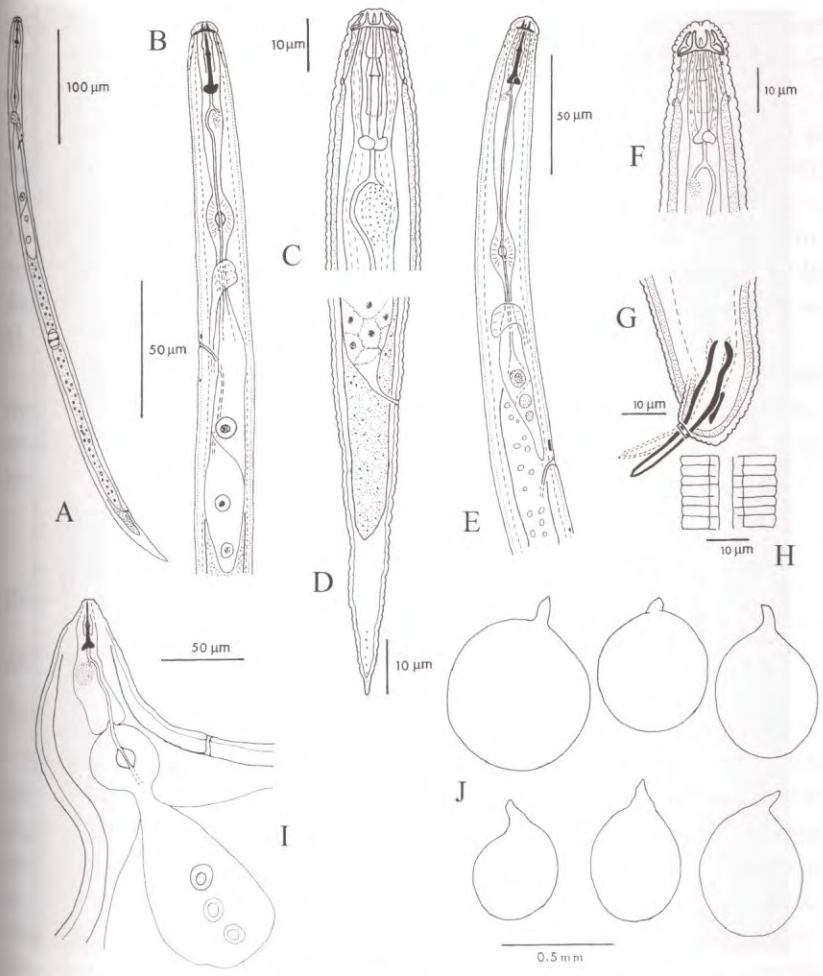


Fig. 54. Globodera pallida. *A-D: J2. A: Entire body; B, C: Anterior region; D: Tail; E-H: Males. E, F: Anterior region; G: Tail; H: Lateral field; I: Anterior region of female; J: Cysts. After Stone (1972).*

and Carpenter (1971) were able to distinguish these females using electrophoresis in polyacrylamide gels. Stone (1973a) described this species as *Heterodera pallida* but considered that it belonged to the subgenus *Globodera*. Measurements and descriptions of different stages were also given by Trudgill *et al.* (1970), Webley (1970), Bowman and Ross (1972), Behrens (1972), Øydvin (1973), Stone (1973c), Hesling and Ellis (1974), Evans and Franco (1977), Hesling (1978), Baldwin

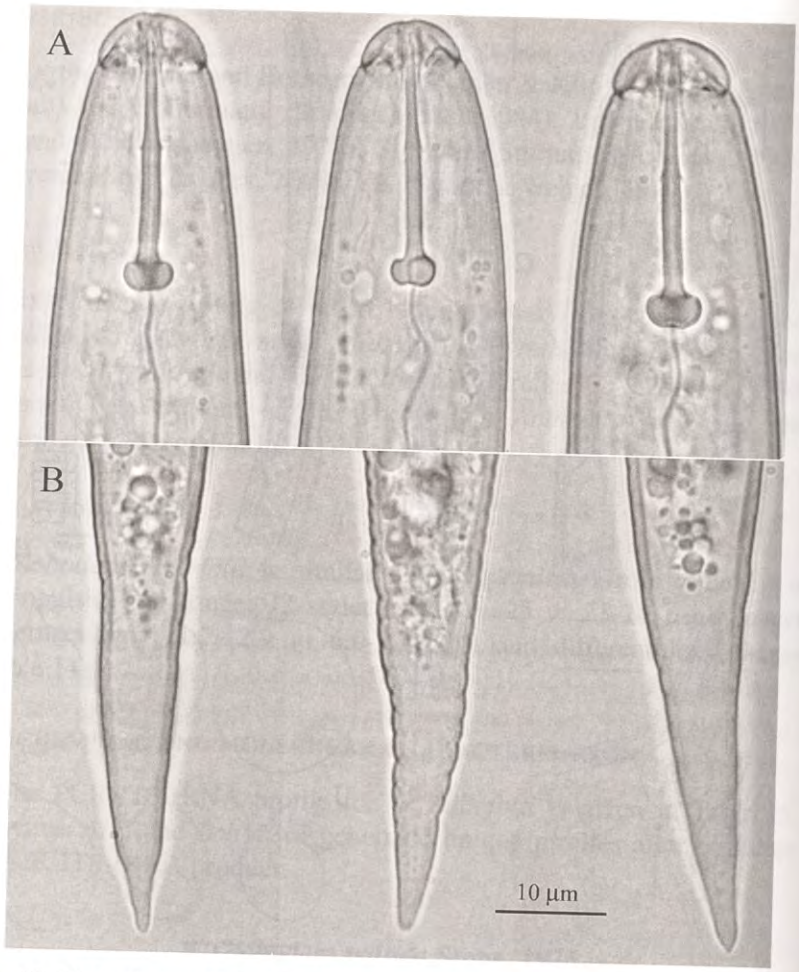


Fig. 55. Globodera pallida. *J2. A: Anterior region; B: Tail. (Courtesy of A.-M. Deeren, ILVO, Belgium.)*

and Mundo-Ocampo (1991), Gonzales *et al.* (1992), Manduric *et al.* (2004), Skantar *et al.* (2007), *etc*. The pale cyst nematode, *G. pallida*, is considered to be a major pest of potato crops in cool temperate climates.

