Journal of Nematology 22(3):247–261. 1990.

Structure of the Cuticle of *Metadasynemoides cristatus* (Chromadorida: Ceramonematidae)

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Abstract: Structure of the cuticle of *Metadasynemoides cristatus* (Chromadorida: Ceramonematidae) is examined by light, scanning, and transmission electron microscopy. The nematode has more than 600 annuli, and each annulus has eight cuticular plates. Eight longitudinal ridges, beginning on the cephalic capsule, extend the whole length of the body. Where a ridge traverses an annulus, it forms a complicated articulating structure of overlapping vanes. Within the electron-dense cortical layer, from which the cuticular plates are formed, there are spaces crossed by fine fibrillae, forming what have been termed "vacuoles" by light microscopists. There is an epicuticle and a continuous lucent basal layer. There appears to be no median layer. The cuticle lining of the esophagus and that forming the circum-oral ridge is of much simpler construction.

Key words: annulation, cuticle, light microscopy, marine nematode, *Metadasynemoides cristatus*, scanning electron microscopy, transmission electron microscopy, ultrastructure, vacuole.

Nematodes of the family Ceramonematidae have strongly annulated cuticles with overlapping plates. The cuticle also has longitudinal ridges extending the length of the body, which are interrupted by the annuli. Structure of the cuticle in this family has been described by Haspeslagh (5), who observed by light microscopy that the annulation is formed by constriction of the superficial and median cuticular layers.

Metadasynemoides cristatus (Gerlach, 1957) Haspeslagh, 1973 is about 1–1.5 mm long with 600–800 annuli. It is common in beaches along the south coast of New South Wales, Australia. Originally described by Gerlach (3) (from a Brazilian sandy beach) as *Dasynemoides cristata,* it was transferred to the genus *Metadasynemoides* by Haspeslagh (4). In this study, the cuticle structure is examined by light, scanning, and transmission electron microscopy.

MATERIALS AND METHODS

The nematodes were extracted from sand, collected near low tide mark, by sedimentation in sea water with a nylon 50-μm-pore sieve. Specimens were selected under a microscope and transferred by pipet to technique-specific fixatives. For light microscopy (LM), unstained specimens were fixed in a 3% formalin-saline solution, washed in distilled water, and transferred to 5% aqueous glycerol, and the water was allowed to evaporate. Specimens were mounted and examined in anhydrous glycerol.

For scanning electron microscopy (SEM), nematodes were fixed for 18 hours at 4 C in 2.5% glutaraldehyde in phosphate buffer, pH 7.2, containing 3% sucrose. After washing with buffer, they were postfixed in 2% osmium tetroxide for 2 hours at 4 C in distilled water, washed again in water, and then freeze dried. Specimens were mounted on metal stubs with nail polish, coated with gold palladium under vacuum, and then examined with SEM. Three males and three females were examined.

Specimens prepared for transmission electron microscopy (TEM) were fixed as in SEM, except for the use of 3% sucrose glutaraldehyde and osmium tetroxide solutions. After postfixation they were washed, dehydrated with graded ethanols, and transferred through mixtures of epoxypropane to Spurr epoxy resin. After hardening in flat molds at 60 C, specimens were examined by LM within the block to positively confirm identification, sex, and orientation before being sectioned with glass knives. Sections were stained with uranyl acetate and Reynolds lead citrate on formvar coated grids for TEM. Sections, 0.5 μm thick, were also prepared from the epoxy resins and stained with toluidine blue for LM. Several male,

Received for publication 2 December 1988.

