

**Cuticle Ultrastructure of**  
*Hemicycliophora arenaria*, *Aphelenchus avenae*,  
*Hirschmanniella gracilis* and *Hirschmanniella belli*<sup>1</sup>

P. W. JOHNSON, S. D. VAN GUNDY AND W. W. THOMSON<sup>2</sup>

*Abstract:* Ultrathin sections of all parasitic stages of *Hemicycliophora arenaria* revealed two major divisions in the body covering. The outermost was a seven-layered sheath and the innermost a five-layered cuticle comprising three zones; an outer trilaminar cortex, a fibrillar matrix and a striated basal layer. The body covering of the nonparasitic males also exhibited two major divisions: the outer, a relatively thin four-layered sheath and the inner, a six-layered cuticle consisting of three zones; an outer trilaminar cortex, a two-layered matrix and a striated basal layer. The cuticles of all stages of *Aphelenchus avenae* were similar, consisting of five layers divisible into three zones; an outer trilaminar cortex, a fibrillar matrix and a striated basal layer. *Hirschmanniella gracilis* and *H. belli* cuticles were also similar in all stages examined, consisting of six layers divisible into three zones; an outer trilaminar cortex, a two-layered matrix and a striated basal layer. *Key Words:* Cuticle, Ultrastructure, *Hemicycliophora arenaria*, *Aphelenchus avenae*, *Hirschmanniella gracilis*, *Hirschmanniella belli*.

Electron microscope techniques have greatly increased our knowledge of the structure and function of the cuticle of animal-parasitic helminths (12). Only recently have these methods been applied in studies of the cuticle of plant-parasitic nematodes (1, 2, 4, 5, 6, 9, 15, 16, 19, 21, 23). The present study was designed to increase our knowledge of the fine structure of the body covering of the sheath nematode, *Hemicycliophora arenaria*, in which all soil inhabiting stages are encased in a second cuticle or sheath. The nematode genera *Aphelenchus* and *Hirschmanniella*, both lacking a sheath and from different habitats, were included for comparison.

#### MATERIALS AND METHODS

*Hemicycliophora arenaria* Raski, was cultured on greenhouse-grown tomato (*Lycopersicon esculentum* Mill. 'Alexander's Homozygous 630818-1, TMV-resistant') at

28–30 C. Populations of *Aphelenchus avenae* Bastian, were reared monoxenically on the fungus *Rhizoctonia solani* Kühn in petri dishes on potato-dextrose agar (PDA). Populations of *Hirschmanniella gracilis* (de Man) Luc and Goodey, were maintained on a *Cyperus* sp. L. in greenhouse pot cultures. A collection of *Hirschmanniella belli* Sher was obtained from a soil sample taken in Culiacan, Mexico.

All nematode specimens were hand picked into groups consisting of various larval (L2, L3, L4) and adult stages and killed and fixed in a 5 : 1 v/v mixture of formal-calcium fixative (8) and 20% dimethyl sulfoxide (DMSO) in distilled water (16) prepared just before use. Initially, fixation time was 24–48 hr at 5 C but this was later modified to 30 min at room temperature. Following fixation, specimens were rinsed three times in tap water and post-fixed at room temperature in phosphate-buffered 1% osmium tetroxide (13) for 2½–3 hr. After osmication, they were rinsed three times in tap water, transferred to tap water in 35 mm plastic petri dishes, and mid-body segments were excised and embedded in 2% agar in 0.9% saline (20). Agar blocks containing nema-

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<sup>2</sup> Department of Nematology and Department of Life Sciences, University of California, Riverside, California 92502. Present address of senior author: Canada Department of Agriculture, Research Station, Harrow, Ontario, Canada.

tode segments were transferred to Beem® capsules and dehydrated in a graded acetone series. The blocks were embedded in a Maraglas® mixture formulated as follows: 9 parts Maraset® resin 655, 1 part Cardolite® resin NC513, and 1.0 ml N,N-dimethylbenzylamine per 50 ml of resin.

Following polymerization, at 65 C for a minimum of 4 days, the blocks were trimmed and thin sections were cut with glass knives on a Sorvall MT-2 Porter-Blum ultramicrotome. Sections were mounted on 200 mesh uncoated copper grids and exposed on the grid to various staining schedules. The primary staining schedule consisted of 20 min in a saturated alcoholic solution of uranyl acetate followed by 5 min in lead citrate (14). Other stains employed were 1% potassium permanganate (14) and 0.2% phosphotungstic acid in 50% ethyl alcohol (14).

Sections were examined and photographed at either 60 or 80 KV in a Phillips 300 electron microscope.

## RESULTS

**HEMICYCLIOPHORA ARENARIA:** Sections of nematode specimens of all parasitic stages (L2, L3, L4 and females) revealed two major divisions in the body covering. The outermost was a seven-layered sheath (Fig. 1-A, a-g) and the innermost a five-layered cuticle (Fig. 1-B, 1-5). The individual layers of the cuticle and sheath were structurally similar in both larvae and adult females, differing only in thickness.

The body covering of the nonparasitic mature male differed from that of the larvae and mature females in possessing a modified four-layered sheath (Fig. 3-A, a-d), well developed lateral fields (Fig. 4-A) with four incisures, and a wider striated basal layer in the six-layered cuticle (Fig. 3-A, B).

**Sheath.**—Light microscope observations of the rather loose-fitting sheath revealed it

TABLE 1. Comparative measurements ( $m\mu$ ) of the sheath layers of female and larval stages of *Hemicycliophora arenaria*.