MEASUREMENTS

After Stone (1973a).

– *Paratype females* (n = 25): Stylet = 27.4 ± 1.1 μm; labial region diam. = 5.2 ± 0.5 μm; DGO = 5.4 ± 1.1 μm; anterior end to: median

bulb valve = 67.2 ± 1
slit = 11.5 ± 1.3 μm
of cuticular ridges on
– *Paratype male*
excretory pore = 28.4
labial region height =
3.4 ± 1.0 μm; anteri
excretory pore = 176
diam. = 13.5 ± 2.1 μ
11.3 ± 1.6 μm.
– *Holotype J2*: L
region diam. = 9 μm;
median bulb valve =
hyaline region = 31 μ
– *Paratype J2*: se

DESCRIPTION

After Stone (1973a).

Female

Body subspherical w
isthmus and anterior p
populations passing, a
glossy brown when dea
or two prominent annu
changing to reticulate
Head framework weak
backward. Very large
large crescentic valve p
displaced anteriad, three
at base of neck. Intern
hyaline secretions on cu
end, set in a slight circu
between anus and vulva
few cross connections. S
much of body surface, n
crescents.

bulb valve = 67.2 ± 18.7 μm, excretory pore = 139.7 ± 15.5 μm; vulval slit = 11.5 ± 1.3 μm; anus to vulval basin = 44.6 ± 10.9 μm; number of cuticular ridges on anal-vulval axis = 12.5 ± 3.1.

– *Paratype males* (n = 50): L = 1198 ± 104 μm; body width at excretory pore = 28.4 ± 1.3 μm; labial region diam. = 12.3 ± 0.5 μm; labial region height = 6.8 ± 0.3 μm; stylet = 27.5 ± 1.0 μm; DGO = 3.4 ± 1.0 μm; anterior end to: median bulb valve = 96.0 ± 7.1 μm, excretory pore = 176.4 ± 14.5 μm; tail = 5.2 ± 1.4 μm; anal body diam. = 13.5 ± 2.1 μm; spicules = 36.3 ± 4.1 μm; gubernaculum = 11.3 ± 1.6 μm.

– *Holotype J2*: L = 510 μm; labial region height = 5 μm; labial region diam. = 9 μm; stylet = 25 μm; DGO = 2 μm; anterior end to: median bulb valve = 74 μm; excretory pore = 106 μm, tail = 53 μm; hyaline region = 31 μm; anal body diam. = 13 μm.

– *Paratype J2*: see Table 21.

Description

After Stone (1973a).

Female

Body subspherical with projecting neck bearing head, pharynx corpus, isthmus and anterior part of pharyngeal glands. White in colour, some populations passing, after 4-6 weeks, through a cream stage, turning glossy brown when dead. Labial region with amalgamated lips and one or two prominent annuli, deep irregular annulations present on neck, changing to reticulate pattern of ridges over most of body surface. Head framework weakly developed, hexaradiate. Stylet knobs sloping backward. Very large median pharyngeal bulb, almost circular with large crescentic valve plates. Pharyngeal gland lobe broad, frequently displaced anteriad, three gland nuclei. Prominent excretory pore situated at base of neck. Internal structures in neck region often obscured by hyaline secretions on cuticle surface. Vulva a transverse slit at posterior end, set in a slight circular depression or vulval basin. Cuticle surface between anus and vulval basin including about 12 parallel ridges with a few cross connections. Subsurface punctations irregularly arranged over much of body surface, may be confused with surface papillae on vulval crescents.

Table 21. *Morphometrics of cysts and J2 of* Globodera pallida *(all measurements in μm).*

Population Characters	Epworth, UK, Paratypes, Stone (1973a)	Duddingston, UK, Stone (1973a)	Münster, BBA, Germany, Wouts & Weischer (1977)	Pukekole, The Netherlands, Wouts & Weischer (1977)	Pakistan, Shahina & Maqbool (1995)	Idaho, USA, Skantar *et al.* (2007)
Cyst (n)	25	25	–	–	20	80
Length (without neck)	579 ± 70	510 ± 69	–	–	675 (555-748)	574 ± 85 (420-700)
Width	534 ± 66	451 ± 76	–	–	648.5 (540-685.2)	534 ± 80 (400-600)
Length/width	–	–	–	–	–	–
Vulval areas (n)	25	25	–	–	20	80
Fenestral diam.	24.5 ± 5.0	20.7 ± 3.2	–	–	22 (20-23.5)	24.8 ± 5.6 (17.5-45.0)
Number of ridges	12	–	–	–	12	12 ± 2.3 (7-17)
Anus-fenestra distance	49.9 ± 13.4	50.2 ± 9.8	–		48 (40-53)	53.8 ± 11.8 (30-80)
Granek's ratio	2.1 ± 0.9	2.5 ± 0.8	–	–	–	2.2 ± 0.5 (1.2-3.6)
J2 (n)	50	50	20	20	20	80
Length	486 ± 23	482 ± 18	457 ± 3.5	455 ± 3.6	479 (456-505)	452 ± 36 (380-533)
Stylet	23.8 ± 1.0	23.4 ± 0.6	23.7 ± 0.2	23.3 ± 0.2	23.7 (23.5-23.9)	23.2 ± 0.7 (22.5-25.0)
Labial region height	5.5 ± 0.1	5.2 ± 0.6	4.8 ± 0.1	4.7 ± 0.1	–	–
Labial region diam.	10.6 ± 0.5	10.9 ± 0.4	9.6 ± 0.1	10.0 ± 0.1	–	–
DGO	2.7 ± 0.9	2.9 ± 0.9	–	–	–	–
Anterior end to excretory pore	108.6 ± 4.1	103.6 ± 3.7	–		–	–
Anterior end to median bulb valve	68.7 ± 2.7	66.9 ± 2.6	68.8 ± 0.5	68.9 ± 0.6	–	–
Anal body diam.	12.1 ± 0.4	12.0 ± 0.6	–	–	–	–
Hyaline region	26.6 ± 4.1	26.6 ± 3.4	26.0 ± 0.6	26.4 ± 0.9	25.2 (24.5-27.5)	25.9 ± 2.4 (20-31)
Tail	51.1 ± 2.8	52.6 ± 4.1	50.3 ± 0.7	52.0 ± 0.8	51.8 (51.7-51.9)	49.6 ± 3.0 (40-57)