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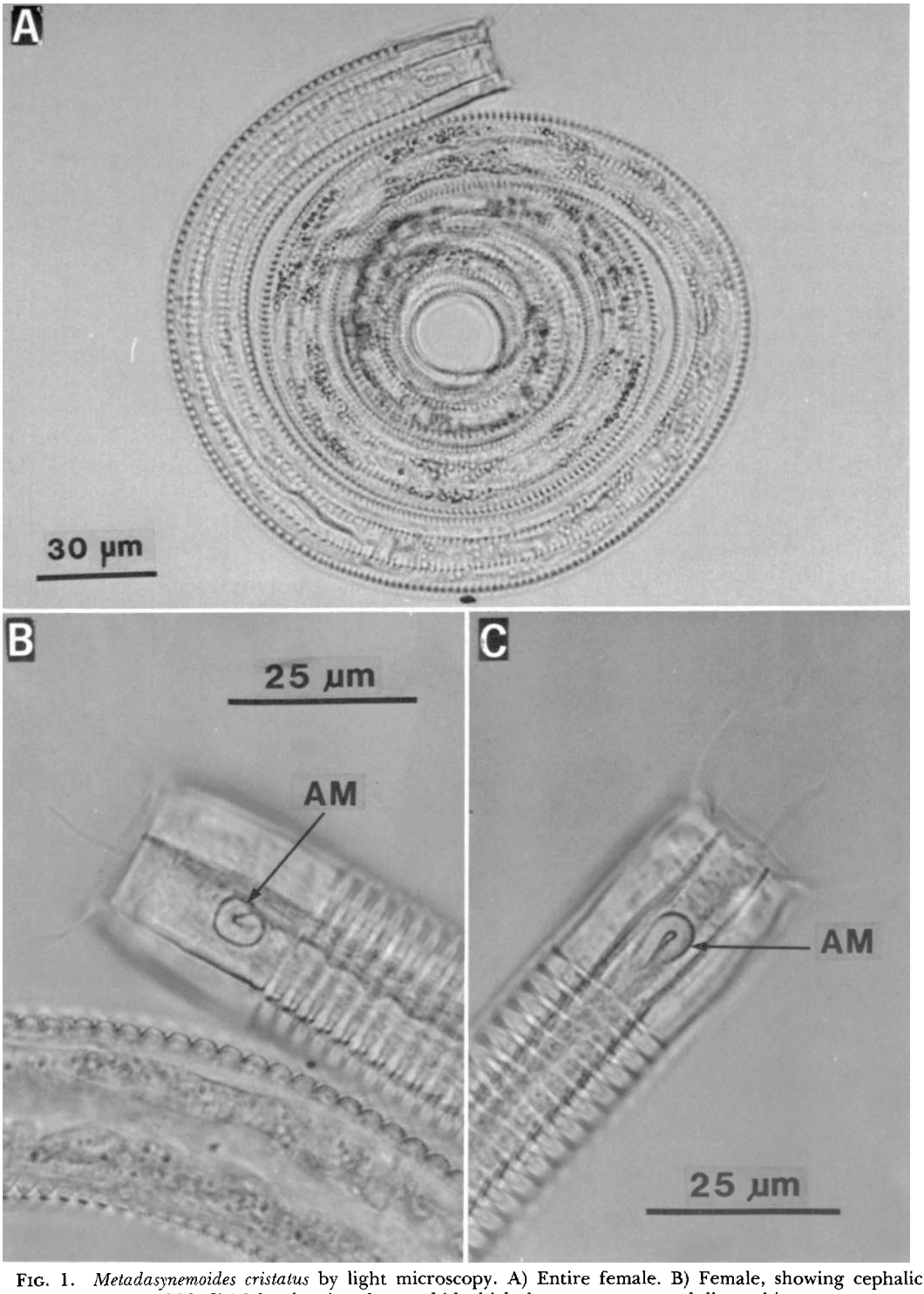

FIG. 1. *Metadasynemoides cristatus* by light microscopy. A) Entire female. B) Female, showing cephalic capsule. AM = amphid. C) Male, showing the amphid which demonstrates sexual dimorphism.

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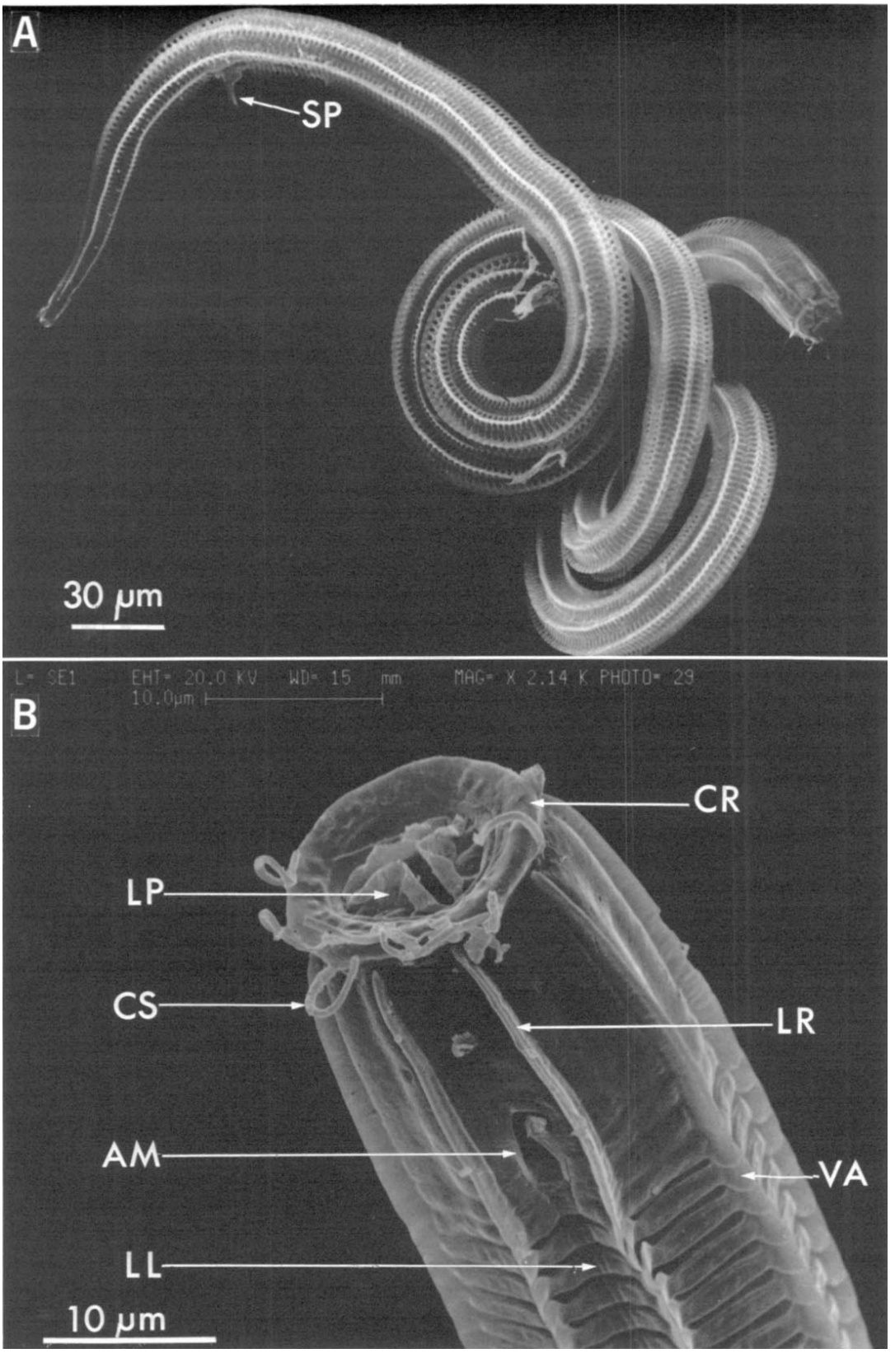
FIG. 2. Scanning electron microscopy of *M. cristatus*. A) Entire male. SP = spicule. B) Male head. LP = lips; CS = cephalic setae; CR = circum-oral ridge; AM = amphid; LL = lateral line; LR = longitudinal ridges; VA = vanes.


