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| ATTN: | SUBMITTED: | 2009-12-09 15:25:22 |
| PHONE: (970) 494-7518 | PRINTED: | 2009-12-11 15:43:35 |
| FAX: | REQUEST NO.: | BOR-20185148 |
| E-MAIL: | SENT VIA: | World Wide Web |

PATRON TYPE: USDA

BOR Regular

| | |
|---------------------|--|
| TITLE: | JAPANESE JOURNAL OF NEMATOLOGY |
| VOLUME/ISSUE/PAGES: | 24 14-19 |
| DATE: | 1995 |
| AUTHOR OF ARTICLE: | Mian IH, Latif MA |
| TITLE OF ARTICLE: | ULTRASTRUCTURE AND MORPHOMETRICS OF DITYLENCHUS ANGUSTUS (BUTLER, 1913) FILIPJEV, 1936 (NEMATODA: ANGUINIDAE). |

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Ultrastructure and Morphometrics of *Ditylenchus angustus* (BUTLER, 1913) FILIPJEV, 1936 (Nematoda: Anguinidae)

I. H. MIAN* and M. A. LATIF*

The morphology and morphometrics of *Ditylenchus angustus* (BUTLER, 1913) FILIPJEV, 1936 were studied under light and scanning electron microscope (SEM). Lip region is cap-like, not distinctly offset and without distinct striations. Body annules are 0.7-1.2 μm wide. Lateral fields with four equally spaced incisures and areas between them are further divided to 6-8 sub-incisures. Vulval slit is less than half way across the body with slightly protruding lips. The measurements of the nematode are compared with those given in previous reports. Dimensions such as b, G₁, H, M, O, S, L', V', MB, length of tail, DGO and esophagus are first reported for *D. angustus*. *Jpn. J. Nematol.* 24(1): 14-19 (1994).

Key words: *Ditylenchus angustus*, morphometrics, ultrastructure, SEM, Bangladesh.

INTRODUCTION

The rice stem nematode, *Ditylenchus angustus* (BUTLER, 1913) FILIPJEV, 1936 is a major pest of rice in Bangladesh. It causes a severe disease of the crop called "Ufra" or "Dak pora". The disease is distributed throughout the country and attack deepwater rice (1), transplanted 'aman', and transplanted 'boro' rice grown under irrigated conditions (5, 6). The disease can cause 40-60% yield losses under Bangladesh conditions (5).

The morphology of *D. angustus* was first reported by BUTLER in 1913 (2). Later, FILIPJEV and SCHUURMANS STECKHOVEN, GOODEY, and SESHADRI and DASGUPTA also recorded its morphology by light microscopic observation (3, 4, 9). They gave only the dimensions of L, a, b, c, c', V, length of spicule, gubernaculum, stylet, distance from excretory pore to anterior end of the nematode, size of eggs and the second stage juveniles (2, 3, 4, 9). However, report on the ultrastructures and other dimensions are not available.

The present paper includes the further measurement and ultrastructure of a population of *D. angustus* observed under both light and scanning electron microscopes.

MATERIALS AND METHODS

An ufra nursery was established with a pure culture of *D. angustus*. Males, females and eggs were collected from ufra infected rice plants in the ufra nursery. The specimens were mounted in water, killed by gentle heat and measured with an oculometer under a compound microscope at 10 \times or 40 \times objective. The abbreviations of measurements used were as follows: L, a, b, c,

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c', V, T, b₁, G₁, H, M, O, S, L', V' and MB (8). The dimension of eggs was also recorded. Twenty nematodes of both sexes and eggs were used for measurements. The surface feature of *D. angustus* at ultrastructural level was observed under a scanning electron microscope (SEM). The nematode specimens were washed with distilled water, prefixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 2 hr and post fixed in 2% osmium tetroxide in the same buffer for 2 hr. Before immersion in osmium tetroxide, nematodes were rinsed thrice with the same buffer. The fixed nematodes were dehydrated with a seven graded ethanol series, viz. 30, 50, 70, 90, 99, 99.5 and 100% for 5, 10, 15, 20, 25, 30 and 60 min, respectively. After dehydration, they were air dried, mounted on SEM stubs with a double adhesive tape, coated with gold ion (20 nm) in an ion sputter (JFC-1100) and observed under a SEM (JSM T-220) at 10-15 kV accelerating voltages.