Cyst

New cysts glossy
region intact or fene
pying all or part of
ened 'vulval bodies'
most specimens, ofte
female but more acc

Male

Heat relaxed spec
180° about longitu
four incisures in lat
rounded with large
and heavily sclerotic
with posteriorly slop
stylet. Ellipsoid pha
plates linked by a
to a narrow, ventrall
annuli long, situated
testis, commencing
head, terminating in
with small raised c
terminating distally
without ornamentatio
with bluntly rounded

J2

Lateral field with f
orly, occasionally co
or eight body annuli.
six annuli. Oral disc
apertures, adjacent d
tour of lips and oral
rotised hexaradiate h
tips in 60% of specir
tinct anterior projecti
riorly for *ca* 35% of t
from anterior end. Di
nulus anterior to excr

Anterior end to excretory pore	108.6 ± 4.1	103.6 ± 3.7	–	–	–	–	–	–
Anterior end to median bulb valve	68.7 ± 2.7	66.9 ± 2.6	68.8 ± 0.5	68.9 ± 0.6	–	–	–	–
Anal body diam.	12.1 ± 0.4	12.0 ± 0.6	–	–	26.4 ± 0.9	25.2 (24.5-27.5)	25.9 ± 2.4 (20-31)
Hyaline region	26.6 ± 4.1	26.6 ± 3.4	26.0 ± 0.6		52.0 ± 0.8	51.8 (51.7-51.9)	49.6 ± 3.0 (40-57)
Tail	51.1 ± 2.8	52.6 ± 4.1	50.3 ± 0.7				

Cyst

New cysts glossy brown, subspherical with protruding neck. Vulval region intact or fenestrated with single circumfenestrate opening occupying all or part of vulval basin. Abullate, but small darkened or thickened 'vulval bodies' sometimes present in vulval region. Anus visible in most specimens, often at apex of a V-shape mark. Cuticular pattern as in female but more accentuated. Subcrystalline layer absent.

Male

Heat relaxed specimens C- or S-shaped, posterior part twisted 90-180° about longitudinal axis. Cuticle with regular annulations and four incisures in lateral field, terminating on tail. Labial region offset, rounded with large oral disc, six irregular lips, six or seven annuli and heavily sclerotised hexaradiate framework. Stylet well developed with posteriorly sloping basal knobs and cone forming *ca* 45% of total stylet. Ellipsoid pharyngeal median bulb with strong crescentic valve plates linked by a narrow isthmus encircled by a broad nerve ring, to a narrow, ventrally situated, pharyngeal gland lobe. Hemizonid two annuli long, situated two or three annuli posterior to excretory pore. One testis, commencing with single cap cell 40-65% of body length from head, terminating in a narrow *vas deferens* with glandular walls. Cloaca with small raised circular lip containing two stout arcuate spicules terminating distally in uni-pointed tips. Small dorsal gubernaculum without ornamentation, slightly wider in dorsoventral aspect. Tail short with bluntly rounded terminus of variable shape.

J2

Lateral field with four incisures but with three anteriorly and posteriorly, occasionally completely areolated. Cuticle thickened for first seven or eight body annuli. Labial region rounded, slightly offset with four to six annuli. Oral disc surrounded by two lateral lips bearing amphidial apertures, adjacent dorsal and ventral submedial lips often fused. Contour of lips and oral disc sub-rectangular (Stone, 1973a). Heavily sclerotised hexaradiate head framework, dorsal and ventral radii bifurcate at tips in 60% of specimens. Stylet well developed, basal knobs with distinct anterior projection as viewed laterally. Gland lobe extending posteriorly for *ca* 35% of body length. Excretory pore *ca* 20% of body length from anterior end. Distinct hemizonid two annuli long, located one annulus anterior to excretory pore; hemizonion five or six annuli posterior

to excretory pore. Genital primordium at *ca* 60% of body length from anterior end. Tail tapering uniformly with a finely rounded point, hyaline region forming about half of tail region.

Type host and locality

Potato, *Solanum tuberosum* L. (Solanaceae, Solanales), from Epworth, Lincolnshire, UK.

Other hosts

Eggplant, *Solanum melongena* L., tomato, *S. lycopersicum*, many species of *Solanum*; black henbane, *Hyoscyamus niger* (Stelter & Engel, 1975; Stelter, 1987; CAB International, 2005a; Sullivan *et al.*, 2007 and others) (Table 17). It was experimentally demonstrated that only a few females can develop on NC-95 flue-cured tobacco (Parrot & Miller, 1977).