A
SP
30 µm
L= SE1 EHT= 20.0 KV WD= 15 mm MAG= X 2.14 K PHOTO= 29
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B
CR
LP
CS
LR
AM
VA
LL
10 µm

female, and juvenile specimens were sectioned for TEM.

Location of voucher specimens: One male (GL10016) and one female (GL10017) have been deposited in the Queensland Museum, Brisbane, Australia, and one male (V4090) and one female (V4091) have been deposited in the South Australian Museum in Adelaide.

Results

Light microscopy: The general appearance of the nematode is marked by more than 600 cuticular annulations (Fig. 1A), which begin posterior to the cephalic capsule and extend to the non-annulated tail tip. Eight longitudinal ridges extend the entire length of the body, beginning just posterior to the lips and extending almost to the tail tip (Fig. 1B, C). The amphids show sexual dimorphism; they are larger with an elongated loop in the male (Fig. 1C), and in the female they are a tight unispiral (Fig. 1B).

Scanning electron microscopy: The total structure of the cuticle is better seen by SEM of an entire specimen (Fig. 2A), but the structure of the ridges is clearer at higher magnification (Fig. 2B). On the non-annulated cephalic capsule, the ridges are simple cuticular elevations that do not quite reach the circum-oral ridge that encircles the three triangular lips.

Where the ridges traverse the body annulations, their structure is much more complex (Figs. 3A, B; 4A, B). Each annulus is separated from the two adjoining annuli by a deep groove, and each is divided by the ridges into eight cuticular plates. At each ridge, the adjacent plates give rise to a protuberance supporting a thin vane, oriented along the longitudinal axis of the body. The two vanes of adjacent plates lie side by side. They are in such close contact that when viewed from above it is difficult to determine that the two are separate structures, but in lateral view they are distinct (Fig. 3A, B). The degree of vane overlap varies slightly, depending upon the degree to which the nematode's body is curved. The cuticular structure is illustrated diagrammatically in Figure 5. The lateral lines appear as three parallel grooves crossing the lateral cuticular plates (Fig. 3A, B), beginning at the amphids (Fig. 2B) and extending almost as far as the anus where they disappear (Fig. 4A). Figure 4A shows two unequal spicules and the gubernaculum projecting from the body of a male specimen. The ridges extend almost to the extreme tip of the non-annulated tail spike (Fig. 4B).

Transmission electron microscopy: A low magnification transverse section shows the eight ridges and the lateral lines (Fig. 6). Because the body becomes gently curved when fixed, the sectioning plane is not absolutely transverse; it cuts through the eight ridges at slightly different planes. Five of the ridges clearly show two separate vanes, but the supporting protuberance is not in the section. On three of the ridges the conical protuberances are partly in the plane of section, with two vanes lying alongside. To illustrate this, the section plane of Figure 6 has been superimposed on the diagram of a SEM photomicrograph in Figure 5.

Higher magnification shows the structure of the cuticle in greater detail (Fig. 7). The cuticular plates are formed by the cortical layer, which has a meshwork or irregular honeycomb-like substructure of electron-dense elements in a slightly granular less dense matrix. It merges into a dense band on its outer surface and is separated by a narrow, less dense gap from the thin dense line of the epicuticle. The cortical meshwork condenses into an obscure line at the base of the cortex. The cortical layer is thicker at the protuberances and extends into the vanes, is thinner in the middle of the plates, and is absent at the lateral lines (Figs. 6, 7) and from the grooves (Figs. 8A–C, 9, 11).

