Observations on *Aphelenchoides hylurgi* Massey, 1974 feeding on fungal pathogens of wheat in Australia

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SUMMARY

Aphelenchoides hylurgi Massey, 1974, which was originally described from Gorham, Maine, USA, is redescribed using abundant material, both male and female, originally from Toowoomba, Queensland, Australia, together with the original type series (three females) of this species. A comparison of measurements of this species when grown on two different genera of plant pathogenic fungi is made.

RÉSUMÉ

Observations sur Aphelenchoides hylurgi *vivant sur des champignons pathogènes du blé en Australie*

Aphelenchoides hylurgi Massey, 1974, décrit à l'origine sur des spécimens provenant de Gorham, Maine, USA, est redécrit à partir d'un matériel abondant provenant de Toowoomba, Queensland, Australie, et de la série type originale (trois femelles). Les caractéristiques biométriques de spécimens issus d'élevages sur deux genres différents de champignons phytopathogènes sont comparées.

It has been shown by Rössner and Urland (1983) that *Aphelenchoides hamatus* Thorne & Malek, 1968 could cause the beak-up of various colonies of foot rot pathogens in agar cultures. In glasshouse experiments, they found initially that *A. hamatus* was able to reduce the attack of *Fusarium culmorum* (W. G. Smith) Sacc. on wheat. The observed effect of *A. hamatus* as an agent of biological control was not obtained in subsequent experiments because of difficulties subsequently experienced in obtaining uniform infections of wheat seedlings with conidial suspension of *F. culmorum*. However, Rössner and Urland have drawn attention to mycophagous nematodes as potential agents of biological control of root-rots of cereals and to the need for a better understanding of the taxonomy and physiology of such species of *Aphelenchoides*.

Nematodes were detected feeding on cultures of *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex. Dastur isolated from the base of stems of wheat (*Triticum aestivum* L. cv. Banks) from Toowoomba, Queensland and originally sent to Mr. J. Harris of CSIRO Division of Soils for mycological identification. We decided to see whether they would feed on other fungal plant pathogens and, subsequently, to study their physiology with a view to testing their potential as agents of biological control. In this paper we identify and redescribe the nematode species involved.

Materials and methods

ISOLATION AND GROWTH OF NEMATODES

Several males and females were selected from the original isolates and washed in 0.5 % hibitane for 20 min, followed by numerous washes in sterile distilled water, and inoculated onto their fungal hosts using a sterilized mounted eye-lash under sterile conditions in a laminar flow cabinet. The fungal hosts used were *Rhizoctonia solani* Kühn and *Fusarium graminearum* Schwabe. These were grown on half strength potato dextrose agar (PDA) containing antibiotics and made up as follows in distilled water : PDA-19.5 g/l, bacto-agar-7.5 g/l, chloramphenicol-250 ppm, neomycin sulphate-50 ppm streptomycin sulphate-50 ppm. Subsequent inoculations were made by transferring small portions of fungi and nematodes to fresh media.

LIVING MATERIAL

Living nematodes were examined in sealed slides and photographed using interference contrast optics after nematode movement had ceased due to anoxia.

FIXATION AND DEHYDRATION

The nematodes were killed, fixed and processed

through to pure glycerol at 23° by evaporation as described by Seinhorst (1959).

These nematodes were placed in small drops of pure glycerol and sealed by a circle of molten paraffin using the method of De Maeseneer and D'Herde (1963).

Eggs were fixed in 4 % buffered (pH 7.3) paraformaldehyde and mounted in this medium in sealed slides. Specimens were drawn using a camera lucida and measured.

Preparation of material for scanning electron microscopy

Specimens were fixed sequentially at 4° using 0.1 M phosphate buffered 4 % paraformaldehyde at pH 7.3 (Stynes & Bird, 1980) as follows : they were chilled in 0.5 ml (12 drops) of water at 4° for 30 min and then sequentially fixed (Eisenback, 1986) by adding 0.5 ml of cold fixative over 1.5 hours (four drops every 30 mn). The material was then washed three times in 0.05 M buffer followed by two washes in distilled water, post-fixed in 1 % osmium tetroxide at 23° for one hour, washed three times in distilled water and then left in freshly made filtered saturated aqueous thiocarbohydrazide for 30 min followed by three washes in distilled water. This process was then repeated. It uses the osmium-bridging properties of thiocarbohydrazide to bind additional osmium to tissue surfaces (Kelley, Dekker & Bluemink, 1973). The specimens were then either air dried on a coverslip attached to an aluminium stub or dehydrated with ethanol through a ten step series, infiltrated with Freon 113 and then air dried. These nematodes were examined in a Cambridge S 250 Mk 3 scanning electron microscope (SEM) operating at 20 kV.