Distribution

It is generally accepted that PCN originated from the Andean region of South America, where these nematodes parasitise wild potatoes and other *Solanum* species (Evans & Stone, 1977). Although a few records of *G. pallida* have been reported from Bolivia (Evans *et al.*, 1975), the main range lies in Peru, Ecuador and Colombia, *i.e.*, to the north of Lake Titicaca (15.6°S), whilst its sibling species, *G. rostochiensis*, is found to the south of this latitude. In South America, this cyst nematode is mainly found between 2000 m and 4000 m a.s.l., with the heaviest infestations between 2900 m and 3800 m a.s.l. (Franco, 1977). From South America PCN has spread, as the result of human activity, into many regions of the world. Although potatoes were introduced into Europe in about 1570, it is thought that PCN probably came with tubers from South America introduced into Europe only in around 1850 (Evans *et al.*, 1975). In Central and North America *G. pallida* has been reported in Panama, USA and Canada, but in the last two countries PCN have a rather restricted distribution with small infested areas, because of rigorous phytosanitary regulations and seed potato certification programmes, compared to the widespread infestations found in European countries. Recently, mtDNA has been used to study genetic relationships among Peruvian populations of *G. pallida*, thus identifying the origin of western

European populations of
al., 2008). Using mtDN
microsatellite loci, Plant
presently distributed in E
the extreme south of Pe
Titicaca and Cusco (Fig. 2

Europe: Austria, Bel
Greece, Iceland, Ireland,
Norway, Poland, Portuga
Ukraine, Yugoslavia (fo
Algeria, Tunisia; *North a*
South America: Bolivia,
Oceania: New Zealand
2005b).

Relationships

Globodera pallida is n
tabacum. Stone (1973a,
species. It differs from G
yellow or gold, smaller r
(mean of 12 *vs* 17-20),
3.0-4.5), stylet knob sha
stylet length (23-24 *vs*
rounded) and presence o
tail (usually 4-7 refractiv
by longer mean body len
knob shape (anteriorly p
of cuticular ridges (12 *vs*

Biology

Globodera pallida dev
This species is adapted t
in the year and develop a
1978; Langeslag *et al.*, 1

European populations of this species (Picard *et al.*, 2007; Plantard *et al.*, 2008). Using mtDNA gene, cytochrome *b* (*cytb*) sequences and microsatellite loci, Plantard *et al.* (2008) showed that the *G. pallida* presently distributed in Europe derived from a single restricted area in the extreme south of Peru, located between the north shore of Lake Titicaca and Cusco (Fig. 29).

Europe: Austria, Belgium, Cyprus, Denmark, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, The Netherlands, Norway, Poland, Portugal, Slovakia, Spain, Sweden, Switzerland, UK, Ukraine, Yugoslavia (former); *Asia*: India, Pakistan, Turkey; *Africa*: Algeria, Tunisia; *North and Central America*: Canada, Panama, USA; *South America*: Bolivia, Chile, Colombia, Ecuador, Peru, Venezuela; *Oceania*: New Zealand (EPPO Quarantine Pest; CAB International, 2005b).

Relationships

Globodera pallida is most closely related to *G. rostochiensis* and *G. tabacum*. Stone (1973a, c) gave the most complete diagnosis of this species. It differs from *G. rostochiensis* by cream coloured females *vs* yellow or gold, smaller number of ridges between the vulva and anus (mean of 12 *vs* 17-20), smaller mean for Granek's ratio (2.1-2.5 *vs* 3.0-4.5), stylet knob shape (anteriorly projected *vs* rounded), longer stylet length (23-24 *vs* 20-22 μm), tail terminus (more pointed *vs* rounded) and presence of refractive bodies on the hyaline part of the tail (usually 4-7 refractive bodies *vs* absence) in J2; from *G. tabacum* by longer mean body length (452-486 *vs* 476-556 μm) and by J2 stylet knob shape (anteriorly projected *vs* rounded) and higher mean number of cuticular ridges (12 *vs* 7-9) for cysts (Table 14).

Biology

Globodera pallida develops one generation for a vegetation season. This species is adapted to cool temperatures and is able to hatch earlier in the year and develop at 2°C cooler than *G. rostochiensis* (Mugniéry, 1978; Langeslag *et al.*, 1982).

PATHOGENICITY

The symptoms of attack by *G. pallida* are similar to those for *G. rostochiensis* and the damage threshold is 1-2 eggs/g soil (EPPO Quarantine Pest).

