Nematologica 22 (1976): 381-389. E. J. Brill, Leiden

PUNCTODERA CHALCOENSIS N. SP. (NEMATODA: HETERODERIDAE) A CYST NEMATODE FROM MEXICO PARASITISING *ZEA MAYS*

BY

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Punctodera chalcoensis n. sp., a cyst-nematode from Mexico parasitising *Zea* spp. is described. Information on distribution, host range and probable significance as an agricultural pest is presented and discussed. A key to the species of *Punctodera* is provided.

Sosa Moss (1968) found what was then thought to be *Punctodera punctata* (Thorne, 1928) parasitising *Zea mays* L. in Mexico, differing in measurements and host range from the Canadian specimens described by Thorne (1928) and English specimens described by Franklin (1938, 1940). Villanueva Rodriguez (1974) also found morphometric differences between Mexican and English populations. The Mexican specimens sufficiently differ from *P. punctata* as described by Thorne and Franklin and from *P. matadorensis* Mulvey & Stone, 1976, the only other species in the genus, to require description as a separate species *P. chalcoensis* n. sp.

MATERIALS AND METHODS

Material used in the description was cultured from cysts collected from the type locality raised on *Z. mays* at ± 25° in a glasshouse at Rothamsted Experimental Station. Specimens of juveniles and males were measured unfixed after heat relaxation and permanent preparations in glycerol or lactophenol made after fixation in 4% formaldehyde, F.P. 4 : 1 (Netscher & Seinhorst, 1969) or TAF (Courtney, Polley & Miller, 1955); cysts were mounted in 'Euparal'. For scanning electron microscopy (SEM) second stage juveniles were infiltrated with epoxy resin after formaldehyde fixation and processing through acetone using the method of Clark & Stone (1975). SEM observations were made with a Cambridge Instruments Stereoscan MK IIA microscope.

Host tests were done at Chapingo, using pots of sterilised soil inoculated with cysts collected from the type and other localities.

Mean measurements are given with their standard deviations.

DESCRIPTION

Mature females

Holotype cyst entire, mounted in glycerol: Length excluding neck = 510 µm;

maximum width = 470 μm; neck length = 88 μm; length excluding neck/width ratio = 1.1.

Entire mature females, n = 20: Length excluding neck = 473 ± 105 μm; width = 429 ± 120 μm; neck length = 142 ± 24 μm; length excluding neck/width = 1.14 ± 0.18.

Anterior portions of mature females, fixed in TAF, mounted in glycerol, n = 13: Stylet length = 25.8 ± 0.9 μm; stylet base to dorsal oesophageal gland duct junction = 5.7 ± 0.9 μm; length of median oesophageal bulb = 25.7 ± 1.9 μm; width of median oesophageal bulb = 24.9 ± 2.4 μm; head tip to median oesophageal bulb valve = 77.4 ± 8.4 μm; head tip to excretory pore = 131.0 ± 0.6 μm.

Perineal portions of mature females, fixed 4% formaldehyde, mounted in 'Euparal', n = 25: Vulval fenestra length = 30.7 ± 7.0 μm, width = 32.1 ± 7.0 μm; length of vulval slit = 4.0 ± 0.5 μm; anal fenestra length = 29.5 ± 5.7 μm, width = 31.1 ± 5.3 μm; length of anal slit = 3.0 ± 1.0 μm; distance between fenestrae = 67.7 ± 14.5 μm.

Mature females white in colour, spherical or sub-spherical with a projecting neck containing the oesophagus; length excluding neck/width ratio close to one. (Fig. 2-B, 4-A). Head with one or two prominent annules. Stylet slender, often curved, with rounded basal knobs. Oesophagus strongly developed with massive, circular median bulb with prominent valve; oesophageal glands lobe with distinct dorsal and subventral gland cells (Fig. 2-A, 4-B). Excretory pore at base of neck. Two large ovaries filling the enlarged body cavity which in mature females is occupied by eggs. Cuticle greatly thickened except in head region, covered with a rugose or lace-like pattern of shallow ridges, with a sub-surface pattern of rows of fine refractive spots — the "punctations". Vulva a short transverse slit (Fig. 5-A, 5-B) situated at the opposite pole of the body to the neck, lying centrally in a circular zone lacking ridges and punctations, with the cuticle of reduced thickness — the vulval fenestra. Vulval slit on slight circular prominence (Fig. 4-E, 5-A, 5-B). The vulva fenestra (Fig. 2-C, 4-C) appears more transparent than the surrounding body wall and is itself surrounded by a narrow zone of less thick cuticle. Anus, a transverse slit smaller than the vulva and lying 'dorsal' to the vulva within a thin-walled circular zone — the anal fenestra (Fig. 2-C, 4-C). The anal fenestra is similar in size and appearance to the vulval fenestra but lacks the surrounding clear zone; the anus does not lie centrally in the fenestra but towards the ventral side. Clusters of elongate bodies are associated with the anal and vulval apertures (Fig. 4-D, 4-E); they appear to be the same as the vulval bodies described by Wilson (1968) and Mulvey (1973) but are not limited to the endocuticle. The size of the vulval aperture precludes extrusion of eggs but a very small gelatinous matrix (egg sac) was observed on some specimens and may have been lost from other specimens in extraction. A thick white sub-crystalline layer is typically present (Fig. 4-A) consisting of polygonal plates and resembling that described from *P. punctata* (Brown *et al.*, 1971).