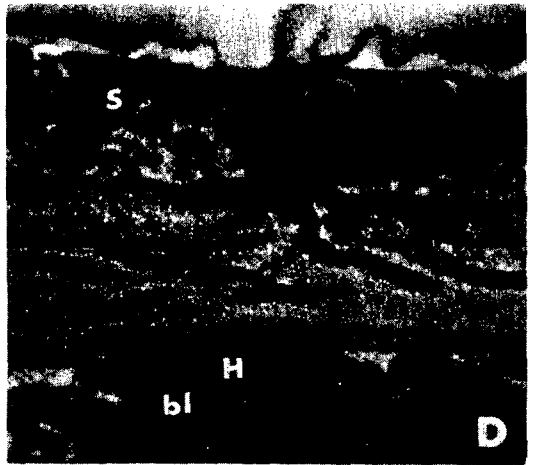
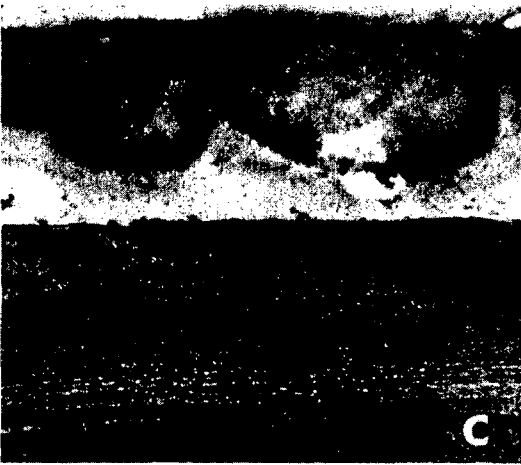
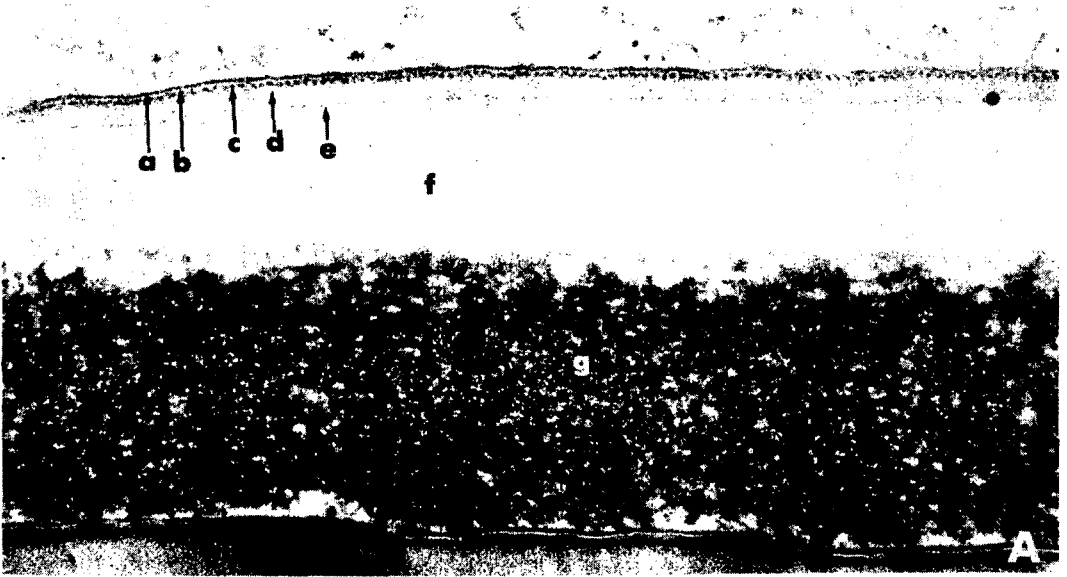
Stage	Sheath layers						
	a	b	c	d	e	f	g
L2	4.6 <sup>a</sup>	4.8	4.6	32.3		50-150 <sup>b</sup>	
L3	4.6	5.4	5.4	34.4		210	0-160
L4	4.8	5.2	5.2	35.0	2.4	220-440	0-410
Female	4.8	6.1	6.1	36.3	2.6	500	0-500
Average	4.7	5.4	5.3	34.5	2.5	50-500	0-500

<sup>a</sup> Data are averages of 10 or more measurements.

<sup>b</sup> Range of measurements from specimens observed.

was attached to the underlying cuticle only at the head and tail which were excised for this study. Consequently, in many sections the sheath was distorted or lost. The modified sheath of the male was not observed under the light microscope.

In all parasitic stages the outer five layers of the sheath (Fig. 1-A, a-e) had constant dimensions; only the inner two layers varied in thickness (Fig. 1-A, f-g). Measurements of the sheath layers of the different stages are presented in Table 1. Outermost was a trilaminar zone consisting of: a thin osmiophilic outer layer (a), an electron-transparent layer (b), and an inner broken osmiophilic layer (c), which averaged 4.7, 5.4 and 5.3  $m\mu$  in thickness, respectively. Inward from this was an electron-transparent layer (d) averaging 34.5  $m\mu$  thick. Bounding this layer on the inner side was a second thin, broken, osmiophilic layer (e), approximately 2.5  $m\mu$  thick and beneath that a thick electron transparent layer (f) of variable thickness (Fig. 1-B). The body annulations, averaging 2  $\mu$  apart, cut deeply into this layer which ranged from as thin as 50  $m\mu$  in L2 larvae to as thick as 500  $m\mu$  in the mature female. The seventh and innermost layer (g) of the sheath was a porous or spongy-appearing electron-dense layer of variable thickness (Fig. 1-B). The thickest point, mid-way between the body annulations, averaged 500  $m\mu$  in mature female



specimens and tapered off to zero at the base of the annulation in some specimens.

The modified four-layered sheath of the male (Figs. 3-A, 4-B) was approximately 55m $\mu$  thick and composed of a trilaminate outer zone consisting of: a thin osmiophilic outer layer (a), an electron-transparent middle layer (b), and a broken osmiophilic inner layer (c). These layers averaged 2.5, 5.2 and 4.3 m $\mu$  in thickness, respectively. The basal layer of the sheath (d) averaged 43.4 m $\mu$  in thickness and appeared as an electron-transparent layer in some micrographs (Figs. 3-B, 4-A) and as a moderately electron-dense layer in others (Figs. 3-A, 4-B).

*Cuticle.*—The cuticle of larval stages and females (Figs. 1–2) consisted of five distinct layers (Figs. 1-B, 2-A) in three zones: cortex, matrix and striated zones (Fig. 2-A).

Outermost was a trilaminate cortical zone consisting of a thin outer osmiophilic layer, an electron-transparent layer and an inner osmiophilic layer. These layers averaged 4.6, 8.1 and 14.6 m $\mu$  in thickness, respectively (Table 2). The inner layer was sharply defined on its outer edge but more diffuse on its inner side.

The next innermost zone was a thick matrix layer, ranging from 218.7–718.1 m $\mu$

TABLE 2. Comparative measurements (m $\mu$ ) of the cuticle layers of female and larval stages of *Hemicyclophora arenaria*.

Stage	Cuticle layers				
	Trilaminate cortex			Matrix	Striated
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5
L2	4.3*	7.5	13.7	218.7	45.0
L3	5.1	8.0	14.0	281.6	64.0
L4	5.0	8.0	14.0	459.2	70.4
Female	4.0	8.8	16.9	718.1	73.8
Average	4.6	8.1	14.6	419.4	63.3

\* Averages of 10 or more measurements.

in thickness, in which two regions could frequently be distinguished. An outer fibrillar one (Fig. 1-C, 4a) with progressively narrower striations toward its base (Fig. 2-A, arrows) and an inner loose fibrillar layer (Figs. 1-C, 4b; 2-A, 4b). In other sections these two regions were not observed and the striations were less obvious (Fig. 1-D). Although these two layers appeared to differ structurally, it was felt that the inner region represented only a less-compacted zone in a single fibrillar matrix layer. Occasionally longitudinal sections through the cuticle revealed a substructure in the outer region of the matrix (Fig. 2-B). Variation in matrix thickness accounted for most of the variation