RESULTS AND DISCUSSION

Morphometrics of D. angustus. The morphometrics of males and females of *D. angustus* are given in Table 1. Among the measurements the values of b₁, G₁, H, M, O, S, L', V', MB, length of tail, DGO, and esophagus were not reported earlier. Therefore, these are new additions to the measurements of *D. angustus*.

Table 1. Measurement of *Ditylenchus angustus* (averages of twenty specimens).

| Character | Female | | | Male | | |
|-------------------------------------|-----------------|---------|---------|----------------|---------|------|
| | Lowest-Highest | Average | SD | Lowest-Highest | Average | SD |
| L (mm) | 1-1.2 | 1.1 | 0.06 | 0.9-1.1 | 1.00 | 0.05 |
| a | 50-60 | 55.3 | 3.25 | 55-60 | 56.7 | 1.42 |
| b | 7-8 | 7.5 | 0.32 | 6.5-7 | 6.7 | 0.17 |
| c | 20-24 | 22.1 | 1.34 | 18-20 | 19.4 | 0.58 |
| c' | 4-4.6 | 4.3 | 0.22 | 4-4.5 | 4.3 | 0.16 |
| V (%) | 72-80 | 76.1 | 2.68 | - | - | - |
| Spear (μm) | 10-11 | 10.6 | 0.31 | 10-11 | 10.6 | 0.29 |
| b ₁ | 17-20 | 18.1 | 1.22 | 15-18 | 16.9 | 0.86 |
| G ₁ (%) | 13-15 | 14.1 | 0.71 | 22-25 | 23.9 | 0.97 |
| H (μm) | 12.5-15 | 14.1 | 0.80 | 10-12 | 11.3 | 0.55 |
| M (%) | 22.5-25 | 23.6 | 0.76 | 22.5-25 | 23.3 | 0.65 |
| O (%) | 100-113 | 107.5 | 3.95 | 100-112 | 106.6 | 3.20 |
| S | 1.2-1.3 | 1.3 | 0.04 | 1.2-1.3 | 1.3 | 0.03 |
| T (%) | - | - | - | 55-60 | 57.1 | 1.53 |
| L' (mm) | 0.9 | 0.9 | 0.01 | 0.8-0.9 | 0.9 | 0.03 |
| V' (%) | 84-89 | 86.8 | 1.48 | - | - | - |
| MB (%) | 39-40 | 39.5 | 0.31 | 38-40 | 38.9 | 0.64 |
| Egg (μm) | 78×16- 87×20 | 82×18 | 2.7×1.3 | - | - | - |
| Spicule (μm) | - | - | - | 14-17.5 | 16.0 | 0.98 |
| Gubernaculum (μm) | - | - | - | 4-6 | 5.2 | 0.53 |
| Tail (μm) | 45-50 | 47.5 | 1.56 | 40-50 | 44.3 | 2.94 |
| DGO (μm) | 10-11 | 10.8 | 0.48 | 10-11 | 10.5 | 0.33 |
| Esophagus (μm) | 140-145 | 142.9 | 1.49 | 135-142 | 138.9 | 1.93 |
| Excretory pore to anterior end (μm) | 90-113 | 101.7 | 7.59 | 89-111 | 99.3 | 6.72 |

It reveals that there are variations among the measurements like a, c, spicule, gubernaculum of *D. angustus* recorded by other investigators and those recorded during the present study (Table 2). They might be due to intraspecific variations in nematodes, the localities and/or those in environmental factors under which they were cultured.

Ultrastructure. Body of the fixed nematode is slightly arcuate. Lip region is sclerotized and hexaradiate. The *en-face* view showed six almost equal size lips. Lip region is flattened and cap like but not distinctly set off. Striations in head region are not distinct (Fig. 1 A, B). Cuticle finely annulated. Annulations are 0.7-1.2 μ m in width and slightly interrupted by lateral lines (Fig. 1 C, D). Lateral fields are with equally spaced four incisures and areas between them are further divided to 6-8 sub-incisures. The lateral fields are extended from the region of median bulb to anal level (Fig. 1 C). Vulva is a distinct transverse slit reaching less than half way across the body, with slightly protruding lips (Fig. 2 A, B). The spicules are curved ventrally, and gubernaculum short and simple (Fig. 2 C, D).