Cysts [1])

Entire dry cysts, n = 20: Length excluding neck = 441 ± 69 µm; width = 416 ± 61 µm; neck length = 95 ± 26 µm; length excluding neck/width = 1.06 ± 0.10.

Perineal portions of cysts, n = 25: Vulval fenestra length = 18.1 ± 2.7 µm, width = 19.8 ± 2.9 µm; diameter of clear zone surrounding vulva fenestra ≏ 30 µm; length of vulval slit = 4.2 ± 0.4 µm; anal fenestra length = 21.1 ± 3.4 µm, width 22.4 ± 3.1 µm; length of anal slit = 2.8 ± 0.4 µm; distance between fenestrae = 42.3 ± 8.8 µm.

Cyst shape as female, colour pale to dark brown, darkening with age. New cysts often retain the subcrystalline layer. In old cysts thin walls of the vulval and anal fenestrae are lost, younger cysts show incomplete fenestration. Some specimens have small scattered bullae in the perineal region (Fig. 5D) or closely sited in an area just below the vulval fenestra (Fig. 5E) but they are lacking from many cysts (Fig. 5C). New cysts typically contain from 200-400 embryonated eggs.

Embryonated eggs

Eggs from cysts collected from type locality, in water, n = 20: Length = 114.4 ± 3.6 µm; width = 43.1 ± 1.7 µm; length/width = 2.7 ± 0.1. Second stage juvenile folded four times within egg.

Males

Killed and fixed in FP 4 : 1, mounted in glycerol, n = 12: L = 985 ± 69 µm; body width = 25.8 ± 1.7 µm; stylet length = 26.8 ± 0.8 µm; stylet base to dorsal oesophageal gland duct junction = 4.0 ± 0.6 µm; head tip to oesophageal median bulb valve = 82.7 ± 3.7 µm; head tip to excretory pore = 131.3 ± 7.7 µm; tail length = 2.8 ± 1.4 µm; spicule length (across chord) = 31.9 ± 1.8 µm; gubernaculum length = 7.3 ± 0.8 µm; overall gonad length = 476 ± 107 µm; G = 48.

Typical heteroderid male morphology. Body vermiform, heat relaxed specimens with strong ventral curvature and tail frequently twisted through 180° (Fig. 2-D). Head offset with 5-7 annules as seen by light microscopy. Tail bluntly rounded, less than one quarter of body width long. Cuticle with regular annulations, four lateral incisures, areolated, lateral field terminating on tail. Heavy hexaradiate cephalic skeleton. Cephalids at level of second and eighth body annules. Stylet well developed with shallow basal knobs, flat to concave anteriorly. Median oesophageal bulb ellipsoidal, not filling body cavity; oesophageal glands lobe overlapping intestine ventrally (Fig. 1-C). Hemizonid extending over two annules, one annule anterior to excretory pore; hemizonion not observed. Single gonad extending for

[1]) Measurements are of cysts collected from field soil in the type locality; field specimens were smaller than the females cultured in the glasshouse.

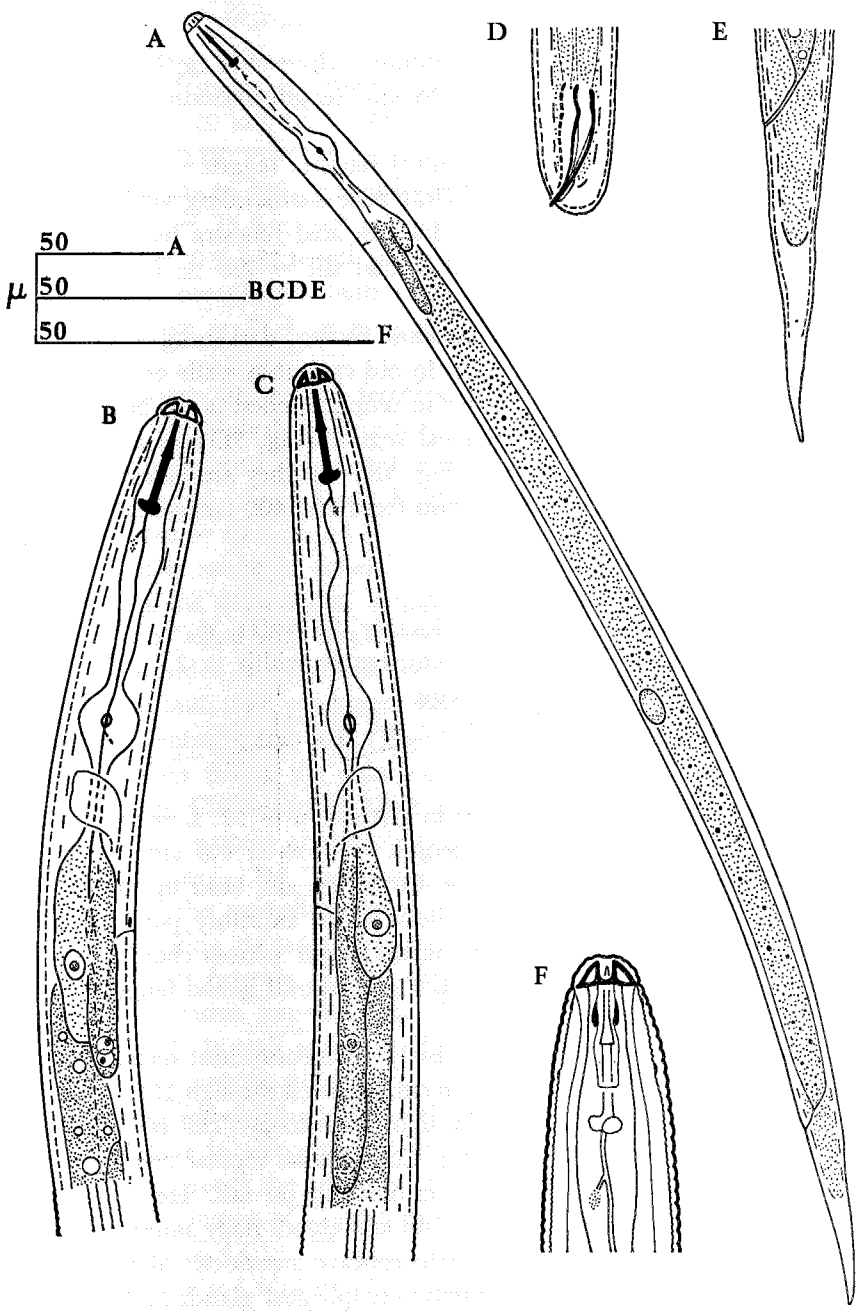
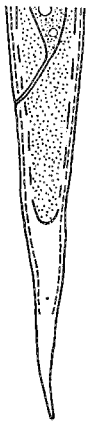