There is no apparent median layer in the cuticle (Fig. 7). The electron-lucent basal layer is of almost uniform thickness and encircles the body, overlying the cells of the hypodermis. At the lateral lines, the epicuticle lies directly on the basal layer (Fig. 7). The lateral field has two low ridges and three grooves (Fig. 7).

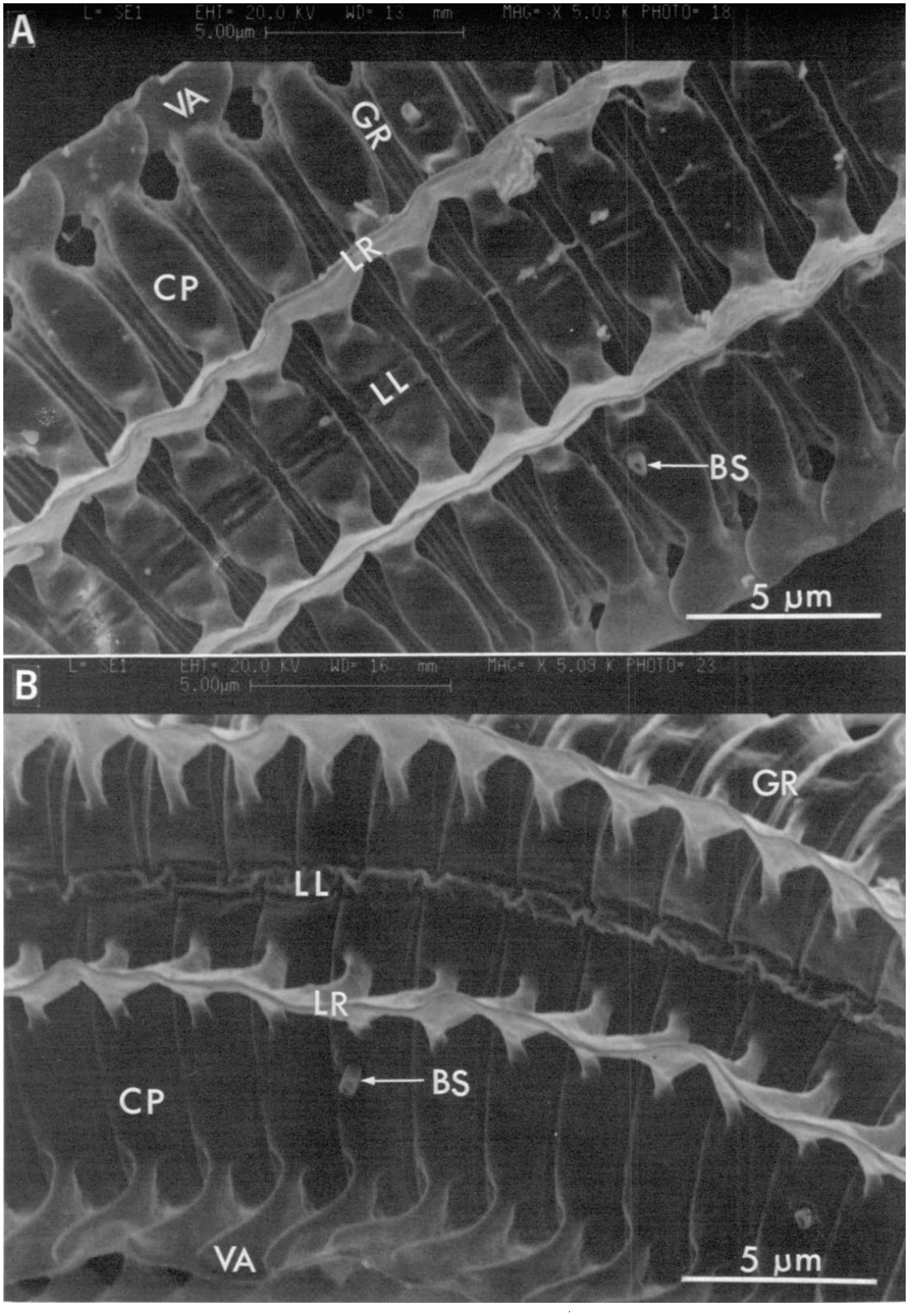


FIG. 3. Annulation and longitudinal ridges by SEM. A) Near head. B) Mid-body. CP = cuticular plates; GR = deep grooves between the annuli; LR = ridges viewed from above, with the two vanes from abutting ridges in very close contact; VA = vanes, lateral view showing how the vanes from adjacent annuli overlap; LL = lateral line; BS = body setae.