Appendage like structures, bacteria and fungus attached to nematode body. In some nematode specimens collected from the infected rice plants grown in ufra nursery, spiny appendage like structures were found to be attached with the cuticle. They were not removable by washing with

Table 2. Comparison of measurements of *Ditylenchus angustus* reported by different authors.

| Character | BUTLER (1913) | | GOODEY (1953) | | SESHADRI (1975) | | Present study | |
|---|------------------|---------|------------------|---------|-----------------|---------|---------------------|---------|
| | Female | Male | Female | Male | Female | Male | Female | Male |
| L (mm) | 0.7-1.1 | 0.6-1.1 | 0.7-1.23 | 0.6-1.1 | 0.8-1.2 | 0.7-1.2 | 1.0-1.2 | 0.9-1.1 |
| a | 47-58 | 36-47 | 36-58 | 36-47 | 50-62 | 40-55 | 50-60 | 55-60 |
| b | 7 | 7 | 7-8 | 6-7 | 6-9 | 6-8 | 7-8 | 6.5-7 |
| c | 15-23 | 18-23 | 17-20 | 18-23 | 18-24 | 19-26 | 20-24 | 18-20 |
| c' | - | - | - | - | 5.2-5.4 | - | 4.0-4.6 | 4-4.5 |
| V (%) | 70-80 | - | 80 | - | 78-80 | - | 72-80 | - |
| Spear (μ m) | 9-10 | 9-10 | 10 | 10 | 10-11 | 10 | 10-11 | 10-11 |
| b ₁ | - | - | - | - | - | - | 16.6-20 | 15-18 |
| G ₁ (%) | - | - | - | - | - | - | 13-15 | 22-25 |
| H (μ m) | - | - | - | - | - | - | 12.5-15 | 10-12 |
| M (%) | - | - | - | - | - | - | 22.5-25 | 22.5-25 |
| O (%) | - | - | - | - | - | - | 100-113 | 100-112 |
| S | - | - | - | - | - | - | 1.2-1.3 | 1.2-1.3 |
| T (%) | - | - | - | - | - | 60-73 | - | 55-60 |
| L' (mm) | - | - | - | - | - | - | 0.90-0.92 | 0.8-0.9 |
| V' (%) | - | - | - | - | - | - | 84-89 | - |
| MB (%) | - | - | - | - | - | - | 39-40 | 38-40 |
| Egg (μ m) | 80-88 × 16-20 | - | 80-84 × 16-20 | - | - | - | 78 × 16- 87 × 20 | - |
| Spicule (μ m) | - | - | - | 20 | - | 16-21 | - | 14-17.5 |
| Gubernaculum (μ m) | - | - | - | 8 | - | 6-9 | - | 4-6 |
| Tail (μ m) | - | - | - | - | - | - | 45-50 | 40-50 |
| DGO (μ m) | - | - | - | - | - | - | 10-11 | 10-11 |
| Esophagus (μ m) | - | - | - | - | - | - | 140-145 | 135-142 |
| Excretory pore to anterior end (μ m) | - | - | - | - | 90-110 | - | 90-113 | 89-111 |

gubernaculum
ent study (Table
and/or those in

sclerotized and
attened and cap
A, B). Cuticle
by lateral lines
between them are
region of median
half way across
ventrally, and

some nematode
appendage like
by washing with

authors.