Fig. 1. *Punctodera chalcoensis* n. sp. A: entire second-stage juvenile; B: anterior of second-stage juvenile; C: anterior of male; D: male tail; E: second-stage juvenile tail; F: second-stage juvenile head.



about half body length, less in small specimens. Paired spicules flask-shaped proximally and tapering distally, curved ventrally with single points; single unornamented gubernaculum (Fig. 1-D). Phasmids not observed.

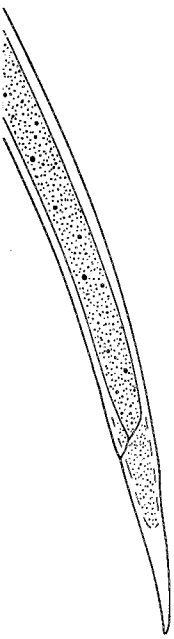
Second-stage juveniles

Unfixed juveniles in water, n = 20: L = 542 ± 26 μm; maximum body width = 21.6 ± 0.7 μm; stylet length = 24.4 ± 0.2 μm; stylet base to dorsal oesophageal gland duct junction = 5.5 ± 0.8 μm; head tip to median bulb valve = 70.4 ± 6.3 μm; head tip to excretory pore = 110.1 ± 7.9 μm; head tip to end of oesophageal glands lobe = 176.0 ± 15.1 μm; tail length = 66.2 ± 3.5 μm; hyaline terminus length = 38.6 ± 3.4 μm; hyaline terminus length/stylet length = 1.6 + 0.1.

Juveniles fixed in 4% formaldehyde, mounted in glycerol, n = 50: L = 533 ± 29 μm; maximum body width = 20.1 ± 0.6 μm; stylet length = 24.7 ± 0. 6 μm; stylet base to dorsal oesophageal gland duct junction = 5.1 ± 0.7 μm; head tip to median bulb valve = 73.7 ± 2.7 μm; head tip to excretory pore = 107.8 ± 4.9 μm; tail length = 63.2 ± 3.3 μm; hyaline terminus length = 38.2 ± 3.1 μm; hyaline terminus length/stylet length = 1.5 + 0.1.

Typical heteroderid second-stage juvenile with vermiform body adopting a slight ventral curve on heat relaxation (Fig. 1-A) tail tapering to a slender point (Fig. 1-E, 3-C). Cuticular annulations distinct; four incisures in lateral field reducing to three anteriorly and posteriorly, areolated intermittently throughout. Cuticle thicker for first eight body annules.

Head offset, apparently three-five, usually four head annules when seen by light microscopy but SEM observation shows usually three (Fig. 3-D), the extra annule seen in the light microscope being part of the lips. (Head defined as the region of the body occupied by the cephalic framework and separated from the body by a deeper annulation.) Oral disc distinct, elongated dorso-ventrally to about 1.5 × its width and surrounded by distinct lateral lips bearing the amphid apertures; the two components of each pair of submedian lips fused into single, distinct arcs. No indication of cephalic or labial papillae. Moderately heavy hexaradiate head skeleton. Cephalids at level of second and seventh-eighth body annules. Well developed stylet with massive basal knobs rounded posteriorly, flat to shallowly concave anteriorly (Fig. 1-F, 3-B). Anterior (prorhabdial) portion of stylet less than half stylet length. Median oesophageal bulb well developed occupying full width of body cavity and often with a somewhat rectangular shape. Oesophageal gland lobes with ventral overlap, usually extending back about one third of body length but sometimes considerably shorter (compare Fig. 1-B and 3-A). No distinct oesophageal-intestinal valve observed. Hemizonid one-two annules anterior to excretory pore; hemizonion not observed. Genital primordium lying at about 60% of body length from anterior end. Phasmids situated two-thirds along tail length; small refractive bodies sometimes present in matrix of hyaline terminus.



anterior of second-stage
second-stage juvenile head.