Preparation of material for transmission electron microscopy

Specimens were treated as described above except that they were left overnight in the cold paraformaldehyde and then cut in half before post fixing in osmium tetroxide. They were dehydrated in an ethanol series, taken into Spurrs resin mixture via propylene oxide and polymerized overnight at 60°. Thin sections were cut with glass knives using an LKB ultramicrotome and further stained in a filtered saturated aqueous solution of uranyl acetate for 20 min at 23°, followed by lead citrate for 10 mn at the same temperature. The sections were examined in a Philips EM 400 transmission electron microscope (TEM) operating at 80 kV.

Aphelenchoides hylurgi Massey 1974
(Figs 1-5)

Measurements

Females : see Table 1

Table 1

Measurements of adult demales of *Aphelenchoides hylurgi* mounted in glycerol. Originally from Toowoomba, Queensland and grown on cultures of *Rhizoctonia solani* (population 1) or *Fusarium graminearum* (population 2). Population 3 consists of type specimens originally described by Massey (1974) and associated with *Hylurgops pinifex* in eastern white pine from Gortham, Maine.

Character µm	*Population 1* n = 23	*Population 2* n = 12	*Population 3* n = 3
L	470-565 (507.2 ± 27.2) [495-519] [11.73]	430-519 (469.5 ± 25.5) [453-486] [16.04]	595-622 (610.7 ± 14.01) [576-645] [34.78]
a	22-31 (28.4 ± 2.5) [27-29] [1.08]	26-30 (27.7 ± 1.1) [27-28] [0.69]	25.9-35.1 (30.7 ± 4.61) [21-40] [11.44]
b	8-9 (8.2 ± 0.4) [8-8.4] [0.17]	7-9 (7.8 ± 0.6) [7-8] [0.38]	10.5-10.9 (10.7 ± 0.2) [8-11] [0.5]
c	16-21 (17.4 ± 1.3) [17-18] [0.56]	15-17 (16.3 ± 0.8) [16-17] [0.50]	16.2-20.1 (18.3 ± 1.97) [14-22] [4.89]
c'	2.6-3.9 (2.9 ± 0.3) 2.8-3.0] [0.13]	2.6-3.5 (3.0 ± 0.3) [2.8-3.2] [0.19]	2.9-3.8 (3.3 ± 0.47) [2-4] [1.17]
V	67-73 (71.0 ± 1.5) [70-72] [0.65]	70-74 (72.0 ± 1.5) [71-73] [0.94]	68.3-69.8 (68.9 ± 0.79) [67-70] [1.96]
Oesophagus	59-64 (62.1 ± 1.5) [61-63] [0.65]	58-63 (59.7 ± 1.6) [59-61] [1.01]	55.5-59 (57 ± 1.8) [53-61] [4.47]
Dist. to end of œsophagal gland		121-141 (130 ± 6.5) [126-134] [4.09]	115-130 (121.8 ± 7.6) [107-137] [18.84]
Dist. ant. end to vulva	327-408 (360.4 ± 21.4) [351-370] [9.23]	311-368 (338.3 ± 19.2) [326-350] [12.08]	408-434 (420.7 ± 13.01) [395-447] [32.30]
Dist. vulva to anus	103-127 (112.1 ± 5.7) [110-115] [2.46]	92-118 (101.8 ± 7.8) [97-107] [4.91]	
Tail	26-31 (29.1 ± 1.3) [29-30] [0.56]	27-31 (28.8 ± 1.1) [28-29] [0.69]	31-38 (33.7 ± 3.78) [26-41] [9.38]
Maximum diameter	16-21 (18.0 ± 1.4) [17-19] [0.60]	16-17 (16.8 ± 17.1) [16.6-17.1] [0.25]	17.5-23 (20.2 ± 2.75) [15-26] [6.83]
Diameter at anus	8-12 (10.0 ± 1.0) [9.6-10.4] [0.43]	8-11 (9.5 ± 0.9) [9-10] [0.57]	10-11 (210.3 ± 0.58) [9-11] [1.44]
Diameter at vulva	12-17 (14.4 ± 1.1) [14-15] [0.48]	12-16 (14.2 ± 1.2) [13-15] [0.75]	17.5-19 (18.2 ± 0.76) [16-19] [1.89]
Stylet	11-13 (12.3 ± 0.5) [12-13] [0.22]	12-13 (12.2 ± 0.4) [12-12.5] [0.25]	10-11.5 (10.7 ± 0.76) [9-12] [1.89]

For each measurement : first line = actual range; second line (between brackets) = mean and standard deviation of the above. Third line (between square brackets) = theoretical range (95 % probability); fourth line (between square brackets) = confidence interval of the mean.

Males : see Table 2

Eggs : (n = 12). L = 51-57 (53.4 ± 2.1) µm; theoretical range (95 % probability) = 52-55 µm; confidence interval of the mean = 1.34; width = 15-21 (17.4 ± 1.5) µm; theoretical range = 16-18 µm; conf. interv. mean = 0.95.