Present study

| Female | Male |
|---------------|---------|
| 0-1.2 | 0.9-1.1 |
| 50-60 | 55-60 |
| 7-8 | 6.5-7 |
| 20-24 | 18-20 |
| 0-4.6 | 4-4.5 |
| 72-80 | - |
| 10-11 | 10-11 |
| 6-20 | 15-18 |
| 13-15 | 22-25 |
| 5-15 | 10-12 |
| 5-25 | 22.5-25 |
| 0-113 | 100-112 |
| 2-1.3 | 1.2-1.3 |
| - | 55-60 |
| 0.90- 0.92 | 0.8-0.9 |
| 84-89 | - |
| 39-40 | 38-40 |
| ×16- ×20 | - |
| - | 14-17.5 |
| - | 4-6 |
| 45-50 | 40-50 |
| 10-11 | 10-11 |
| 0-145 | 135-142 |
| 0-113 | 89-111 |

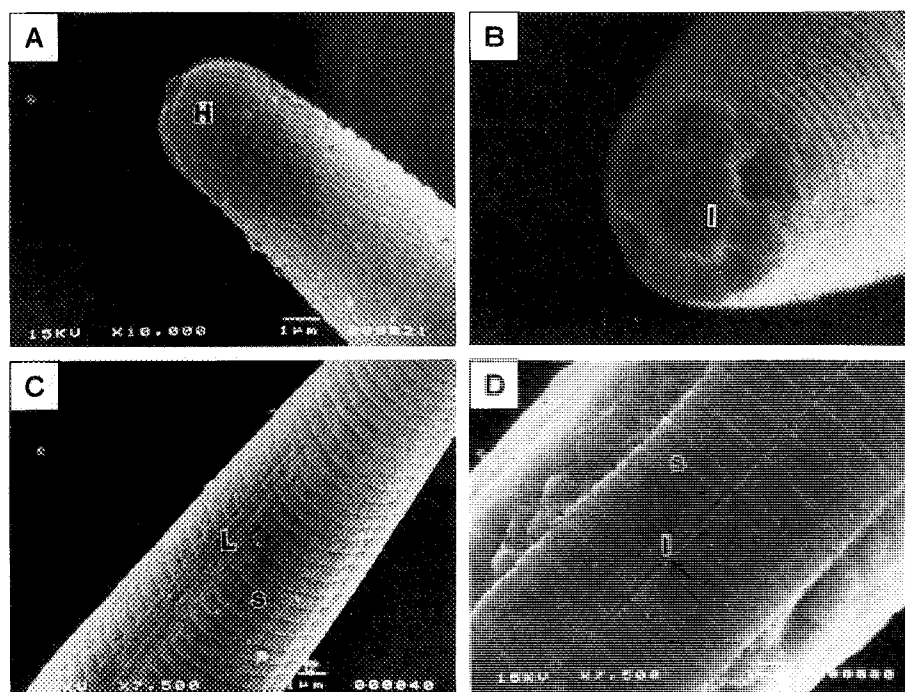


Fig. 1. Scanning electron micrographs showing ultrastructures of head region and body cuticle of *Ditylenchus angustus*.

A. Sclerotized head with finely annulation (H).

B. Head with hexaradiate lip region (l).

C. Body cuticle showing striation (s) and lateral field (L).

D. Enlarge view of body cuticle showing striation (s) and lateral field (l).

distilled water or 1.0% solution of NaOCl. The structures were pointed anteriorly and 1.4-4.5 μm long. Many of them were inserted into the cuticle (Fig. 3 A). Some kinds of minute appendage like structures were also attached to the spicules of adult males (Fig. 3 B). Similar types of appendage like structures were also observed on leaf sheath of rice plants. They were not found on nematodes obtained from *in-vitro* culture. These structures are probably some kinds of trichome of rice plants which were attached with the nematode body during their movement on the rice plants.

At the time of observation under SEM, rod shaped bacteria as well as mycelium of a nematode trapping fungus were observed on the surface of nematode body collected from infected rice plants from the ufra nursery. The fungus was identified as *Dactylaria* sp.

ACKNOWLEDGEMENTS

The authors wish to thank to the National Science and Technology Division, Ministry of Education, the Government of the People's Republic of Bangladesh for awarding a fellowship to the second author during this study.