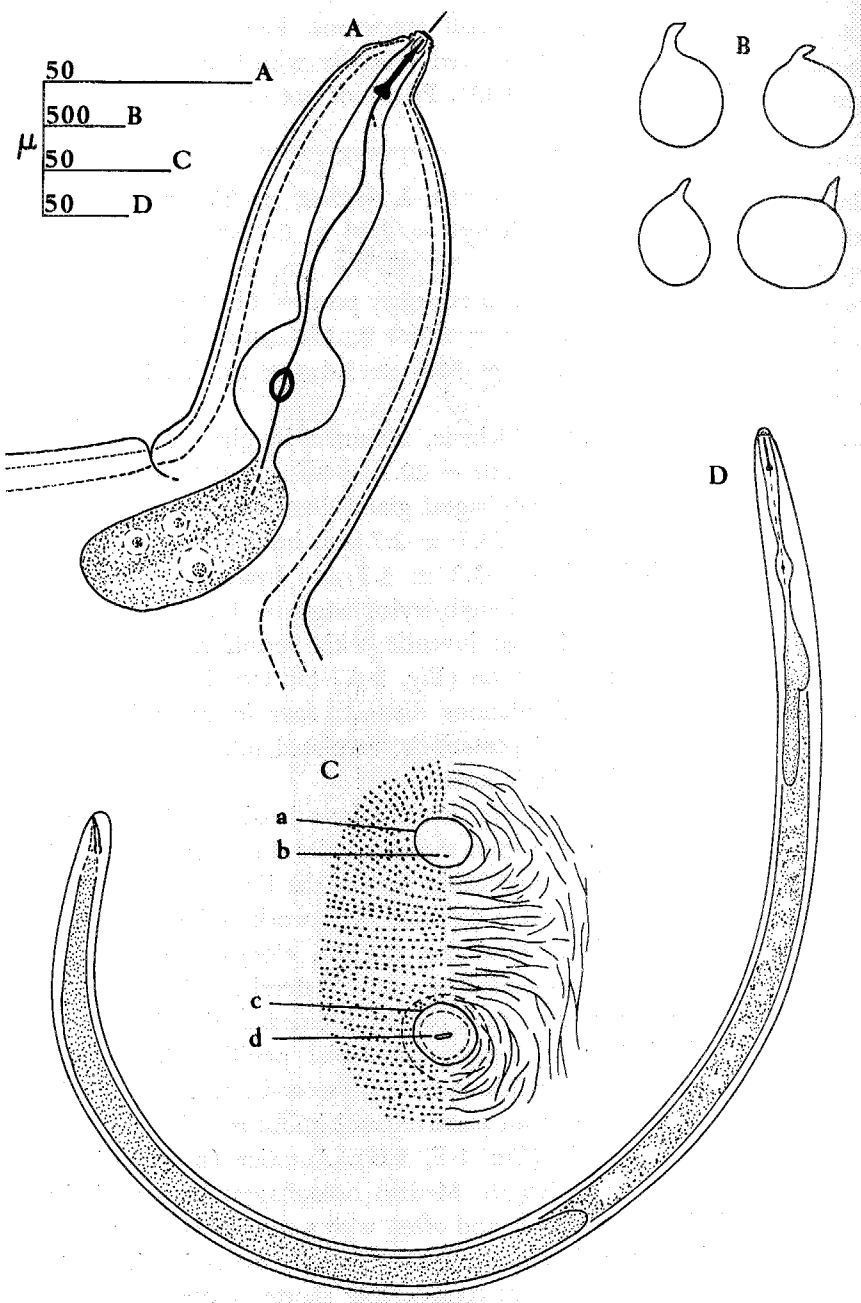
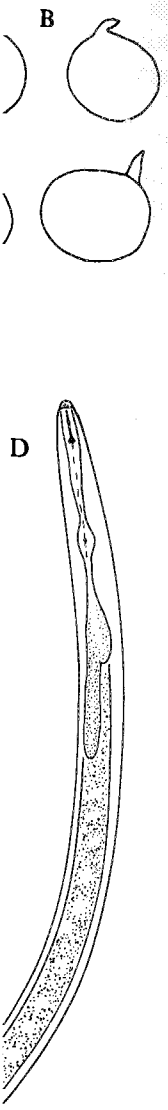


Fig. 2. *Punctodera chalcoensis* n. sp. A: head and neck of female; B: entire females; C: perineum of female, a = anal fenestra, b = anus, c = vulval fenestra, d = vulval slit; D: entire male.



nales; C: perineum of
; D: entire male.