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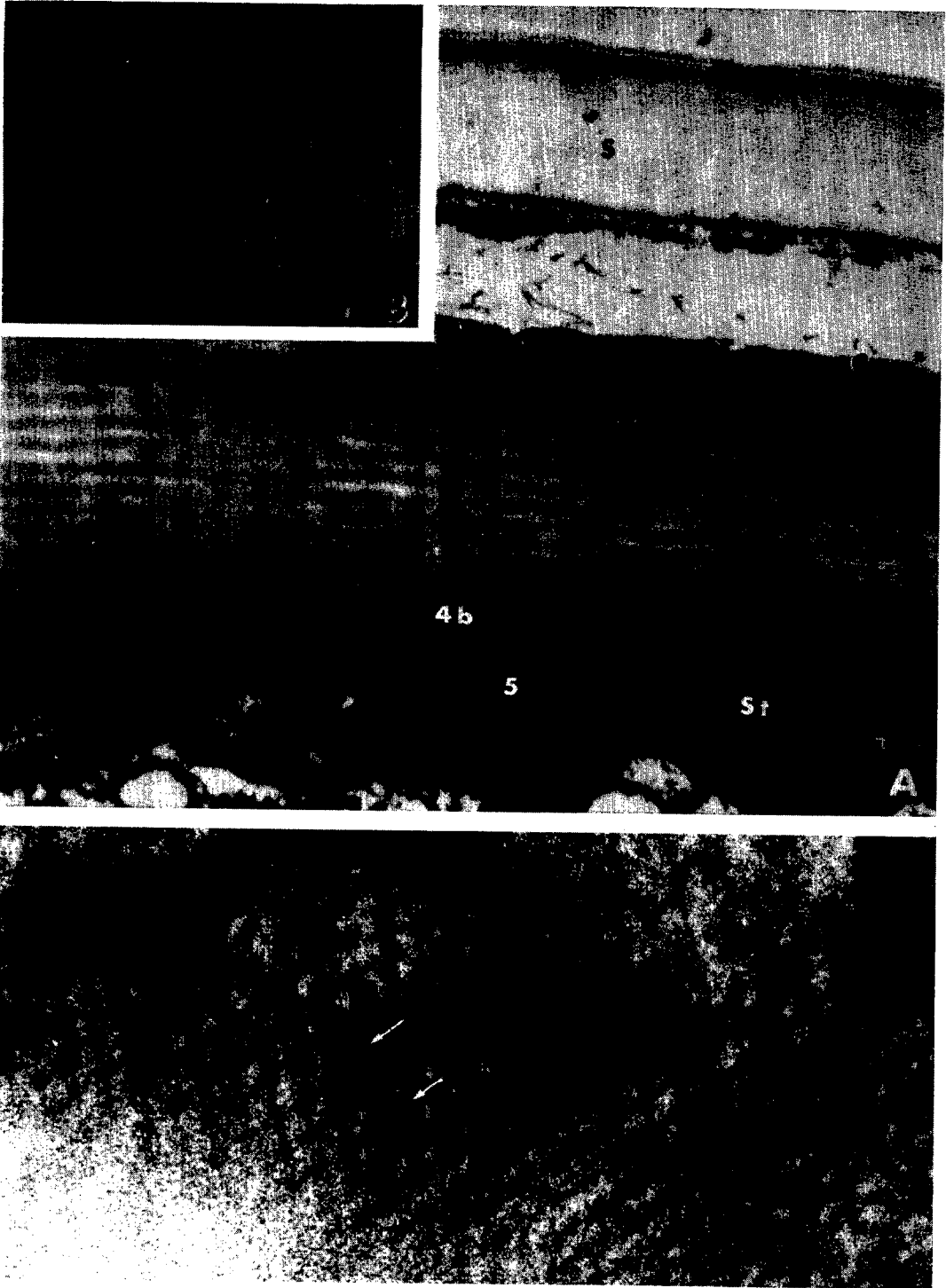
FIG. 1. Electron micrographs of *H. arenaria* L4 and female specimens. **A.** Transverse section of L4 larvae sheath.  $\times 90,000$ ; **B.** Longitudinal section of L4 larvae sheath and cuticle. Note variation in thickness of layers f and g.  $\times 31,500$ ; **C.** Transverse section of female showing cuticle and deformed sheath. Stained with phosphotungstic acid, uranyl acetate and lead citrate.  $\times 29,000$ ; **D.** Transverse section of female. Stained with potassium permanganate and uranyl acetate.  $\times 56,000$ .

#### KEY TO LETTERING OF FIGURES

A. annulation  
 a,b,c, etc. layers of sheath, signified in centripetal order.  
 1,2,3, etc. layers of cuticle, numbered in centripetal order.  
 bl. basal lamella  
 C. cortex  
 Cu. cuticle  
 F1,F2, etc.—Fibrillar layers (in centripetal order)

H. hypodermis  
 M. matrix  
 Mi. mitochondria  
 Ms. modified sheath of *H. arenaria* male  
 Mu. somatic musculature  
 S. sheath  
 St. striated layer

All sections stained with uranyl acetate and lead citrate, unless otherwise indicated.



in cuticle thickness of the different stages (Table 2). Matrix thickness was only slightly reduced at the base of the annulations in the sheath (Fig. 1-B).

The third and innermost zone was a striated layer (Fig. 2-A, St) averaging 63  $m\mu$  in thickness. The striae, arranged perpendicular to the nematode surface, showed a periodicity of approximately 20  $m\mu$  in transverse sections (Fig. 2-A), and 16.0  $m\mu$  in longitudinal sections (Fig. 1-B) and appeared to be embedded in less electron-dense material. Oblique sections through this layer (Fig. 2-C) revealed a network of electron-dense spots and less dense interconnecting lines.

The membrane-bounded hypodermis (Fig. 1-D) internal to the striated layer varied in thickness and bulged into the body cavity forming chords at four places: the two lateral chords were larger than the dorsal and ventral chords. The hypodermis was separated from the somatic musculature in interchordal zones and from the body cavity in chordal zones by a membrane-bound basal lamella (Fig. 1-D). Mitochondria and other intracellular structures were absent in the interchordal hypodermis but present in chordal areas. Nuclei were observed only in the large lateral chords.

The cuticle of the male consisted of six distinct layers divisible into three zones: a trilaminar cortex, a two-layered matrix and a striated basal zone (Fig. 3-A). The trilaminar cortex consisted of: a thin osmiophilic outer layer, an electron-transparent layer and an osmiophilic inner layer. These layers averaged 5.2, 7.7 and 27.9  $m\mu$  in width, respectively. The inner layer was

sharply defined on its outer edge but more diffuse at its inner boundary.

Below the cortex was found the two-layered matrix zone. The outermost layer, layer four of the cuticle, was a moderately electron-dense fibrillar layer (Figs. 3-A, B; 4-A) ranging from 70  $m\mu$  in thickness at the base of the annulation to 160  $m\mu$  mid-way between annulations. Basally in the matrix, layer five was an electron-transparent layer, 0–83  $m\mu$  thick, appearing similar to Wisse and Daems' (19) "fluid-filled space" in the cuticle of L2 *Heterodera rostochiensis* larvae. This layer was crossed by strands of material apparently identical to the fibrillar strands in layer four with which they merged (Figs. 3-A, B; 4-A) and similar to those in the matrix of the outer stages.

The innermost zone of the male cuticle was a striated layer ranging from 130–150  $m\mu$  in thickness, nearly double the thickness found in the female. As in the other stages, the striae were arranged perpendicular to the nematode surface and showed a periodicity of approximately 18  $m\mu$  in transverse sections and 15  $m\mu$  in longitudinal sections. In oblique sections through this layer (Fig. 4-C) a network of electron-dense spots and less dense interconnecting lines embedded in less electron-dense material were present.

In longitudinal section (Fig. 3-B) body annules averaged 1.1  $\mu$  wide. The sheath and the outer five layers of the cuticle were bent inward at the annulations, layer four was reduced in thickness by about one-half and layer five appeared to be absent.