Table 2

Measurements of adult males of *Aphelenchoides hylurgi* mounted in glycerol. Originally from Toowoomba, Queensland and grown on cultures of *Rhizoctonia solani* (population 1) or *Fusarium graminearum* (population 2).

Character (µm)	*Population 1* *n = 12*	*Population 2* *n = 12*
L	295-375 (340.2 ± 21.6) [327-354] [13.59]	305-373 (331.0 ± 25.8) [315-347] [16.2]
a	25-37 (29.0 ± 4.0) [26-32] [2.52]	22-30 (25.3 ± 2.70) [24-27] [1.70]
b	5.8-7.1 (6.6 ± 0.4) [6.4-6.9] [0.25]	6-7 (6.3 ± 0.5) [6-7] [0.31]
c	11-13 (12.3 ± 0.- [12-13] [0.38]	11-14 (12.3 ± 0.9) [12-13] [0.57]
c'	2.1-3.9 (2.8 ± 0.5) [2.5-3.1] [0.31]	2.6-3.1 (2.8 ± 0.4) [2.6-3.1] [0.25]
Oesophagus	51-53 (51.8 ± 0.8) [51-52] [0.50]	47-58 (51.6 ± 3) [50-54] [1.9]
Dist. to end of oesophageal gland		103-122 (118.8 ± 6.1) [108-116] [3.8]
Tail	26-29 (27.6 ± 1.0) [27-28] [0.63]	25-30 (27.0-1.4) [26-28] [0.88]
Maximum diameter	9-14 (11.9 ± 1.7) [11-13] [1.07]	11-14 (13.2 ± 1.1) [12-13] [0.69]
Diameter at anus	7-13 (10.0 ± 1.5) [10-11] [0.94]	9-11 (9.8 ± 0.9) [9-10] [0.57]
L of spicules (median)	12-16 (14.2 ± 1.0) [14-15] [0.63]	13-16 (14.6 ± 1.2) [14-15] [0.75]
Stylet	10-12 (10.7 ± 0.6) [10-11] [0.38]	10-12 (10.8 ± 0.6) [10-11] [0.38]

For each measurement : first line = actual range; second line (between brackets) = mean and standard deviation of the above; third line (between square brackets) = theoretical range (95 % probability); fourth line (between square brackets) = confidence interval of the mean.

Description

Female : Body slightly ventrally arcuate when relaxed. Body annuli about 1.2 µm wide, interrupted by lateral field about 15 % of mid-body width, with four lines (Fig. 2 A, B) sometimes difficult to see. Labial region about a third as high as wide slightly offset from body by a depression. Stylet with cone a little shorter than shaft (m = 45 to 48 %); small swellings present at the base of the stylet, 1.25 to 1.50 µm wide. Median bulb oval 10.5 to 11.5 µm by 12 µm, with valvular apparatus somewhat posteriorly situated at 58 to 63 % of the bulb length. Excretory pore usually at level of the posterior margins of the nerve ring, sometimes at the level of the middle of the nerve ring. Hemizonid seen in one specimen just anterior to the nerve ring. Hemizonion not seen. Tail slightly convex dorsally, conoid with a rounded tip bearing a star shaped mucro with four processes (Fig. 2 C, D). Ovary usually outstretched and staying short of oesophageal glands, rarely flexed and reaching into the oesophageal region, with oocytes in one row except for the multiplication area. Oviduct an empty chamber 20 to 25 µm long. Spermatheca elongate 20 to 40 µm long, empty in the American specimens but with 27 (SE ± 0.7) sperms in the Australian specimens. Vagina oblique forward oriented. Vulval lips slightly raised. Post uterine sac short, about one body diameter long, sometimes containing rounded bodies resembling sperm cells.

Male : Generally resembling female, tail end curved, conoid, with a star shaped mucro at tail tip. Spicules 14 to 15 µm long, as measured along the median line.

Eggs

Eggs cylindrical with rounded ends. Egg shell hyaline and without markings.

Diagnosis

A. hylurgi is characterized by head slightly offset, stylet about 12 µm long with basal knobs, excretory pore at the posterior edge of nerve ring, oocytes in one row, post uterine sac short, tail conoid about three times as long as wide with terminal star-shaped mucro, and lateral field with four lines (not seen by Massey, 1974).