BIOCHEMICAL AND MOLECULAR CHARACTERISATION

Characterisation of *G. pallida* populations using IEF of proteins and PCR-ITS-RFLP have been given by several authors (Tables 9, 12). The IEF profile for *G. pallida* is presented in Figure 33. After studying PCR-ITS-RFLP generated by 16 restriction enzymes for seven European countries and 24 populations from Peru, Ecuador and Colombia, Rumpenhorst and Ayub (2001) revealed three types of RFLP profiles for all studied samples: *i*) type A, or European type; *ii*) type B, or South American type; and *iii*) Peruvian/Ecuadorian type with an RFLP pattern that is a combination of types A and B. Digestion with *Bsh*1236I, *Taq*I, *Rsa*I, *Mva*I, *Pst*I, *Nla*III and *Mse*I can separate the European populations from those from South America. Grenier *et al.* (2001) provided a list of restriction enzymes distinguishing these types of *G. pallida* from each other and from other species parasitising Solanaceae. Differences between European and South American populations of *G. pallida* have been found in PCR-RAPD analyses and IEF of proteins, and Rumpenhorst and Ayub (2001) proposed the presence of a third species among PCN populations that are presently identified as *G. pallida*. The presence of at least two groups within *G. pallida* was also revealed by several other studies using simple repeat primers (Blok & Phillips, 1995), RAPDs (Bendezu *et al.*, 1988; Blok *et al.*, 1997; Grenier *et al.*, 2001), 2-DGE of proteins (Grenier *et al.*, 2001), satellite DNA analysis (Grenier *et al.*, 2001), PCR-ITS-RFLPs and sequences (Blok *et al.*, 1998), and *cytb* sequence analysis (Plantard *et al.*, 2008; Pylypenko *et al.*, 2008; Madani *et al.*, 2010). Madani *et al.* (2010) suggested that clade II and the clade III in the mtDNA tree (Fig. 29) might represent a third and fourth PCN species, respectively. PCR-ITS-rRNA profiles for two types of *G. pallida* are given in Table 15. Enzyme *Pst*I discriminates *G. pallida* type A from *G. mexicana* **sp. n.** Species-specific primers are given in Table 13.

9. *Globodera t*

Common name: tobac

In 1951, a cyst ne
potato cyst nematode,
ardville, Connecticut,
this nematode was no
has been shown in sev
not develop on tobacc
exception of a *G. rosto*
reproduce on *Nicotian*

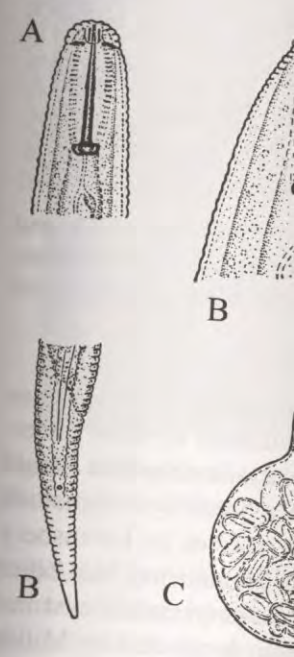


Fig. 56. Globodera tabacum.
female; C: Cyst; D: Pharyng
Tail of male. After Lownsbery

RELATIONSHIPS

Punctodera punctata differs from other *Punctodera* species by the pear-shaped cysts and the absence of bullae (Table 24).

BIOCHEMICAL AND MOLECULAR CHARACTERISATION

Rumpenhorst (1985) provided the IEF protein profile for several populations of *P. punctata*. Fleming *et al.* (2000) showed that PCR-ITS-RFLP can be used routinely to discriminate among cyst nematodes of the genera *Heterodera*, *Punctodera* and *Globodera*. Subbotin *et al.* (2001a) and Sabo *et al.* (2002) sequenced the ITS-rRNA gene and studied the relationships of *P. punctata* with other cyst nematodes.

2. *Punctodera chalcoensis* Stone, Sosa Moss & Mulvey, 1976
(Figs 12C; 63, 64)

Common name: Mexican corn cyst nematode.

The Mexican corn cyst nematode was first observed in the late 1950s in maize fields in Huamantla, Tlaxcala, Mexico, and was identified as *H. punctata* and a Mexican race of this species (Sosa Moss & Gonzales, 1973; Vazquez, 1976; Baldwin & Mundo-Ocampo, 1991). Further detailed morphological and morphometric observations revealed differences between these populations and *P. punctata* and *P. matadorensis* and led to the description of a new species, *P. chalcoensis*, by Stone *et al.* (1976).

MEASUREMENTS

After Stone *et al.* (1976).

– *Holotype cyst*: L (excluding neck) = 510 μm; W = 470 μm; neck = 88 μm; L/W ratio = 1.1.

– *Paratype cysts* (n = 20): L (excluding neck) = 441 ± 69 μm; W = 416 ± 61 μm; neck = 95 ± 26 μm; L/W ratio = 1.06 ± 0.1; vulva to anus plates (n = 25): vulval fenestral length = 18.1 ± 2.7 μm; vulval fenestral width = 19.8 ± 2.9 μm; diam. of clear zone surrounding vulval fenestra ≈ 30 μm; vulval slit = 4.2 ± 0.4 μm; anal fenestra length = 21.1 ± 3.4 μm; anal fenestral width = 22.4 ± 3.1 μm; length of anal slit = 2.8 ± 0.4 μm; distance between fenestrae = 42.3 ± 8.8 μm.