A modification of the basic cuticle structure was seen in the area of the lateral field (Fig. 4-A). The lateral field, averaging 3.2

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FIG. 2. Electron micrographs of *H. arenaria* L4 and female specimens: A. Transverse section of female.  $\times$  50,000; B. Longitudinal section of female showing substructure in the matrix layer.  $\times$  68,500; C. Oblique section through the striated layer of an L4 larva. Note electron dense spots (arrows) with less dense interconnecting lines.  $\times$  220,000.

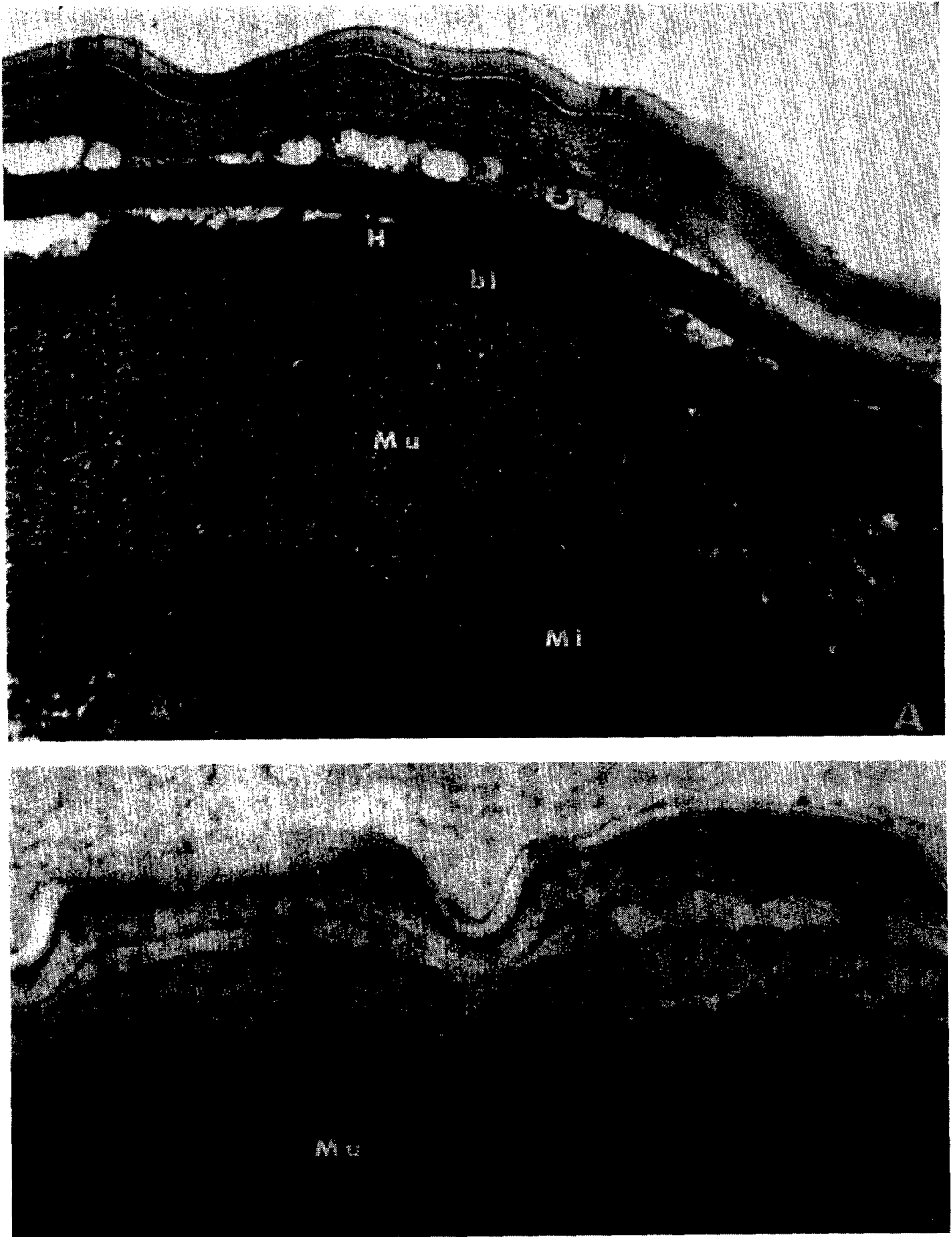


FIG. 3. Electron micrographs of *H. arenaria* males. A. Transverse section showing layers of the modified sheath and cuticle.  $\times 60,000$ ; B. Longitudinal section.  $\times 60,000$ .