Discussion

The Australian population studied here is very similar to the type material of the species except for a few measurements and for the presence of males. The type population is longer, has a shorter oesophagus (thus a greater b ratio), and has a vulva more anterior than the Australian population. No males were found in the type population of *A. hylurgi*, and the three females that constitute the entire type series have empty spermathecae. The type specimens of *A. hylurgi* have a very short ovary, while in the Australian specimens this organ is longer, reaching to the region of the oesophageal glands. Both Sanwal (1965) and B'chir (1979) have commented on the great variability of the ovary length in *Aphelen-*

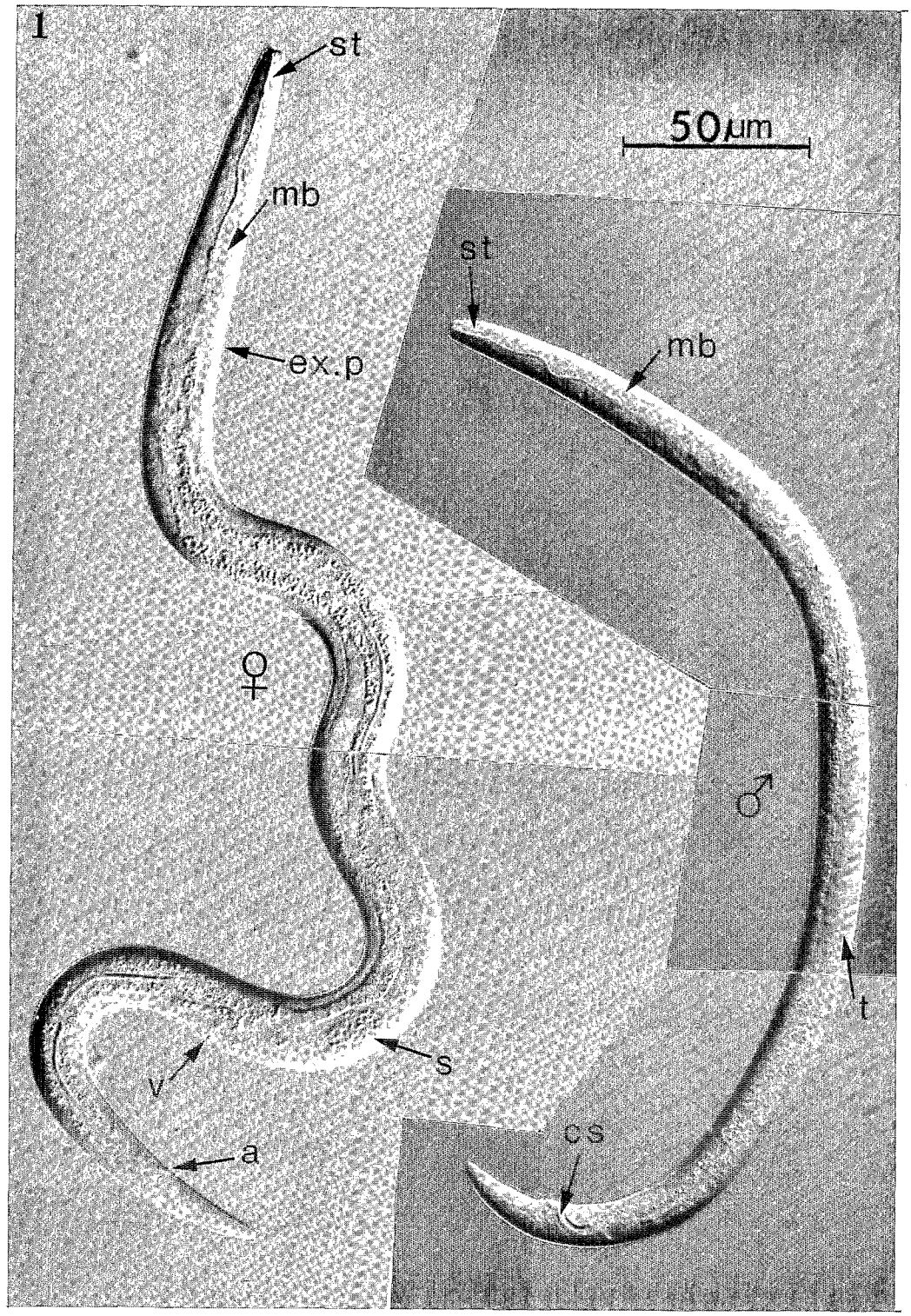

Fig. 1. Montages of a living female and male of *Aphelenchoides hylurgi* from Australia viewed under differential interference contrast optics and both at the same magnification. These photographs illustrate many of the structures measured and shown in Tables 1 and 2. Note buccal stylet (st), median bulb (mb), excretory pore (ex.p), spermatheca (s), testis (t), vulva (v), copulatory spicules (cs) and anus (a).