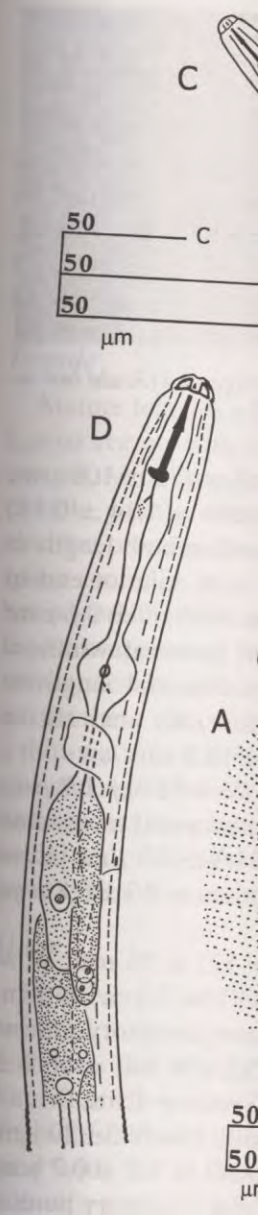


Fig. 63. Punctodera chal
D: Pharyngeal region of
Stone et al. *(1976).*

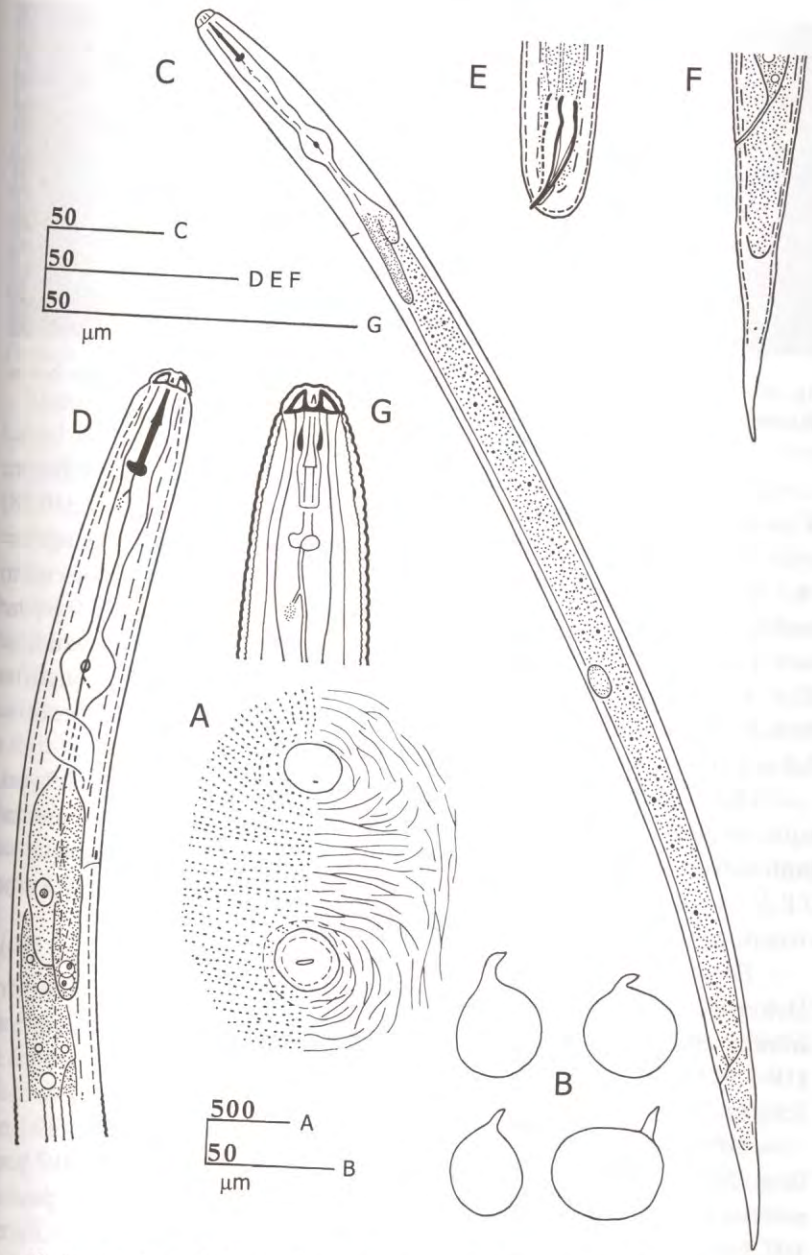


Fig. 63. Punctodera chalcoensis. *A: Perineum of female; B: Females; C: J2; D: Pharyngeal region of J2; E: Male tail; F: Tail of J2; G: Head of J2. After Stone* et al. *(1976).*

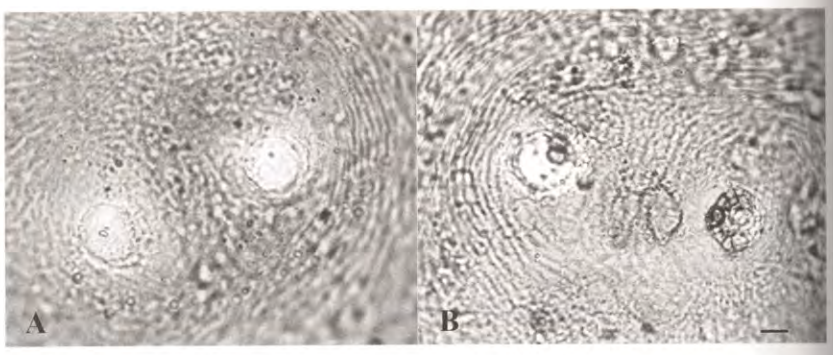


Fig. 64. Punctodera chalcoensis. *A, B: Vulval plates. (Original.) (Scale bar = 10 μm.)*

– *Paratype females* (n = 20): L (excluding neck) = 473 ± 105 μm; W = 429 ± 120 μm; neck = 142 ± 24 μm; L/W ratio = 1.14 ± 0.18; stylet = 25.8 ± 0.9 μm; DGO = 5.7 ± 0.9 μm; median bulb length = 25.7 ± 1.9 μm; median bulb width = 24.9 ± 2.4 μm; anterior end to median bulb valve = 77.4 ± 8.4 μm, excretory pore = 131.0 ± 0.6 μm; vulval fenestral length = 30.7 ± 7.0 μm; vulval fenestral width = 32.1 ± 7.0 μm; vulval slit = 4.0 ± 0.5 μm; anal fenestral length = 29.5 ± 5.7 μm; anal fenestral width = 1.1 ± 5.3 μm; anal slit = 3.0 ± 1.0 μm; distance between fenestrae = 67.7 ± 14.5 μm.