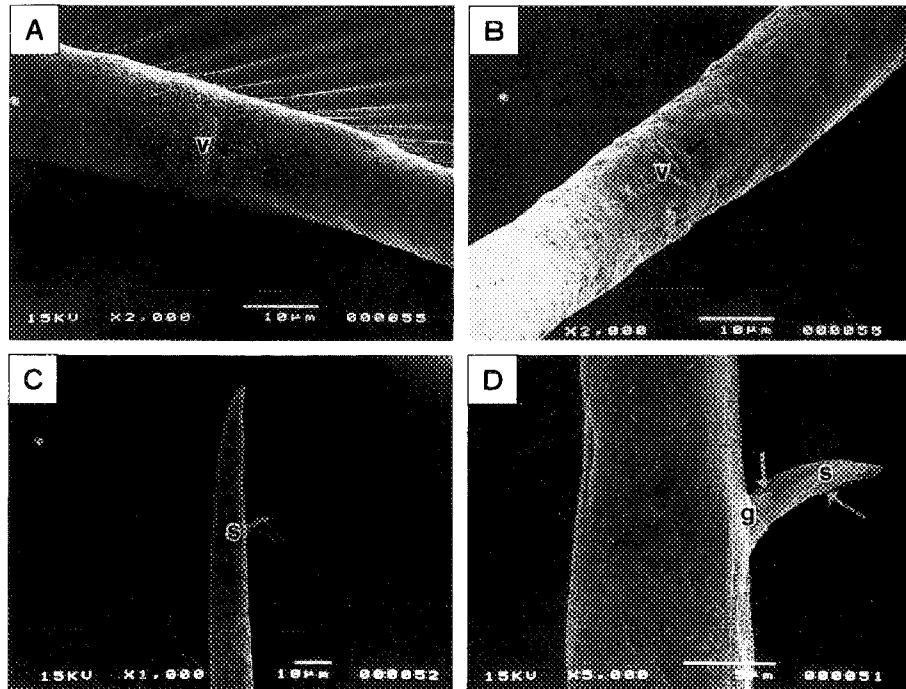


Fig. 2. Scanning electron micrographs showing ultrastructures of genital organs of *Ditylenchus angustus*.

A. Vulva with flappy lips (V).

B. Vulval slit covering <33% of body diameter (V).

C. Tail region of a male showing protruding spicule (s).

D. Enlarge view of genital organ of a male showing spicule (s) with gubernaculum (g).

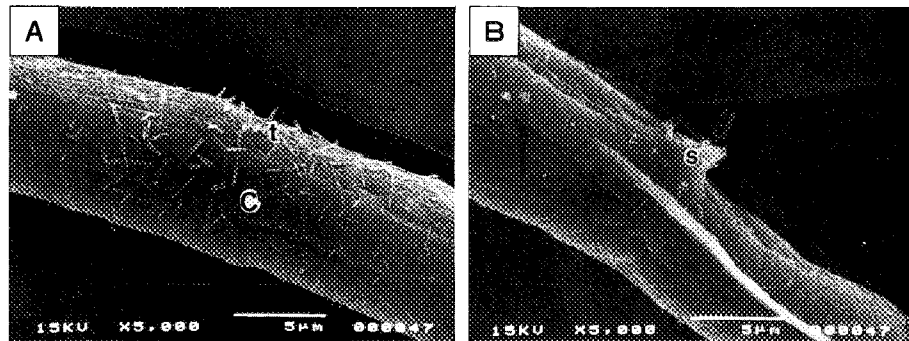


Fig. 3. Scanning electron micrographs showing rice trichome like structures attached to body of *Ditylenchus angustus*.

(A) Body cuticle (C) with trichome like structures (t).

(B) Spicule (s) with trichome like structure (t).

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Accepted for publication : August 1, 1993

和文摘

*Ditylenchus angustus*の微細構造と形態計測値

I. H. MIAN • M. A. LATIF

*Ditylenchus angustus*の微細構造と形態計測値を、光学及び走査電子顕微鏡下で調べた。唇部はcap状を呈し、体部から明瞭にはくびれず、条溝を欠いている。体環の幅は0.7-1.2 μ mである。側帯には、4本の等間隔の側帯溝があり、それらの間は、さらに6-8の副側帯溝で分かれている。陰門隙は、体の半分以下を横切っており、その開口部はわずかに隆起している。本線虫の計測値を、すでに報告されているものと比較した。本線虫としてはじめての計測部分の値も報告する。