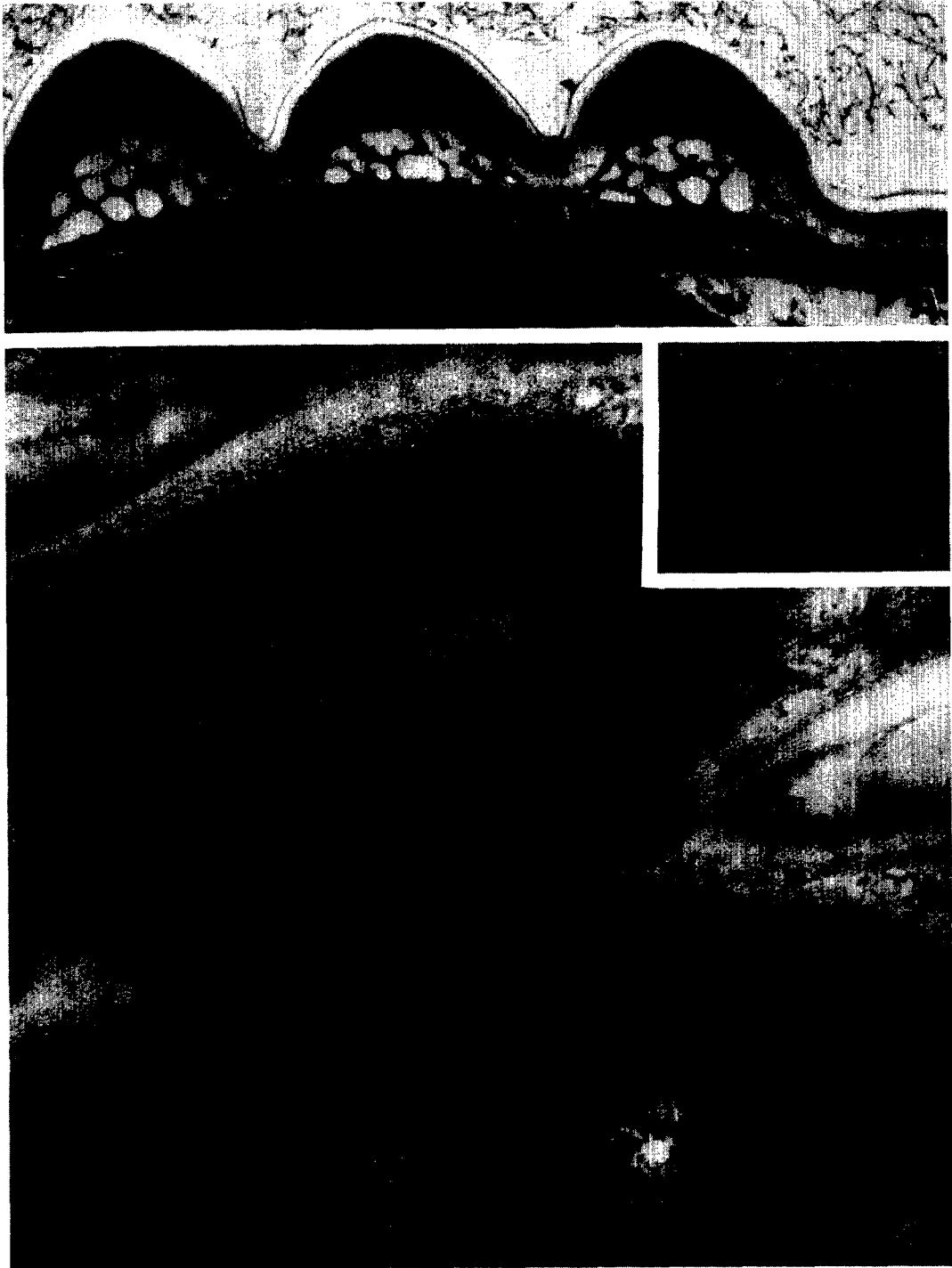


FIG. 4. Electron micrographs of *H. arenaria* males. **A.** Transverse section through the lateral field showing forking (arrows) in the striated layer and its replacement by two fibrillar layers.  $\times 38,000$ ; **B.** Enlarged transverse section through modified sheath and outer cuticle layers.  $\times 144,000$ ; **C.** Oblique section through striated basal layer.  $\times 60,000$ .



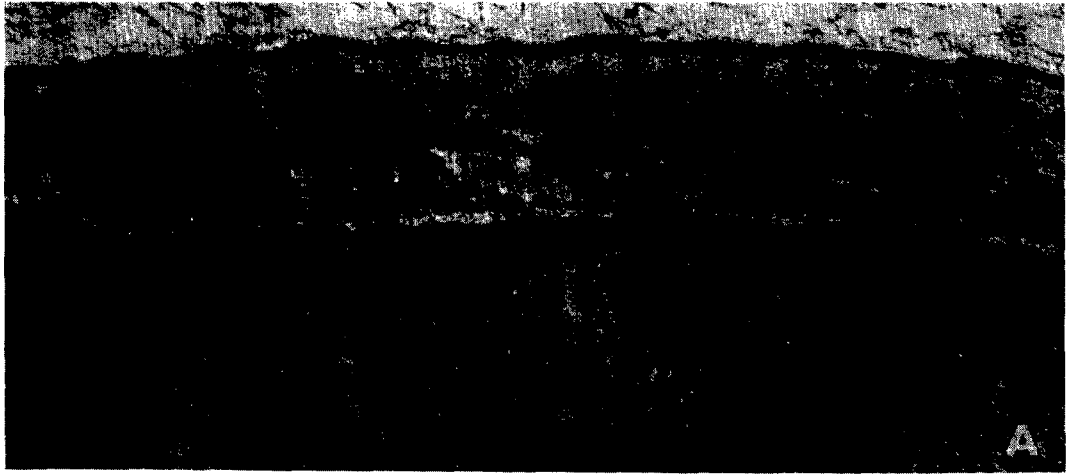


TABLE 3. Measurements ( $m\mu$ ) of the cuticle layers of the adult and larval stages of *Aphelenchus avenae*.

Stage	Cuticle layers				
	Trilaminar cortex			Matrix	Striated
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5
L2	4.0 <sup>a</sup>	7.6	12.0	41.3–67.6 <sup>b</sup>	84.6
L3	4.0	7.6	12.0	67.6–100.0	110.7
L4	4.1	7.6	12.6	75.3–116.9	146.1
Male	4.5	8.0	13.8	61.5–146.1	207.6
Female	4.2	8.0	13.8	124.6–156.9	193.8
Average	4.1	7.7	12.8	41.3–156.9	150.0

<sup>a</sup> Averages of 10 or more measurements.

<sup>b</sup> Range of measurements from specimens observed.

$\mu$  in width, consisted of three ridges extending along the lateral wall of the nematode. At four places the "fluid-filled space" was interrupted by incisures. Under these ridges the two matrix layers were thicker; layer four about twice as thick, and layer five about two and one-half times thicker than in locations away from the lateral field. The striated sixth layer showed considerable modification. It became forked at both sides of the lateral field (Fig. 4-A, arrows), and a wedge composed of two layers of unstriated fibrous material (Fig. 4-A, F1, F2) appeared between the two tongues. In all cases observed, the innermost tongue appeared to extend deeper into the area under the lateral field. Strands of material from the outermost fibrous layer crossed the fluid-filled space and merged with layer four of the matrix. These two new layers, situated in place of the striated sixth layer, made the cuticle of the lateral field a seven-layered structure. As in the other stages, the striated basal layer was underlain by the membrane-bound hypodermis and basal lamella.

*APHELENCHUS AVENAE*: The cuticle struc-

ture of *A. avenae* was similar in all stages consisting of five layers (Fig. 5-A) which could be subdivided in three visibly different zones: an outer trilaminar cortical zone, a fibrillar matrix zone and a striated basal zone (Fig. 5-A). Measurements of the cuticular layers are given in Table 3.

The cortex consisted of two osmiophilic components separated by an electron-transparent layer. These layers averaged 4.1, 7.7 and 12.8  $m\mu$  in width, respectively. The innermost layer was sharply defined on its outer edge but more diffuse at its inner boundary.

Below the cortex was a fibrillar matrix layer ranging from 41  $m\mu$  in thickness in L2 larvae (Fig. 6-A) to 157  $m\mu$  in mature females (Fig. 5-A). The body annulations, .71 to .86  $\mu$  apart (Fig. 5-B), intruded into this layer resulting in considerable variation in its thickness.

The basal zone of the cuticle was a striated layer ranging from 85  $m\mu$  in thickness in L2 larvae (Fig. 6-A) to 208  $m\mu$  in adult worms (Fig. 5-A, B, C). The striae showed a periodicity of approximately 18.5  $m\mu$  in transverse sections and 15.5  $m\mu$  in longitudinal sections. Oblique sections through this layer (Fig. 6-B) showed a network of electron-dense spots and less dense interconnecting lines as in *H. arenaria*.

A modification of the basic cuticle structure was present in the lateral fields of all stages (Fig. 5-C). The striated layer tailed off at the outer edges of the field (Fig. 5-C, arrows) and was replaced under the ridges by two fibrillar layers which made the cuticle in the lateral field a six-layered structure.

Located below the cuticle was a membrane-bound hypodermis and basal lamella similar to that in *H. arenaria*.

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FIG. 5. Electron micrographs of *A. avenae* male and females. A. Transverse section of female.  $\times 60,000$ ; B. Longitudinal section of female.  $\times 60,000$ ; C. Transverse section of male at edge of lateral field showing striated layer tapering off (arrows) to form two fibrillar layers.  $\times 53,000$ .