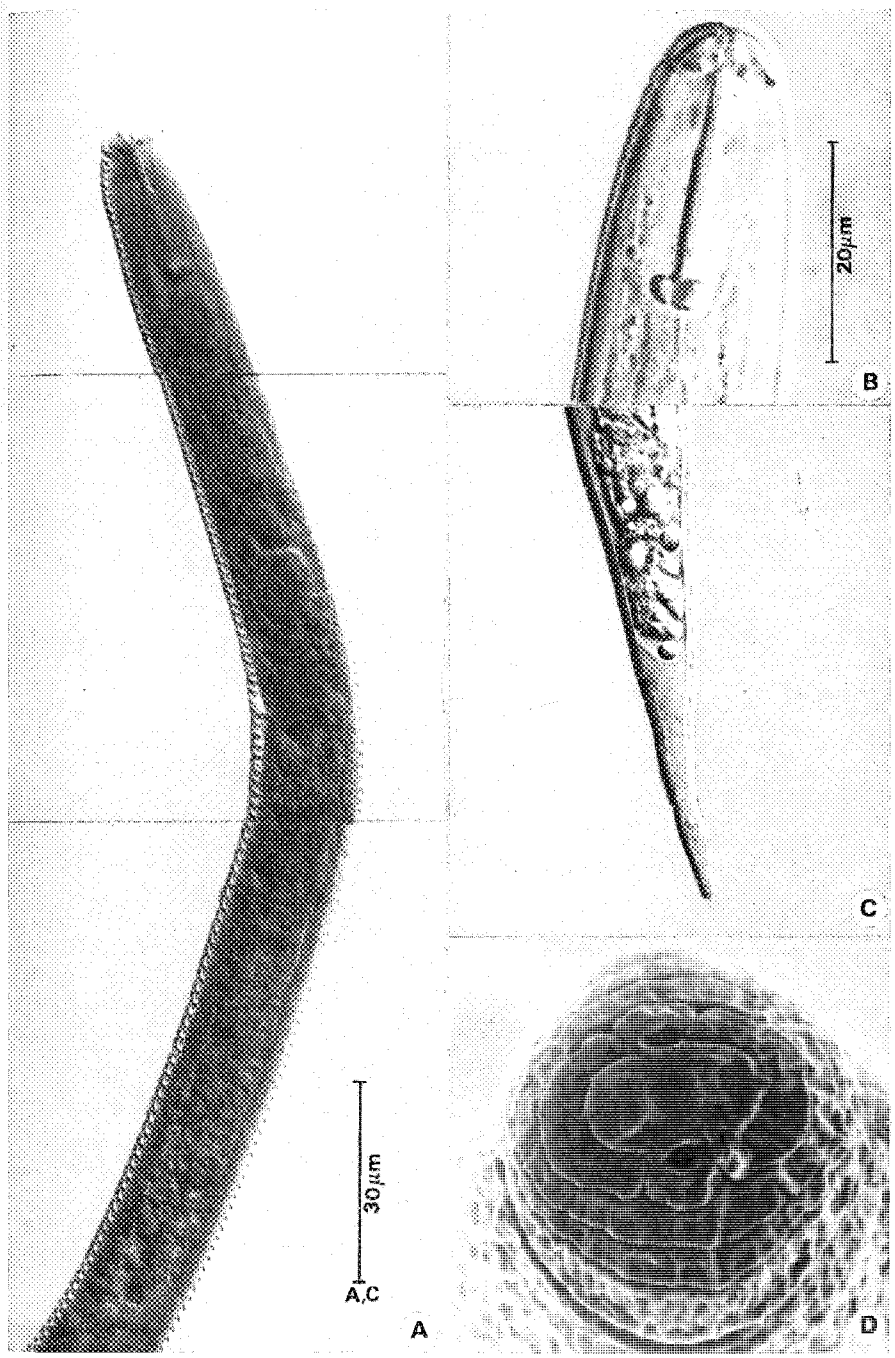


Fig. 3. *Punctodera chalcoensis* n. sp. Second-stage juvenile. A: anterior; B: head; C: tail; D: head (approx. × 9,400) (A-C, Nomarski interference contrast micrographs; D, scanning electron micrograph.)