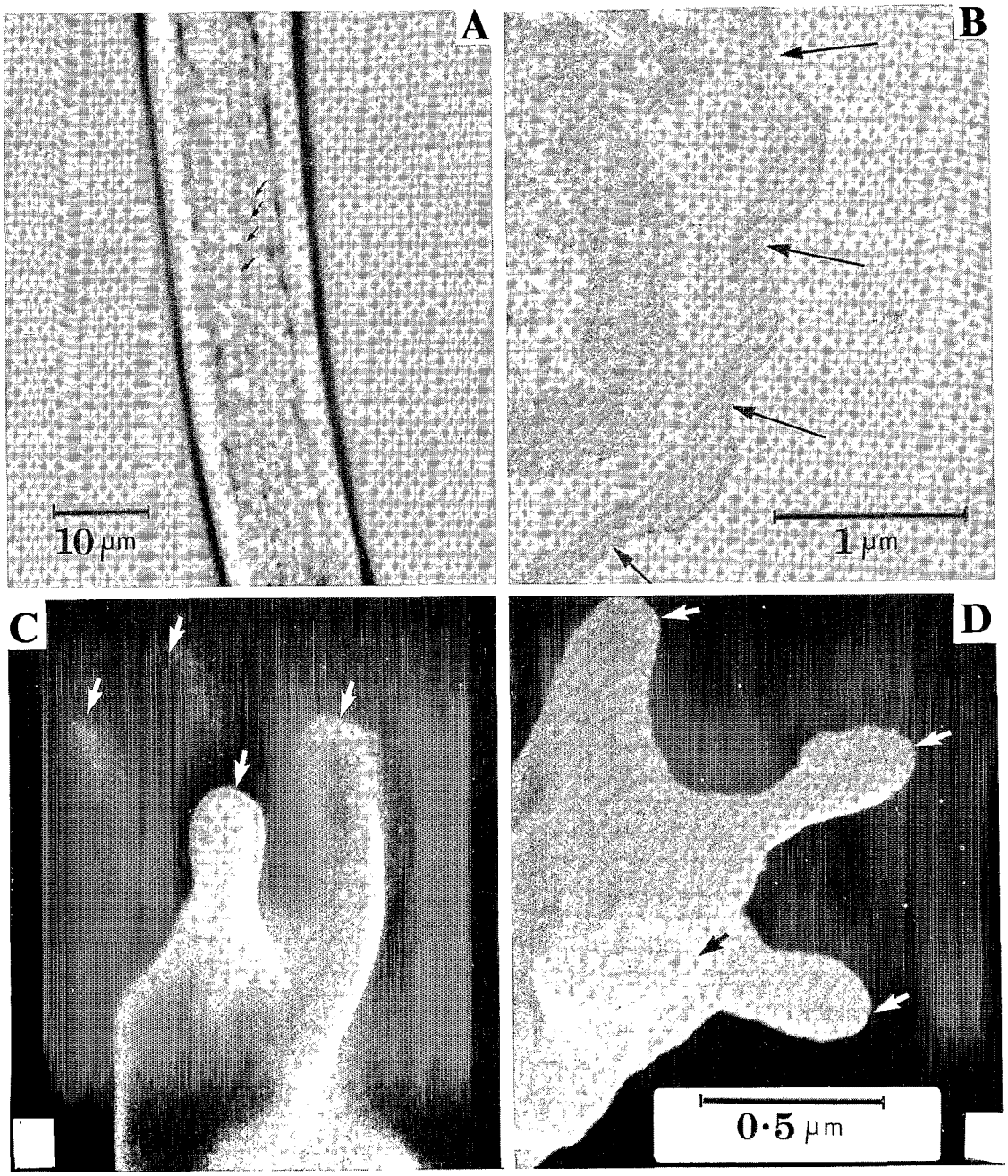

Fig. 2. A : Bright field, oil immersion surface micrograph of a living female *Aphelenchoides hylurgi* from Australia showing lateral alae with four lines; B : Transmision electron micrograph of a cross section through the mid region of a female *Aphelenchoides hylurgi* from Australia. Showing lateral alae with four incisures or lines (arrows); C, D : Scanning electron micrographs of tail regions of female *Aphelenchoides hylurgi* from Australia. Showing mucro with four processes.