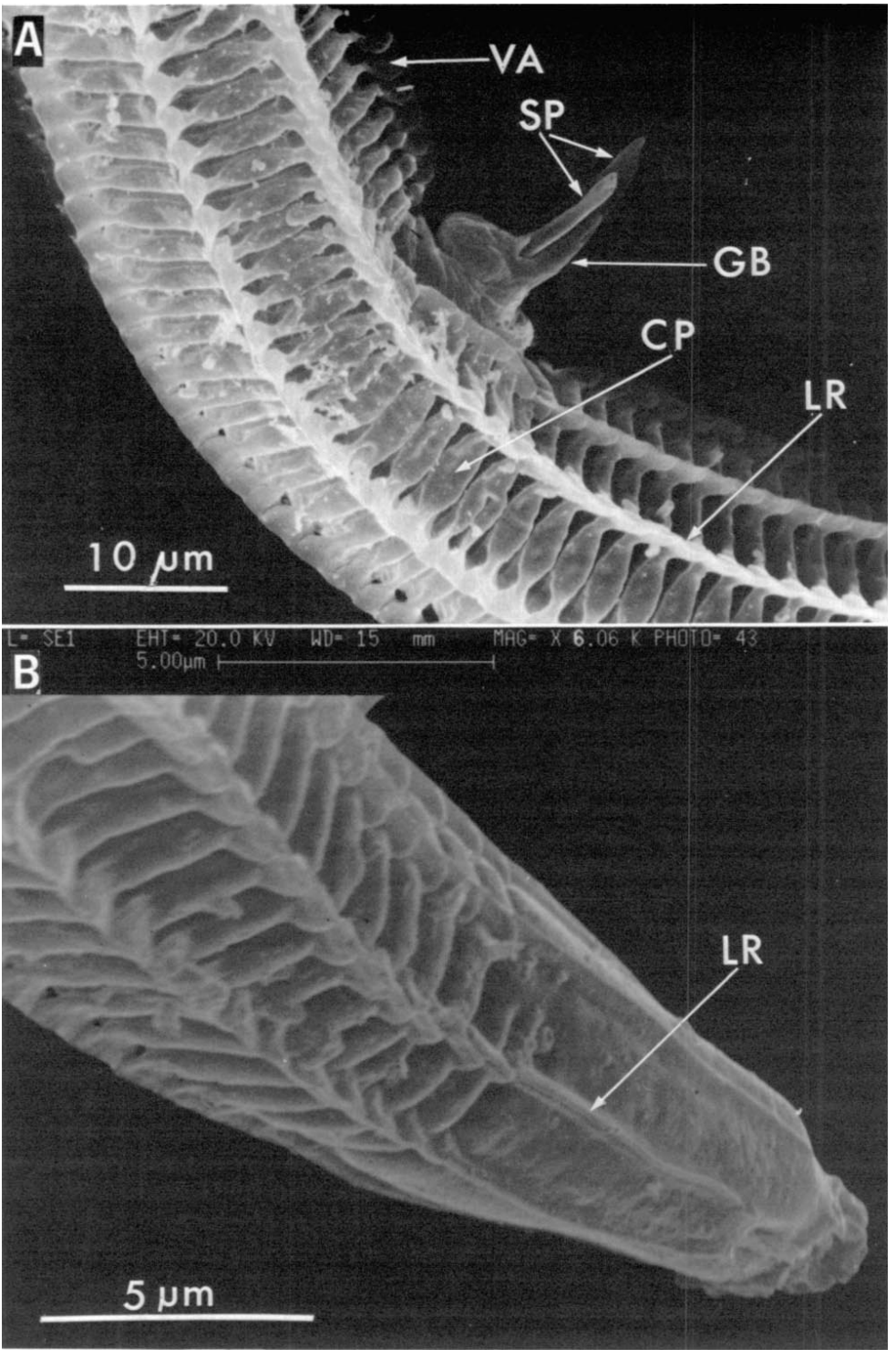


FIG. 4. Posterior region of the male by SEM. A) Spicular area. SP = spicules; GB = gubernaculum; CP = cuticular plates; LR = longitudinal ridge; VA = vane. B) Non-annulated terminus. LR = ridges.

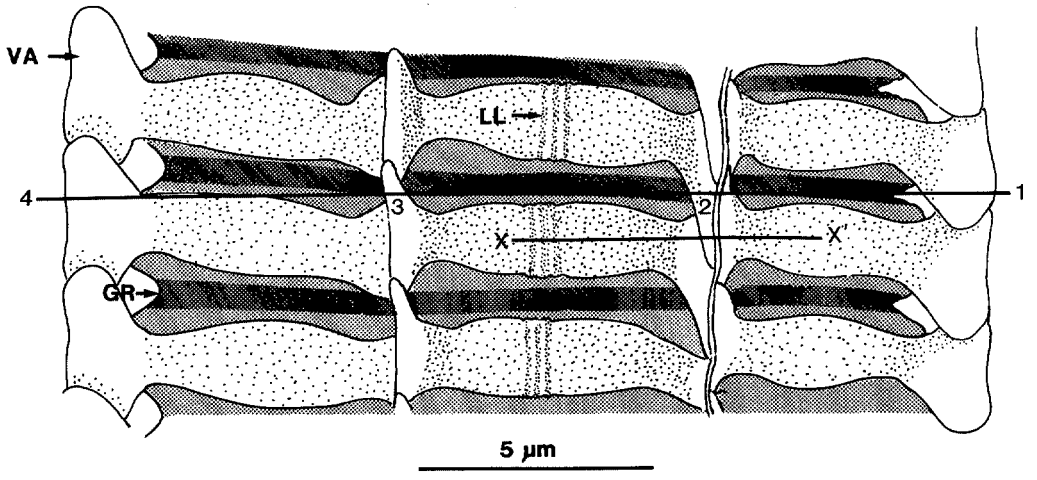


FIG. 5. Diagrammatic illustration of the cuticle by scanning electron microscopy with the planes of the sections shown in Figures 6 and 7 superimposed. Numbers 1 to 4 correspond to the numbers of the ridges in Figure 6. Line X–X′ refers to Figure 7. VA = vane; LL = lateral line; GR = groove between annuli.