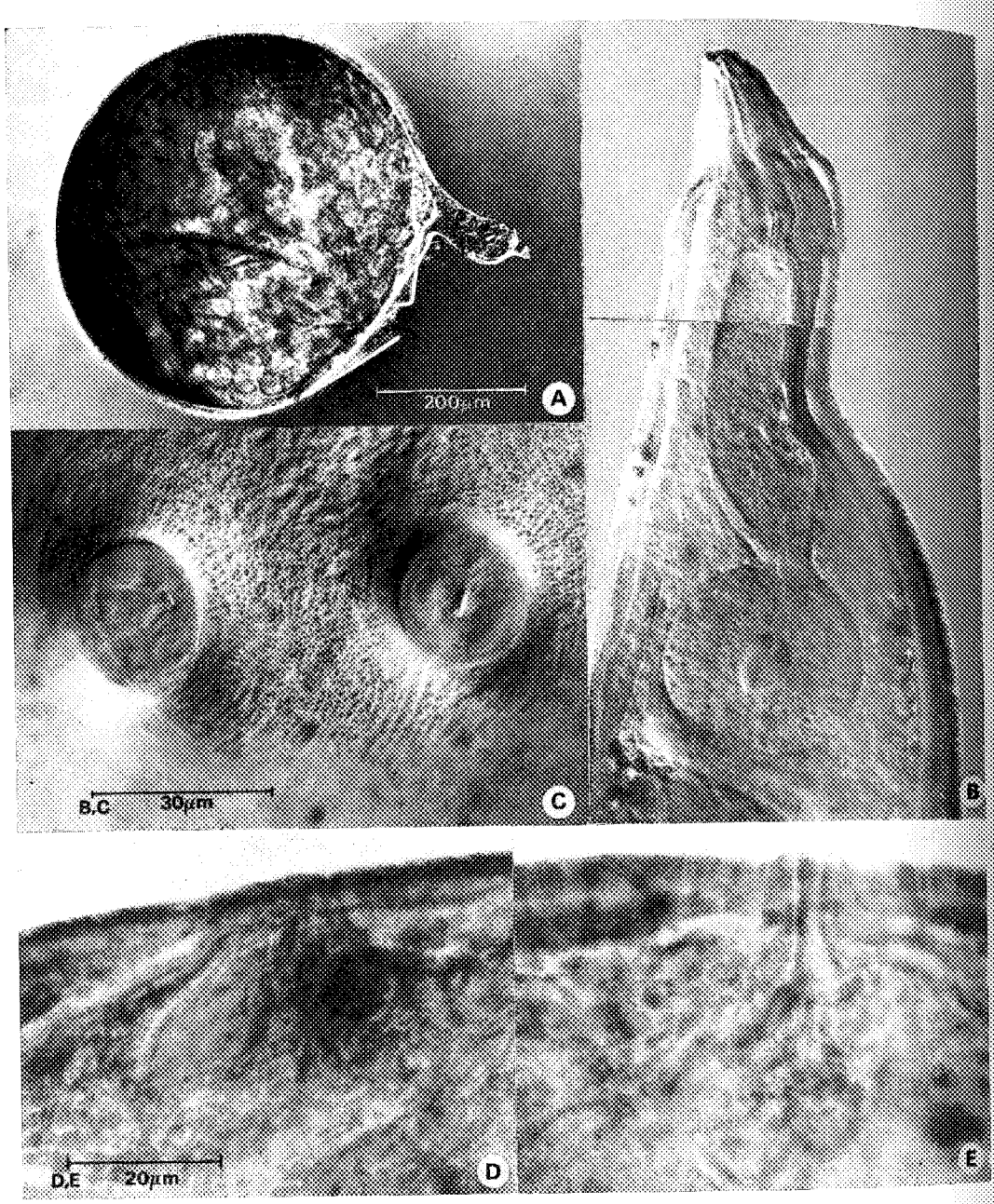
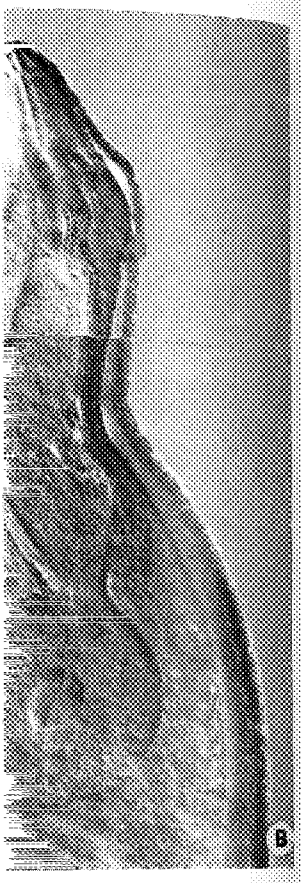


Fig. 4. *Punctodera chalcoensis* n. sp. A: entire female showing detached sub-crystalline layer; B: female head and neck; C: perineum of female showing sub-surface punctations; D: optical section of anal fenestra, showing anus; E: optical section of vaginal fenestra, showing vagina. (A-E, Nomarski interference contrast micrographs.).



:d sub-crystalline layer; B:
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a, showing vagina. (A-E,