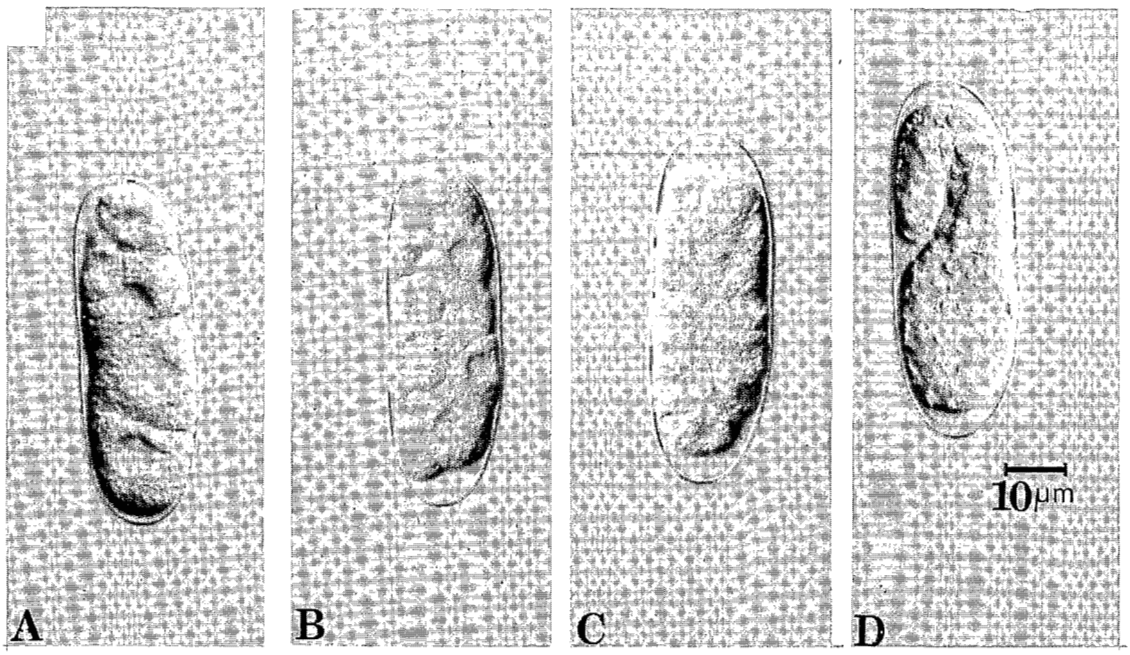

Fig. 3. A living egg of *Aphelenchoides hylurgi* from Australia viewed under differential interference contrast optics and showing development at different times. A = 1 hour after laying; B = 2 hours; C = 6 hours and D = tadpole stage 21 hours after laying.

choides. This measurement was said to be very dependant on age and external factors such as host and temperature. In all the populations described here, the oocytes are arranged in a single row (except for the multiplication area). This character was described as fairly stable by Sanwal (1965). In view of the remarkable similarity of all other morphological characteristics, it is the considered opinion of the taxonomist among us that these slight differences are not sufficiently great to warrant the establishment of a new species.

A. hylurgi is very close to *A. asterocaudatus* Das, 1960 described from *Sorghum vulgare* and *Ricinus communis* from Hyderabad, India except for the shorter post uterine sac. The PUS is about two body widths long in *A. asterocaudatus*. The description of *A. asterocaudatus* is incomplete by modern standards, e.g. measurements are given for only one specimen, and details of the genital branch structure are not given, particularly the arrangement of oocytes in the ovary and the description of the spermatheca and its contents. The structure of the lateral field with two lines has been challenged by Siddiqi and Franklin (1967) who concluded from a study of the holotype that it " probably has four incisures on the lateral line ".

Repeated requests for loan of type material made to Dr. V. M. Das and the Curator of the museum of the Zoology Department, Osmania University, by the taxonomist among us, have not been answered. Until *A. asterocaudatus* can be redescribed from type material, it is best to consider this species as a *species inquirenda*. When it is revalidated it is most likely that it will prove to be a senior synonym of *A. hylurgi*.

A. hylurgi also is close to *A. nonveilleri* Andrássy, 1959, the only difference being the length of the post uterine sac, about three body widths long in the latter species. *A. nonveilleri* is said to have " three longitudinal fields " (drei Langsfelder tragend), which probably means four lines, as in *A. hylurgi*.

It is interesting that a nematode from material from Gorham in Maine (USA) found associated with the insect *Hylurgops pinifex* in eastern white pine should be so similar to nematodes found feeding on cultures of *Cochliobolus sativus* isolated from the base of stems of wheat grown at Toowoomba in Queensland (Australia). Clearly this nematode is cosmopolitan and, from our observation, appears to be a fungal feeder. We have cultured the Australian population on a range of different fungi in addition to *R. solani* and *F. graminearum*. These include *Periconia macrospinosa* Lefebvre & A. G. Johnson, *Phoma* sp., *Microdochium bolleyi* (Sprague) de Hoog & Harmanides-Nijhof, *Gaeumannomyces* sp., *Fusarium esquiseti* (Corda) Sacc., *Periconia* sp., *Embellisia* sp., *Fusarium acuminatum* Ellis & Everh., *Embellisia chlamydospora* (Hoes, Bruehl & Shaw) Simmons and *Curvularia* sp.