– *Paratype males* (n = 12): L = 985 ± 69 μm; W = 25.8 ± 1.7 μm; stylet = 26.8 ± 0.8 μm; DGO = 4.0 ± 0.6 μm; anterior end to: median bulb valve = 82.7 ± 3.7 μm, excretory pore = 131.3 ± 7.7 μm; tail = 2.8 ± 1.4 μm; spicules = 31.9 ± 1.8 μm; gubernaculum = 7.3 ± 0.8 μm; overall gonad length = 476 ± 107 μm.

– *Paratype J2* (unfixed in water, n = 20): L = 522 ± 26 μm; W = 21.6 ± 0.7 μm; stylet (S) = 24.4 ± 0.2 μm; DGO = 5.5 ± 0.8 μm; anterior end to: median bulb valve = 70.4 ± 6.3 μm, excretory pore = 110.1 ± 7.9 μm, pharyngeal glands = 176.0 ± 15.1 μm; tail = 66.2 ± 3.5 μm; hyaline region (H) = 38.6 ± 3.5 μm; H/S ratio = 1.6 ± 0.1.

– *Paratype J2* (fixed in 4% formaldehyde, n = 50): L = 533±29 μm; W = 20.1 ± 0.6 μm; stylet = 24.7 ± 0.6 μm; DGO = 5.1 ± 0.7 μm; anterior end to: median bulb valve = 73.7 ± 2.7 μm, excretory pore = 107.7 ± 4.9 μm; tail = 63.2 ± 3.3 μm; hyaline region = 38.2 ± 3.1 μm; H/S ratio = 1.5 ± 0.1.

– *Paratype eggs* (n = 20): L = 114.4 ± 3.6 μm; W = 43.1 ± 1.7 μm; L/W ratio = 2.7 ± 0.1.

DESCRIPTION

Cyst

Spherical or subsp
darkening with age.
cysts losing thin wa
showing incomplete
bullae in perineal regi
be lacking from many

Female

Mature females wh
Labial region with o
curved, with rounde
Cuticle greatly thick
circular prominence.
lying centrally in a
having cuticle reduce
fenestra appearing m
surrounded by a narr
a transverse slit, shor
a thin-walled cuticula
size and appearance to
Anus located toward
associated with anal
layer typically presen

Male

Body vermiform, h
ture, tail frequently t
7 annuli. Tail bluntly
diam. Cuticle with re
Cephalids at level of
oped with flat to cor
bulb ellipsoidal, not f
annuli, located one a
flask-shaped proximal
gle distal point. Gube
served.

riginal.) (Scale bar =

k) = 473 ± 105 μm;
ratio = 1.14 ± 0.18;
edian bulb length =
μm; anterior end to
e = 131.0 ± 0.6 μm;
l fenestral width =
l fenestral length =
.3 μm; anal slit =
: 14.5 μm.
W = 25.8 ± 1.7 μm;
erior end to: median
1.3 ± 7.7 μm; tail =
lum = 7.3 ± 0.8 μm;

522 ± 26 μm; W =
O = 5.5 ± 0.8 μm;
m, excretory pore =
1 μm; tail = 66.2 ±
ratio = 1.6 ± 0.1.
)): L = 533 ± 29 μm;
GO = 5.1 ± 0.7 μm;
m, excretory pore =
on = 38.2 ± 3.1 μm;

W = 43.1 ± 1.7 μm;

Description

Cyst

Spherical or subspherical with protruding neck, pale to dark brown, darkening with age. New cysts often retaining subcrystalline layer. Old cysts losing thin walls of vulval and anal fenestrae, younger cysts showing incomplete fenestration. Some specimens with small scattered bullae in perineal region or just anterior to vulval fenestra, but bullae may be lacking from many cysts. Cyst typically containing 200-400 eggs.

Female

Mature females white, spherical or subspherical with protruding neck. Labial region with one or two prominent annuli. Stylet slender, often curved, with rounded basal knobs. Excretory pore at base of neck. Cuticle greatly thickened except in head region. Vulval slit on slight circular prominence. Vulva a short transverse slit situated posteriorly, lying centrally in a circular zone lacking ridges and punctations and having cuticle reduced in thickness to form the vulval fenestra. Vulval fenestra appearing more transparent than surrounding body wall and surrounded by a narrow zone of cuticle with reduced thickness. Anus a transverse slit, shorter than vulval slit, lying 'dorsal' to vulva within a thin-walled cuticular zone, the anal fenestra. Anal fenestra of similar size and appearance to vulva fenestra but lacking surrounding clear zone. Anus located toward ventral side of fenestra. Clusters of elongate bodies associated with anal and vulval apertures. Thick white subcrystalline layer typically present.

Male

Body vermiform, heat-relaxed specimens with strong ventral curvature, tail frequently twisted through 180°. Labial region offset with 5-7 annuli. Tail bluntly rounded, length less than one-quarter of body diam. Cuticle with regular annulations, four lateral areolated incisures. Cephalids at level of second and eighth body annuli. Stylet well developed with flat to concave anteriorly basal knobs. Median pharyngeal bulb ellipsoidal, not filling body cavity. Hemizonid extending over two annuli, located one annulus anterior to excretory pore. Paired spicules flask-shaped proximally and tapering distally, curved ventrally with single distal point. Gubernaculum single, unornamented. Phasmids not observed.

J2

Vermiform body adopting a slight ventral curve on heat relaxation. Cuticle annulations distinct, lateral field with four intermittently areolated incisures reducing to three anteriorly and posteriorly (Fig. 9H). Labial region offset, apparently with 3-5, usually four annuli as seen by light microscopy. Labial disc elongated dorsoventrally by *ca* 1.5 times its diam., surrounded by distinct lateral lips bearing amphidial apertures, adjacent submedial lips fused with each other. Cephalids at level of second and seventh or eighth body annulus. Well developed stylet with massive basal knobs, knobs rounded posteriorly, flat to shallowly concave anteriorly. Pharyngeal gland lobes with ventral overlap, usually extending posteriorly *ca* one-third of body length but sometimes considerably shorter. Hemizonid 1-2 annuli anterior to excretory pore. Phasmid openings situated in posterior two-thirds of tail, small refractive bodies sometimes present in matrix of hyaline terminus.