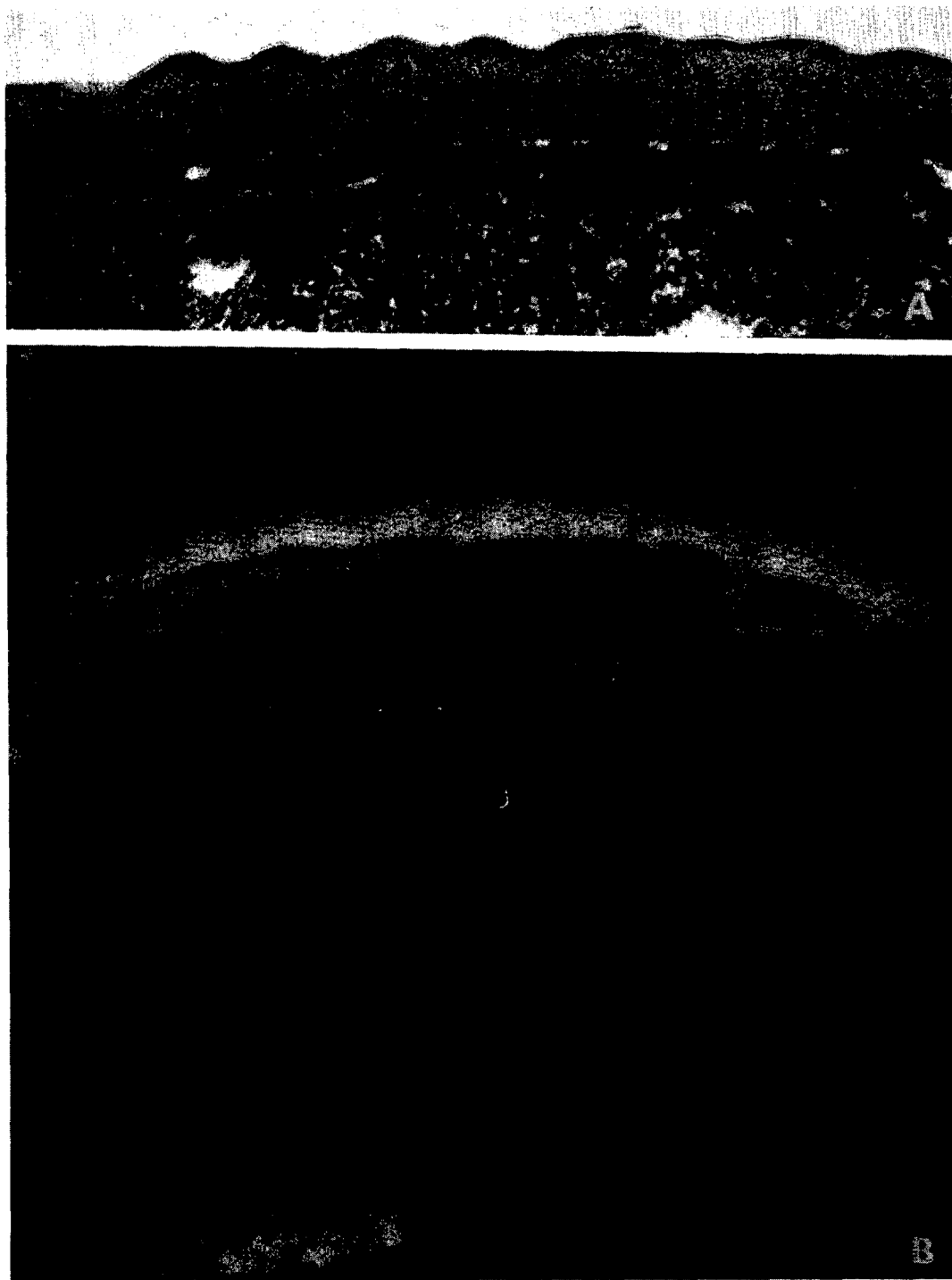


FIG. 6. Electron micrographs of *A. avenae* larval stages. **A.** Transverse section of L2 larvae.  $\times 76,000$ ; **B.** Oblique section through striated layer of L3 larva.  $\times 76,000$ .

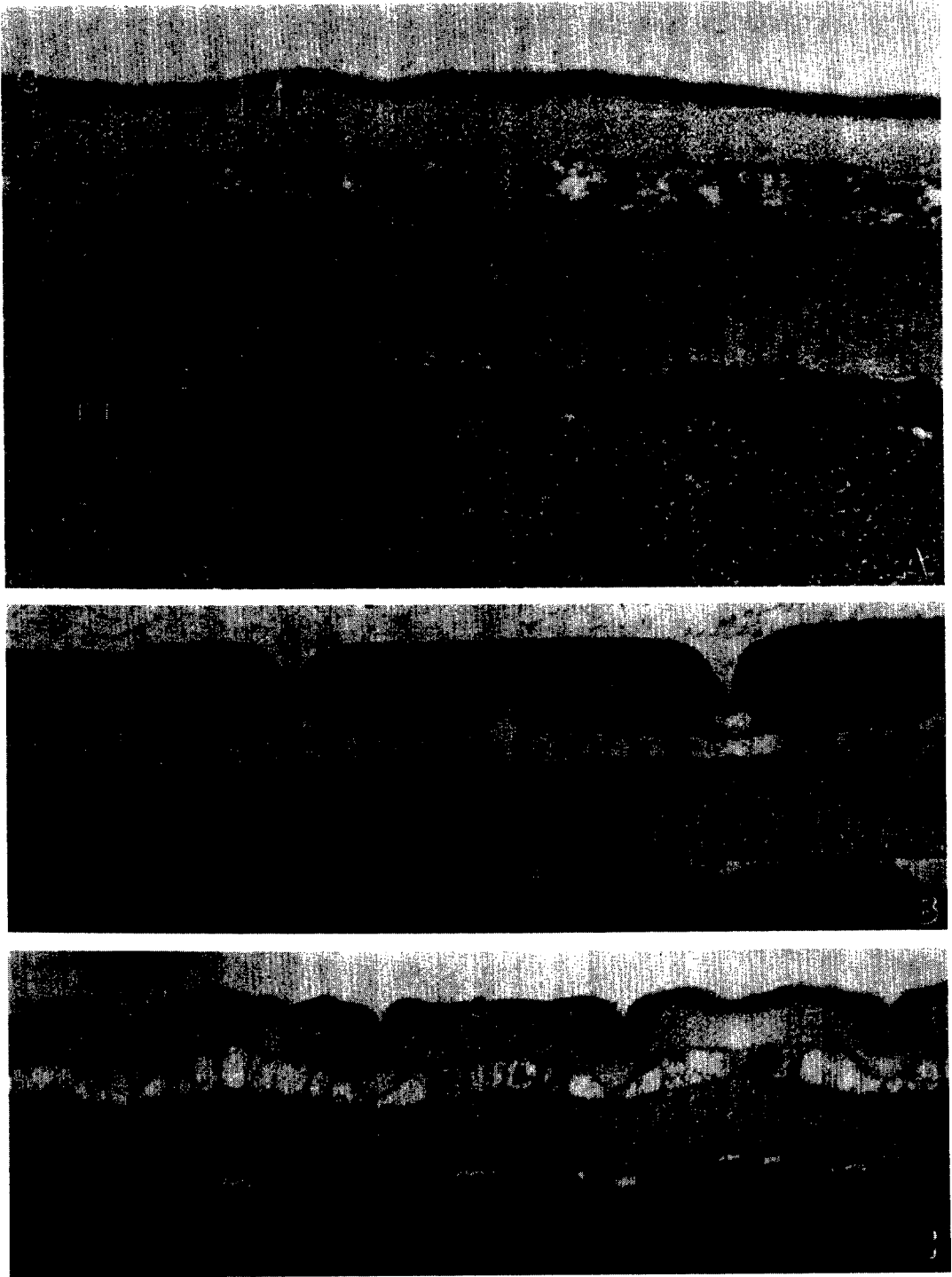


FIG. 7 Electron micrographs of *H. gracilis* and *H. belli*. A. Transverse section of *H. gracilis* male.  $\times 75,000$ ; B. Longitudinal section of *H. belli* male.  $\times 51,000$ ; C. Longitudinal section of *H. gracilis* larval stage.  $\times 51,000$ .