No significant differences were detected in the me-

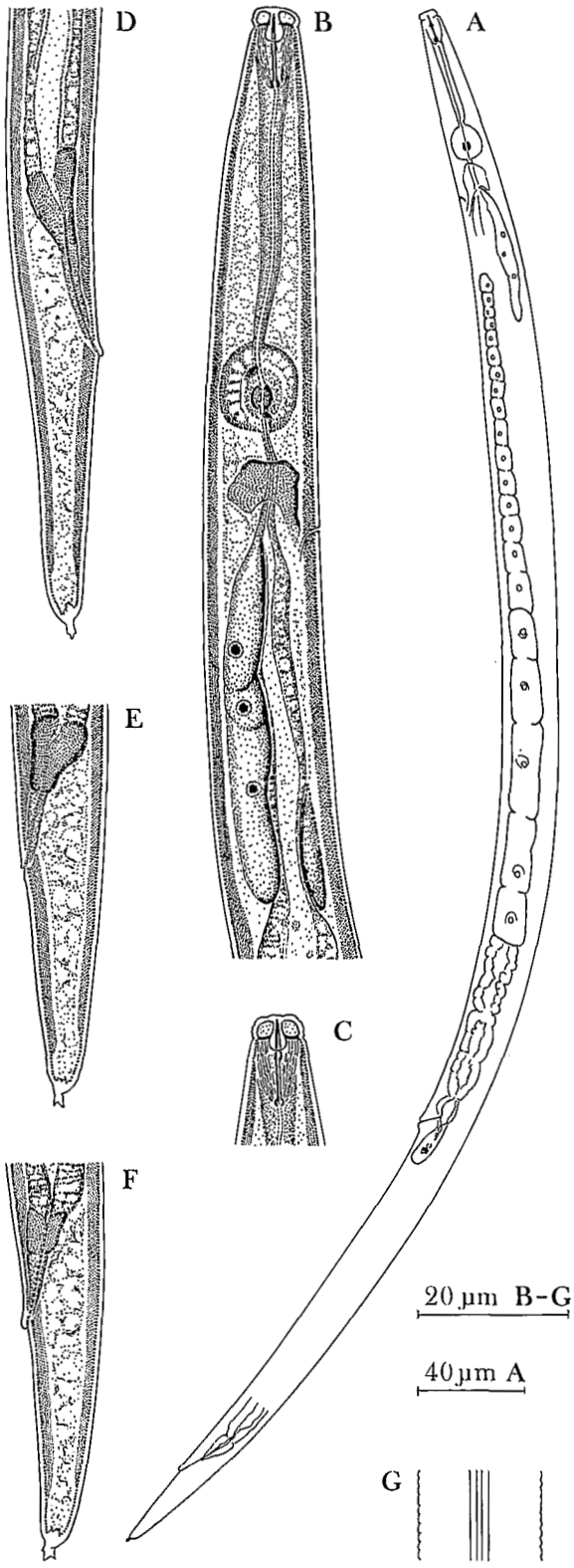

Fig. 4. *Aphelenchoides hylurgi*, paratype females. A : Body, thermal death position; B, C : Anterior ends; D-F : Posterior ends; G : Lateral field and body annulation.

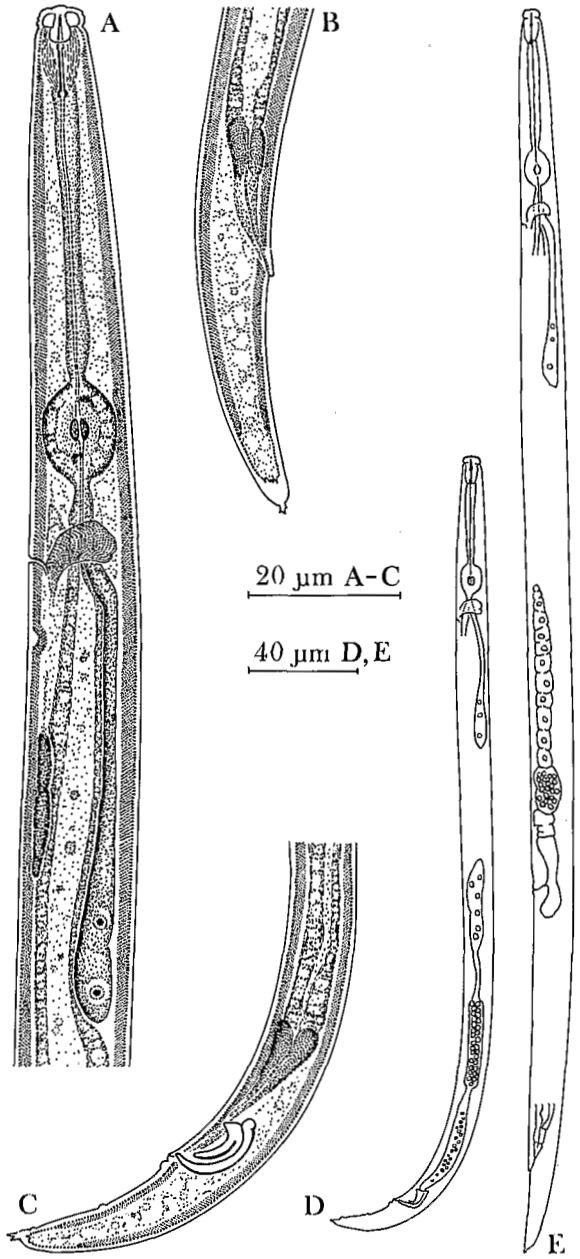

Fig. 5. *Aphelenchoides hylurgi*, specimens from Australia. A, B, E : females; C, D : males. A : anterior end; B, C : posterior ends; D, E : body thermal death position.