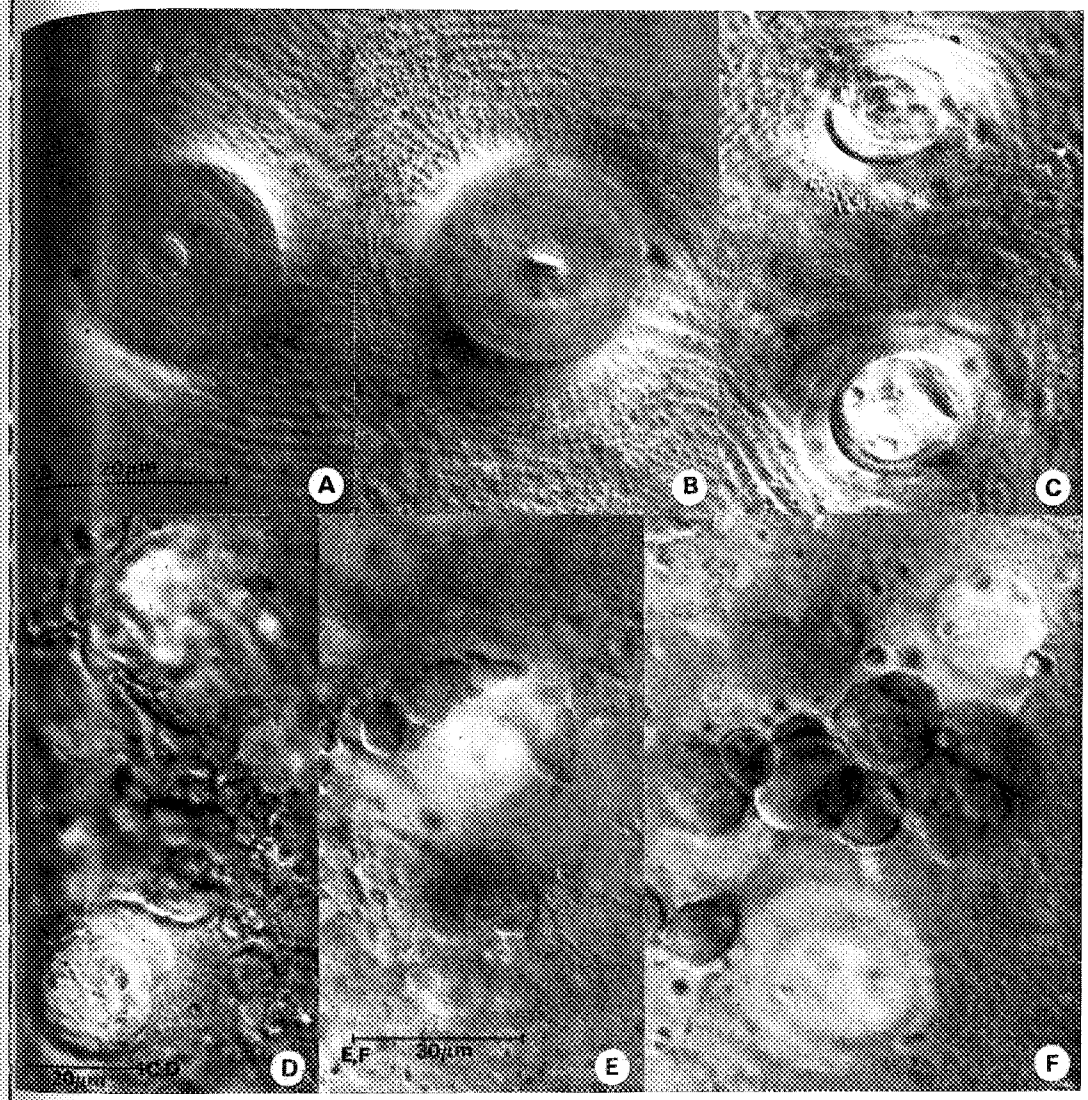

Fig. 5. *Punctodera chalcoensis* n. sp. A, B: mature female, vulval fenestra showing vulval slit, at high (A) and low (B) planes of focus; C: cyst anal and vulval fenestrae, bullae absent; D: cyst perineum, focal plane below fenestrae, with small, scattered bullae between fenestrae; E: cyst perineum, focal plane below fenestrae, with bullae below vulval fenestra; F: *Punctodera matadorensis*, cyst perineum showing massive bullae between vulval and anal fenestrae. (Nomarski interference contrast micrographs).

DIAGNOSIS

Punctodera chalcoensis n. sp. is placed in the genus *Punctodera* Mulvey & Stone, 1976, because of the spherical to sub-spherical shape of the mature female and cyst; the absence of a vulval cone; the absence of perineal tubercles in the female and the presence of separate, sub-equal circular fenestrae surrounding the vulva and anus. In having an elongate oral disc surrounded by discrete sub-median and lateral lips in the second stage juvenile *P. chalcoensis* resembles other *Punctodera* spp. (Stone, 1975; Mulvey & Stone, 1976). *P. chalcoensis* differs from *P. punctata* (Thorne, 1928) in having spherical to sub-spherical mature females and cysts, (pear-shaped in *P. punctata*); in having second-stage juveniles of greater body length, mean length >500 μm compared with 350-470 μm in *P. punctata,* and in host range (see below). *P. chalcoensis* differs from *P. matadorensis* Mulvey & Stone, 1976 in the shape of the second-stage juvenile stylet basal knobs — flat to slightly concave anteriorly in *P. chalcoensis*, strongly concave anteriorly in *P. matadorensis*; in the extension of the oesophageal glands lobe of the second-stage juvenile — about 30% of body length in *P. chalcoensis*, about 50% in *P. matadorensis*; in the distribution of bullae in the cyst perineum — small scattered or absent in *P. chalcoensis*, massive and always present in *P. matadorensis* (Fig. 5-F), and in host range.

Type locality: The vicinity of the town of Chalco in the Valley of Mexico, Mexico State, Mexico, altitude 2300 m, latitude 19°15′53″N, longitude 98°54′14″ W.

Type host: *Zea mays* L.

Holotype: Mature cyst, slide No. 76D/3/1, type slide collection, Nematology Department, Rothamsted Experimental Station.