Longitudinal sections through the cuticle at varying distances from the ridges illustrate the way ridges are formed by expansions of the cortical layer and how the meshwork is expanded at the ridges (Fig. 8). The grooves between the annuli have a basal layer and epicuticle but no cortical layer (Fig. 8). Figure 9 shows a longitudinal section of a series of annuli and the esophagus at low magnification. The cuticle lining of the esophagus has a single uniform, moderately dense layer, with a slightly denser border (Fig. 10). Hemidesmosomes lie between the esophageal muscle fibrils and the cuticle (Fig. 10). The esophagus is anchored to the cuticle by bands of fibrils (Fig. 11). Structure of the body cuticle extends through the cephalic capsule but not into the lip region (Fig. 12A). Cuticle on the lip region resembles the lining of the esophagus. We have not attempted here to describe the cuticle of the buccal cavity.

Higher magnifications show the epicuticle as a dense layer, 10–15 nm thick, which is separated from the cortical layer by a narrow lucent layer (Fig. 12B). Within the cortical layer, less dense cavities are traversed by a fine lattice of fibrils.

DISCUSSION

Two different systems, proposed by Bird (1) and Maggenti (6), are in current use for naming the successive layers, strata, or zones that make up the ultrastructure of the cuticle. We have chosen to use Bird's nomenclature. In *Metadasynemoides* the outermost layer is composed of a thin electron-dense homogeneous layer, 10–15 nm thick, which, as defined by Wright (8), represents the epicuticle, which is always present in nematode cuticles. We were unable to resolve the dense layer into a trilaminar membrane, as done by Wright (8). The epicuticle is separated from the underlying structured layers by a lucent zone of about the same thickness as the epicuticle.

Beneath the epicuticle and lucent zone, there is a clearly defined layer of electron-dense material of varied thickness that in its electron density (after staining with lead and uranium) resembles the cortical layer of other nematodes, where it is usually the most mechanically rigid layer (2). In *Metadasynemoides* it forms the cuticular plates, the conical protuberances where the plates abut, and the vanes. It is thinner in the middle of the plates. This layer is absent beneath the grooves between the plates that define the annuli and at the lateral lines, but it is present in the cephalic capsule. Its substructure varies from an expanded framework, with less dense interstices at the ridges, to a solid plate enclosing spaces in the middle of the cuticular plates. These