dian length of the spicules between males feeding on *R. solani* and those feeding on *F. graminearum* (Tab. 2), nor were there significant differences in tail and stylet length and in the ratios a, c and V (Tab. 1 and 2). The USA population of *A. hylurgi* does appear to have longer tails than those of the two Australian populations but this was not quite significant at the 5 % level due to the

high variability in the three (USA) specimens of *A. hylurgi.* The stylet of this (USA) population is significantly shorter than that of the two Australian populations ($P < 0.001$) and there is a slight though significant difference ($P < 0.01$) between the USA and Australian populations for the ratio V which is greater in the Australian population with means of 71.0 and 72.0 compared with a mean of 68.9 for the USA population (Tab. 1).

The USA population of *A. hylurgi* is significantly longer than either Australian population ($P < 0.001$). However the length of the Australian population feeding on *R. solani* is significantly greater ($P < 0.001$) than that feeding on *F. graminearum.* Thus these nematodes grow larger on *R. solani* than they do on *F. graminearum.* Furthermore oesophageal glands of both males and females are easier to detect and resolve on nematodes grown on *F. graminearum* than on ones grown in *R. solani.* These differences in size and oesophageal gland morphology due to nutrition are clearly not important in taxonomy. Generally these nematodes grow and develop equally well when feeding on either of these two species of plant pathogenic fungi.

Measurements of males and eggs were made for the first time and observation of egg development up to the tadpole stage (Fig. 3) was made. This was achieved at 23° after 21 hours compared with four days for *Meloidogyne javanica* and five days for *Anguina agrostis* under optimum conditions (Bird, 1972; Bird & Stynes, 1981).

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REFERENCES

ANDRÁSSY, I. (1959). Neue und wenig bekannte Nematoden aus Jugoslawien. *Ann. Hist.-nat. Mus. nat. Hung.,* 51 : 259-275.

B'CHIR, M. M. (1979). Description et étude biométrique, morphologique et biologique d'*Aphelenchoides tuzeti* n. sp. (Nematoda : Aphelenchoidea) et discussion des critères spécifiques appliqués au genre *Aphelenchoides. Nematologica,* 25 : 22-31.

BIRD, A. F. (1972). Influence of temperature on embryogenesis in *Meloidogyne javanica. J. Nematol.,* 4 : 206-213.

BIRD, A. F. & STYNES, B. A. (1981). The life cycle of *Anguina agrostis :* Embryogenesis. *Int. J. Parasitol.,* 11 : 23-33.

DAS, V. M. (1960). Studies on the nematode parasites of plants in Hyderabad (Andhra Pradesh, India). *Z. ParasitKde,* 19 : 553-605.

DE MAESENEER, J. & D'HERDE, J. (1963). Méthodes utilisées pour l'étude des anguillules libres du sol. *Revue Agric. Bruxelles,* 16 : 441-447.

EISENBACK, J. D. (1986). A comparison of techniques useful for preparing nematodes for scanning electron microscopy. *J. Nematol.,* 18 : 479-487.

KELLEY, R. O., DEKKER, R. A. F. & BLUEMINK, J. G. (1973). Ligand-mediated osmium binding : Its application in coating biological specimens for scanning electron microscopy. *J. Ultrastruct. Res.,* 45 : 254-258.

MASSEY, C. L. (1974). *Biology and taxonomy of nematode parasites and associates of bark beetles in the United States.* Agric. Handbook, Forest Service, USDA No. 446, 233 p.

RÖSSNER, J. & URLAND, K. (1983). Myceophagous nematodes of the genus *Aphelenchoides* on the stem base of cereals and their action against agents of cereal foot rot. *Nematologica,* 29 : 454-462.

SANWAL, K. C. (1965). Appraisal of taxonomic characters of " *parietinus* group " of species of the genus *Aphelenchoides* Fischer, 1984 (Nematoda : Aphelenchoidea). *Can. J. Zool.,* 43 : 987-995.

SEINHORST, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anydrous glycerin. *Nematologica,* 4 : 67-69.

SIDDIQI, M. R. & FRANKLIN, M. T. (1967). *Aphelenchoides goodeyi* n. sp. (Nematoda : Aphelenchoidea), a mycophagous nematode from south India. *Nematologica,* 13 : 125-130.

STYNES, B. A. & BIRD, A. F. (1980). Effects of methods of killing, fixing and mounting on measurements of *Anguina agrostis. Nematologica,* 26 : 467-474.

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