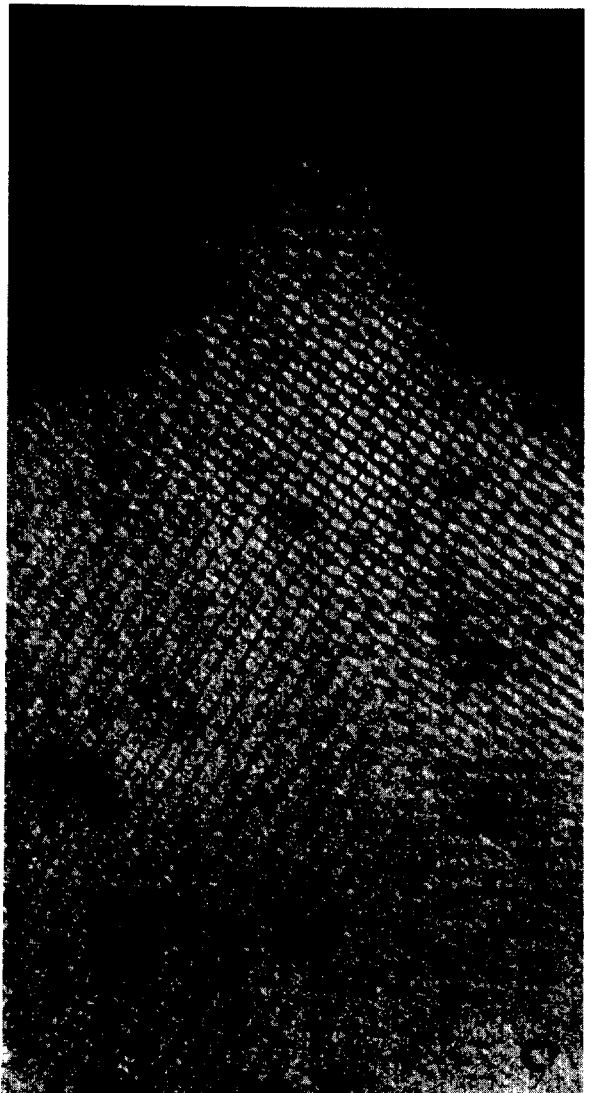
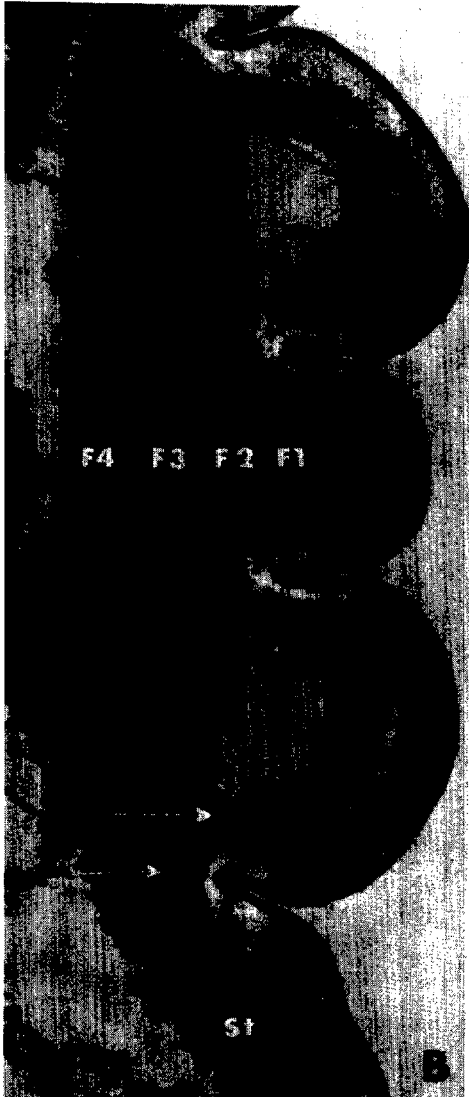
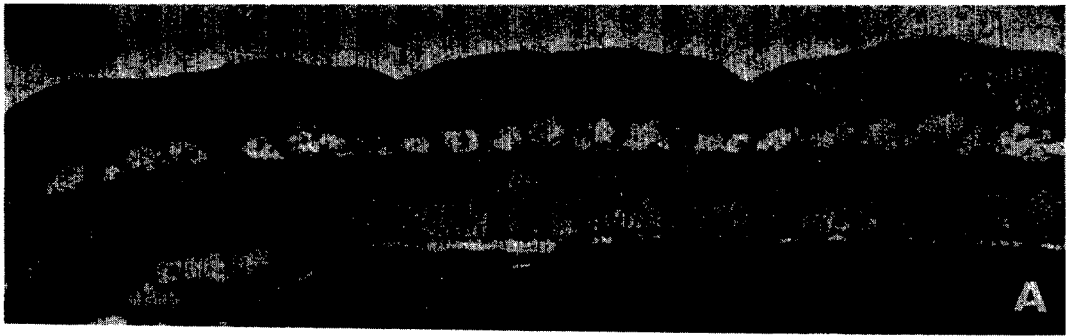


TABLE 4. Measurements ( $m\mu$ ) of the cuticle layers of different stages of *Hirschmanniella gracilis* and *Hirschmanniella belli*.

Stage	Cuticle layers					
	Trilaminar cortex			Matrix		Striated
	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5	Layer 6
<i>H. gracilis</i>						
Male	4.0 <sup>a</sup>	7.0	25.0	156.2	83.9	310.0
Female	4.5	7.6	24.3	169.2	86.1	280.0
Larvae (L4)	4.1	7.5	24.0	150.7	78.4	209.0
Average width	4.3	7.4	24.4	158.7	82.8	266.3
<i>H. belli</i>						
Male	4.0	7.6	23.6	169.2	101.5	310.7
Female	4.1	7.6	18.5	175.7	107.4	270.9
Larvae (L4)	4.1	7.4	23.6	153.8	78.4	216.9
Average width	4.1	7.5	21.9	166.2	95.4	266.1

<sup>a</sup> Averages of 10 or more measurements.

*HIRSCHMANNIELLA GRACILIS* AND *HIRSCHMANNIELLA BELLI*: Examination of the cuticle of male, female and an unidentified larval stage (probably L4) of these two species showed that in all cases the cuticle structure was similar in both species and consisted of six layers which could be divided into three regions: a trilaminar cortex, a two-layered matrix and a striated basal region (Fig. 7-A). Measurements of the cuticle layers in the stages observed are given in Table 4. Except for the presence of an apparent fluid-filled space, similar to that in *H. arenaria* males, which may or may not represent a true layer, the cuticle structure was similar to that of *A. avenae*.