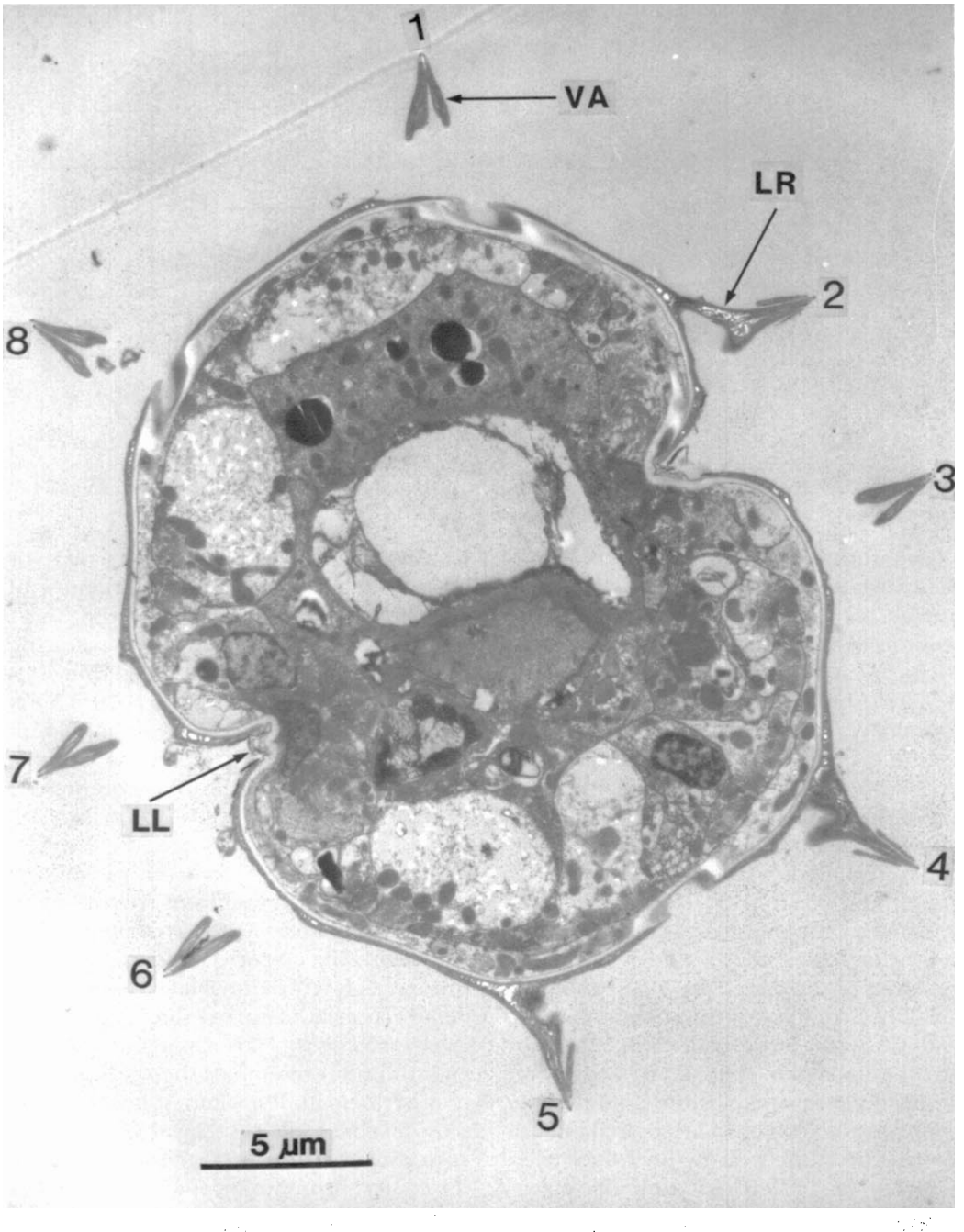


FIG. 6. Transverse section through mid-body showing eight ridges. Numbers locate the plane of section on Figure 5. At 3, the section cuts only through the vanes. At 4, the conical protuberances as well as two vanes are also sectioned. VA = vane; LR = longitudinal ridge; LL = lateral line.

spaces, containing a delicate lattice of fibrils, correspond to the vacuoles in the cuticle described by light microscopists in the Ceramonematidae (5).

The next layer, just preceding the hypodermis, completely encloses the body, with very little variation in thickness. Since this is the only other distinctive layer, we