Type host and locality

Maize, *Zea mays* L. (Poaceae, Poales), near Chaco, Valley of Mexico, Mexico state, Mexico, at an altitude of 2300 m a.s.l.

Other hosts

Punctodera chalcoensis also parasitises teosinte, *Zea mexicana* (Schrad.) Kuntz, 1976. Under experimental conditions, several species of grasses and cereals were not infected by the nematode (Stone *et al.*, 1976).

Distribution

North America: Mexico. Nematological surveys indicated a wide distribution of *P. chalcoensis* at elevations between 1500 and 2800 m a.s.l. throughout the maize-producing regions of Central Mexico from the Pacific Ocean to the Gulf of Mexico and in the following states: Mexico, Tlaxcala, Puebla, Veracruz, Michoacan and Jalisco (Baldwin & Mundo-Ocampo, 1991; Tovar Soto *et al.*, 2006). Climatic conditions at high altitude are temperate with marked seasonal rainfall. The nematode is most abundant in sandy soils (Stone *et al.*, 1976).

BIOLOGY

The life cycle spans the growing season of maize so that only one generation occurs per year (Baldwin & Mundo-Ocampo, 1991).

PATHOGENICITY

It is highly damaging to maize in Mexico, causing severe yellowing, stunting and even death of young seedlings (Baldwin & Mundo-Ocampo, 1991). Heavily attacked plants have stunted root systems with many short laterals, giving a bottle-brush effect, and the aerial parts of the plant appear unthrifty. Pathogenicity studies of *P. chalcoensis* on maize in the glasshouse showed an inverse correlation between root and top weight of mature plants and the number of nematode cysts in the inoculum (Hernandez Aragon, 1965).

RELATIONSHIPS

Punctodera chalcoensis most resembles *P. matadorensis* and *P. stonei*. It differs from *P. matadorensis* by the shape of the J2 stylet knobs, which are anteriorly flat to slightly concave in *P. chalcoensis vs* strongly concave. They also differ in the distribution of bullae in the cyst perineum, these being small scattered or absent in *P. chalcoensis vs* massive and always present in *P. matadorensis*. *Punctodera chalcoensis* differs from *P. stonei* by the shorter hyaline region of the J2 tail (38-39 *vs* 50 μm) (Table 24) and by its distinctive host range.

BIOCHEMICAL AND MOLECULAR CHARACTERISATION

Sabo *et al.* (2002) sequenced the ITS-rRNA gene and studied the relationships of *P. chalcoensis* with other cyst nematodes. The ITS-rRNA gene sequence distinguishes this species from *P. punctata*.

3. *Punctodera matadorensis* Mulvey & Stone, 1976
(Figs 65, 66)

In 1971, several cores of soil containing native grass roots from the Matador Ranch, Matador, Saskatchewan, Canada, were found to be infected with white females and cysts. These nematodes were tentatively identified as *Heterodera punctata* by Mulvey (1972, 1973). Further

Volume 8 in the series, appearing in two parts, A and B, deals with the systematics of cyst nematodes of the subfamily Heteroderinae. Cyst nematodes are important pathogens causing extensive damage and significant yield loss to many crops in both temperate and tropical regions. Part A presents summarised information on various aspects of morphology, biology, ecology, pathology, biogeography, control measures and diagnostics of these nematodes. Molecular diagnostic tables and comprehensive tabular and dichotomous keys for species identification, together with descriptions of protocols for extraction, light and electron microscopy studies, and molecular diagnostics are also included. The taxonomic section of part A provides diagnoses for six genera, *viz.*, *Globodera*, *Punctodera*, *Cactodera*, *Dolichodera*, *Betulodera* and *Paradolichodera*, and includes descriptions and morphometrics of 30 valid species. The work is illustrated by 101 drawings and photos. The genus *Heterodera* is mainly covered in part B, complete descriptions and morphometrics of the 80 known valid species being provided and the species illustrated by 159 line drawings and photos. Identification of the species using morphological and molecular techniques is also covered. Each part includes an extensive bibliography.

Readership: All those interested in biology, ecology and morphological and molecular diagnostics of plant parasitic nematodes. The book is aimed at nematologists, plant pathologists and specialists in plant protection.

SERGEI A. SUBBOTIN, Ph.D. (1987) in Helminthology (Nematology), Institute of Helminthology, Russia, is presently serving as Senior Nematologist at the California Department of Food and Agriculture, USA, and has associate positions as Guest Professor at Ghent University, Belgium, and as Scientist at University of California, USA and Center of Parasitology, Russia. He has published more than 90 articles in nematological and molecular journals.

MANUEL MUNDO-OCAMPO, Ph.D. (1982) in Plant Pathology (Nematology), University of California, Riverside, USA. Currently a Research Associate and Curator of the UCR-Nematode Collection and is in the process of moving to CIIDIR-IPN-Sinaloa in Mexico. He has published more than 50 papers and technical reports in peer-reviewed journals.

JAMES G. BALDWIN, Ph.D. (1972) in Plant Pathology (Nematology), North Carolina State University, Raleigh, NC, USA, is Professor and Chair of the Department of Nematology at the University of California, Riverside. He has published more than 120 refereed articles that emphasise nematode systematics and phenotypic evolution.

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