Outermost was the trilaminar cortex consisting of a thin outer osmiophilic layer, an electron-transparent layer and an inner osmiophilic layer. These layers averaged

4.2, 7.4 and 23.1  $m\mu$  in width, respectively. The inner layer was sharply defined at its outer edge but more diffuse at its inner boundary.

Basal to the cortex was a two-layered matrix consisting of an outer fibrillar layer averaging 162  $m\mu$  in width. This layer was about twice as thick midway between annulations than at the base of the annulations (Fig. 7-B, C). The inner layer ranged from 0.0  $m\mu$  in thickness at the base of the annulation to approximately 107  $m\mu$  midway between them (Fig. 7-C) and appeared to be a fluid-filled space. This layer was traversed by strands of fibrillar material which merged with the outer matrix.

The innermost layer of the cuticle was a striated layer ranging from 209  $m\mu$  in width in larval stages (Figs. 7-C, 8-A) to 311  $m\mu$  in adult worms (Fig. 7-A, B). The striae showed a periodicity of approximately 18.5  $m\mu$  in transverse sections and 15.5  $m\mu$  in longitudinal sections. In oblique section (Fig. 8-C) this layer was composed of electron dense spots and less dense interconnecting lines.

In the region of the lateral field the cuticle was modified similar to that previously described except four (instead of two) fibrillar layers replaced the striated layer which tailed off (Fig. 8-B, arrows). Thus the six-layered cuticle becomes a nine-layered structure in the region of the lateral field.

The hypodermis and basal lamella were the same as previously described.

#### DISCUSSION

The presence of a seven-layered sheath in female, L2, L3 and L4 stages and a modified four-layered sheath in the male clearly dis-

FIG. 8. Electron micrographs of *H. gracilis* and *H. belli*. A. Transverse section of *H. gracilis* larval stage.  $\times 51,000$ ; B. Transverse section through lateral field of *H. belli* females showing tailing off of the basal striated layer (arrows) and its replacement by four fibrillar layers.  $\times 32,500$ ; C. Oblique section through basal striated layer of *H. gracilis* female.  $\times 164,000$ .

tinguishes the outer covering of *H. arenaria* from that of any other nematode yet reported. Eckert and Schwarz (3) reported the sheath of the third stage larvae of trichostrongylids and *Bunostomum trigonocephalum* was structureless, but in some species, *Haemonchus contortus* and *Trichostrongylus colubriformis*, an outer and inner zone of electron dense (osmiophilic) material was detected. Johnson (9) reported that the sheath of *H. arenaria* was composed of a single, apparently porous layer. This work has shown that the sheath of *H. arenaria* and probably of all *Hemicycliophora* species, has a much more complex multi-layered structure.

The five-layered cuticle of females and L2, L3, and L4 stages appears structurally similar to that of *Panagrellus silusiae* as reported by Samoiloff and Pasternak (17). The cuticle does lack the punctated basal lamella between the striated layer and the hypodermis reported in *P. silusiae*.

*H. arenaria* males have a six-layered cuticle in addition to a modified four-layered sheath. The male cuticle differs from that of the other stages by the presence of what may be a two-layered matrix and a striated layer which is approximately twice as thick as in the other stages. The increase in width of the striated basal layer is accompanied by a decrease in width of the matrix. Whether or not the apparent fluid-filled layer of the inner matrix region should be considered a separate layer, as is the case with a similar-appearing layer in L2 *H. rostochiensis* larvae (19), could not be determined. A similar region is seen in L2 *Meloidogyne javanica* larvae (1) but it is not mentioned in the text. Rather than representing a distinct layer, it may be a further modification of the less compacted area of the matrix resulting from a separation of the matrix from the striated layer due to stretching and compaction of the fibrils of the matrix. Additional support

for this interpretation is found in the fact that fibrillar material does cross this area from the striated layer and merge with the matrix. The structure of the male cuticle closely resembled those of *Hirschmanniella*, L2 *H. rostochiensis* (19) and L2 *M. javanica* (1).

The cuticle structure of *A. avenae* is similar in all stages and differs from that of the *Hirschmanniella* species examined only in the absence of the apparent fluid-filled space. The cuticle structure of these two nematodes is very similar to the basic cuticle structure of the following nematodes: larval stages of *Trichostrongylus*, *Bunostomum*, *Haemonchus* and *Strongyloides* (3), L3 *Nippostrongylus brasiliensis* (7, 11), L2 *M. javanica* (1), L2 *H. rostochiensis* (19), female *Tylenchorhynchus martini* (4, 5) and *Ditylenchus dipsaci* (23).

With the exception of *H. arenaria* males, the cuticle structure was similar in all stages of any nematode species examined and with *Hirschmanniella* it appears to also be true with regards to different species of the same genus. This similarity in the cuticle structure of all stages does not hold for *N. brasiliensis* (7, 10, 11) and *Meloidogyne* sp. (1, 2, 4, 6); it should, however, be noted that stages which show this difference characteristically occupy different habitats. With *Nippostrongylus* there is a change in environment from the soil to within an animal host and with *Meloidogyne* a change from the soil to within a plant root. It is probably safe to assume that the cuticle structure of all stages of a given species and possibly even of a given genus will be similar provided the environment remains the same. One case contrary to this suggestion occurs between L2 *M. javanica* larvae (1) and L2 *M. hapla* larvae (6). This point should be reinvestigated.

The striated layer is common to all the nematodes mentioned above as well as to

*Capillaria hepatica* and *Trichuris myocastoris* (22) and *Turbatrix aceti* (18). Transverse, longitudinal and oblique sections indicate that these striae are probably electron dense interconnected rods lying perpendicular to the nematode surface and embedded in a less electron dense material. This interpretation agrees with that of Watson (18) and Wisse & Daems (19). For a full discussion on the structure of this layer, the reader is referred to Wisse & Daems (19).

According to Wright (22) the degree of elasticity required for the cuticle to oppose the somatic muscles via a hydrostatic skeleton has been attributed to the basal layers of the cuticle. It appears that two possible mechanisms have evolved to provide the rigidity required in the basal layers of nematode cuticle (22). Fibre layers have been shown by electron microscopy in a number of nematode cuticles (22) while a striated layer has been found in many others as was pointed out previously. In *N. brasiliensis* and *Meloidogyne* sp., both types of possible strengthening mechanisms are found but in different stages (1, 2, 4, 6, 7, 10, 11).

Results in the present study suggest that the striated layer may be a modification of the fibre layers observed in other nematodes. The striated layer is replaced under the lateral field by at least two, and in the case of *Hirschmanniella*, by as many as four fibrillar layers. This has also been observed in L2 *H. rostochiensis* by Wisse & Daems (19). These observations support the idea that the striated layer can be viewed as a modified type of fibre layer and that it serves the same proposed function as has been suggested by Wright (22). Which of these structures is more "primitive" is not known; however if ontogeny recapitulates phylogeny the studies of Lee (10, 11), Jamuar (7) and Ibrahim (4), would indicate that striated zones are more primitive and are replaced in adult and larger nematodes

by fibers. The only nematode reported in the literature to possess neither fibre nor striated layers is *Trichodorus allius* (15). With this one exception, the nematode cuticle is basically a three-layered structure consisting of an outer cortex, a middle matrix and an inner basal layer any of which may be further subdivided. The sheath in *H. arenaria* must be considered as an addition to the basic cuticle pattern illustrated by most nematodes.

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