Paratypes: Second-stage juveniles, females, cysts and males, Nematology Department, Rothamsted Experimental Station. Second-stage juveniles and cysts, Rama de Fitopatologia, Colegio de Postgraduados, Escuela Nacional de Agricultura, Chapingo, Mexico; Canadian National Nematode Slide Collection, Ottawa, Canada; U.S.D.A. Nematode Collection, Beltsville, Maryland, U.S.A. and Laboratorium voor Nematologie, Landbouwhogeschool, Wageningen, the Netherlands.

BIOLOGY

Distribution: *P. chalcoensis* has been found above 2000 m in the Mexico, Tlaxcala and Pueblo States of Mexico but not in other maize growing regions of the country. Climatic conditions at this altitude are temperate, with marked seasonal rainfall. The nematode is most abundant in sandy soils.

Host-range: In pot tests only the type host and teosinte, *Zea mexicana* (Schrad.) Kuntz, were found to support reproduction of the nematode. No resistance was found in a wide range of maize varieties or in thirteen separate isolates of teosinte. Graminae tested which proved to be non-hosts were: *Avena sativa* L. (7 varieties), *A. fatua* L., *Triticum aestivum* L. (14 varieties), *Secale cereale* L. (11 varieties), triticale (12 varieties), *Sorghum vulgare* Pers. (119 varieties), *Agropyron* spp.

(6), *Bromus* spp. (2), *Dactylis* sp., *Elymus* sp., *Festuca* spp. (3), *Lolium* spp. (2) and *Phleum* sp.

Damage to maize field crops is associated with *P. chalcoensis*; heavily attacked plants have stunted root systems with many short laterals, giving a bottle brush effect and the aerial parts of the plants appear unthrifty. In pot tests with maize plants grown in sterile soil using a range of inocula Hernandez Aragon (1965) found an inverse correlation between root and top weight of mature plants and inoculum size: with the largest inocula, 625 and 3125 cysts/pot at a mean of 250 eggs/cyst, the plants were stunted and chlorotic with characteristic pale stripes on the leaves.

DISCUSSION

Maize is thought to have originated in Mesoamerica and has a history of cultivation in Mexico dating to pre-historic times (Usher, 1974). This together with the apparently limited distribution of *P. chalcoensis* and the occurrence of the only other host teosinte, a close relative and possible progenitor of cultivated maize (Usher, 1974), as a weed in the area where the nematode is found, suggest that *P. chalcoensis* is indigenous to Central Mexico and that it has there co-evolved with maize.

Field observations and the limited pot tests done indicate that *P. chalcoensis* is a potentially serious pest in maize cultivation. The effect of infestations apparently are masked in crops receiving fertiliser but in unfertilised crops the symptoms of stunting and necrosis appear (Sosa Moss & Gonzalez, 1973) and it is probable that well fertilised crops suffer some yield loss. Field trials to establish damaging population levels are lacking. In the Valley of Mexico maize is planted in March/April in the latter part of the dry season. The plants develop slowly until the rainy season begins, usually at the end of May, and then vigorous growth occurs.

In such years damage apparently due to *P. chalcoensis* is less than in years when the rain begins earlier, in late April or early May. The greater damage associated with early start of the rainy season may be due to greater hatch, mobility and invasion of juveniles in the moister soil; a similar effect which occurs with *H. avenae* Woll. on autumn-sown wheat in Australia, following earlier start of the rainy season, has been attributed to increased juvenile emergence and mobility (Meagher, 1970).

KEY TO *PUNCTODERA* SPECIES

1. Cyst pear shaped, second stage juvenile length 350-470 μm .... *Punctodera punctata* (Thorne)
— Cyst spherical or sub-spherical shaped, second stage juvenile length >500 μm 2
2. Massive bullae between vulval and anal fenestrae, second stage juvenile with oesophageal gland lobe extending to about 50% of body length and stylet knobs strongly concave anteriorly, host grasses . . . . . *P. matadorensis* Mulvey & Stone
— Bullae small, scattered or absent, second stage juvenile with oesophageal gland lobe extending to about 30% of body length and stylet knobs flattish to slightly concave anteriorly, host maize . . . . . *P. chalcoensis* n. sp.

Part of this work was done by the senior author while a guest of the Rama de Fitopatologia, Colegio de Postgraduados, Escuela Nacional de Agricultura and

Direccion General de Sanidad Vegetal, Mexico, under the Royal Society Latin American Programme, and part at Virginia Polytechnic Institute & State University, U.S.A., supported by North Atlantic Treaty Organisation Research Grant No. 686. Some material was collected by Dr. L. I. Miller under auspices of the same grant. Mrs. J. A. Rowe is thanked for technical assistance.

RÉSUMÉ

Punctodera chalcoensis *n. sp.* (*Nematoda: Heteroderidae*), *nématode à kyste du Mexique parasitant* Zea mays

Les auteurs donnent la description de *Punctodera chalcoensis* n. sp., un nématode à kyste parasitant *Zea mays* au Mexique. Des données sont présentées et discutées concernant sa répartition, son éventail d'hôtes et son rôle probable de déprédateur. Une clé des espèces de *Punctodera* est donnée.

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Accepted for publication: April 13, 1976.