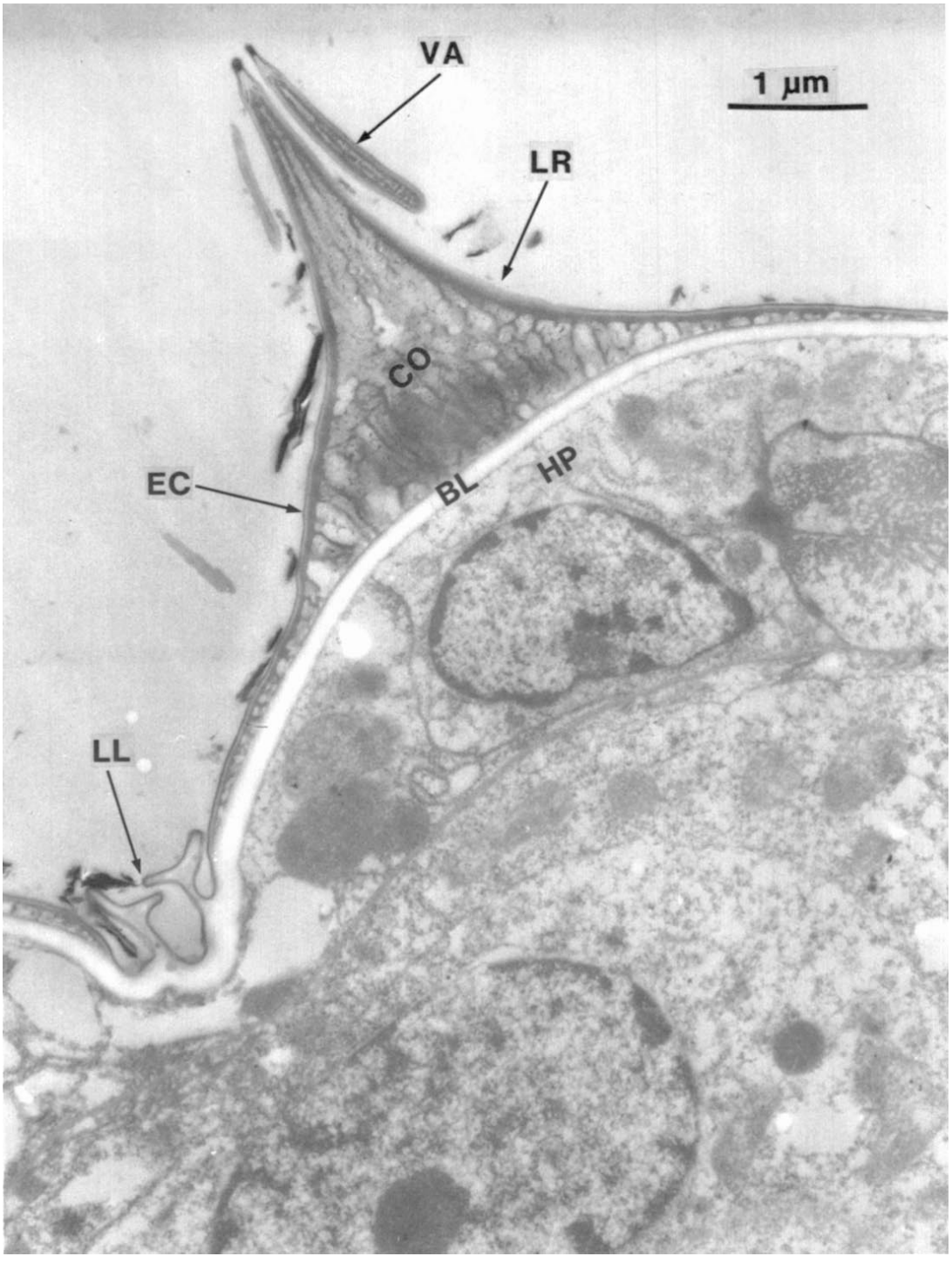


FIG. 7. Transverse section through a ridge. EC = epicuticle; CO = cortical layer; BL = basal layer; LL = lateral line; LR = longitudinal ridge; HP = hypodermal cell; VA = vane.

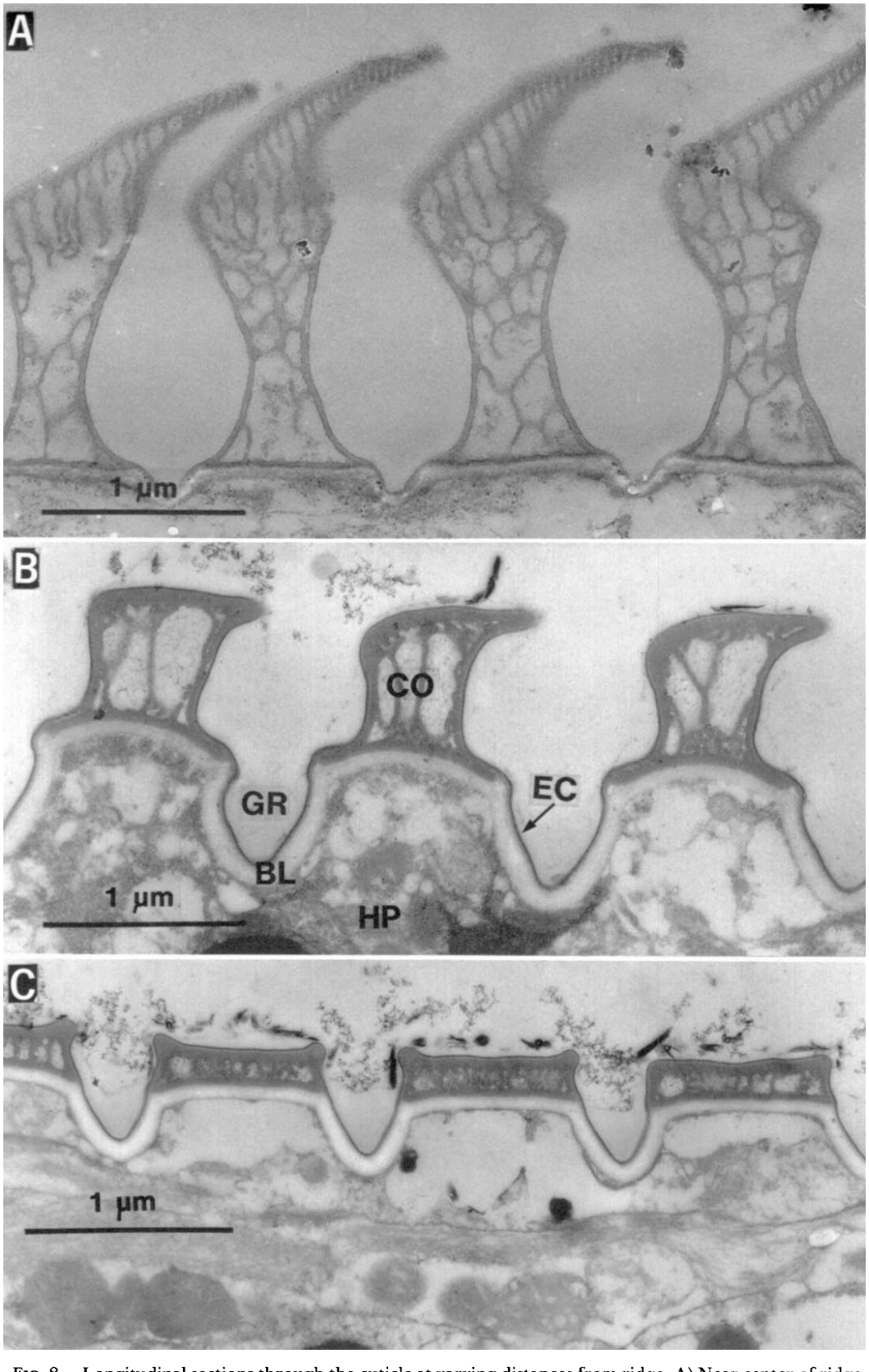


FIG. 8. Longitudinal sections through the cuticle at varying distances from ridge. A) Near center of ridge. B) Adjacent to ridge. C) Near middle of cuticular plate. EC = epicuticle; CO = cortical layer; BL = basal layer; HP = hypodermis; GR = groove between annuli.

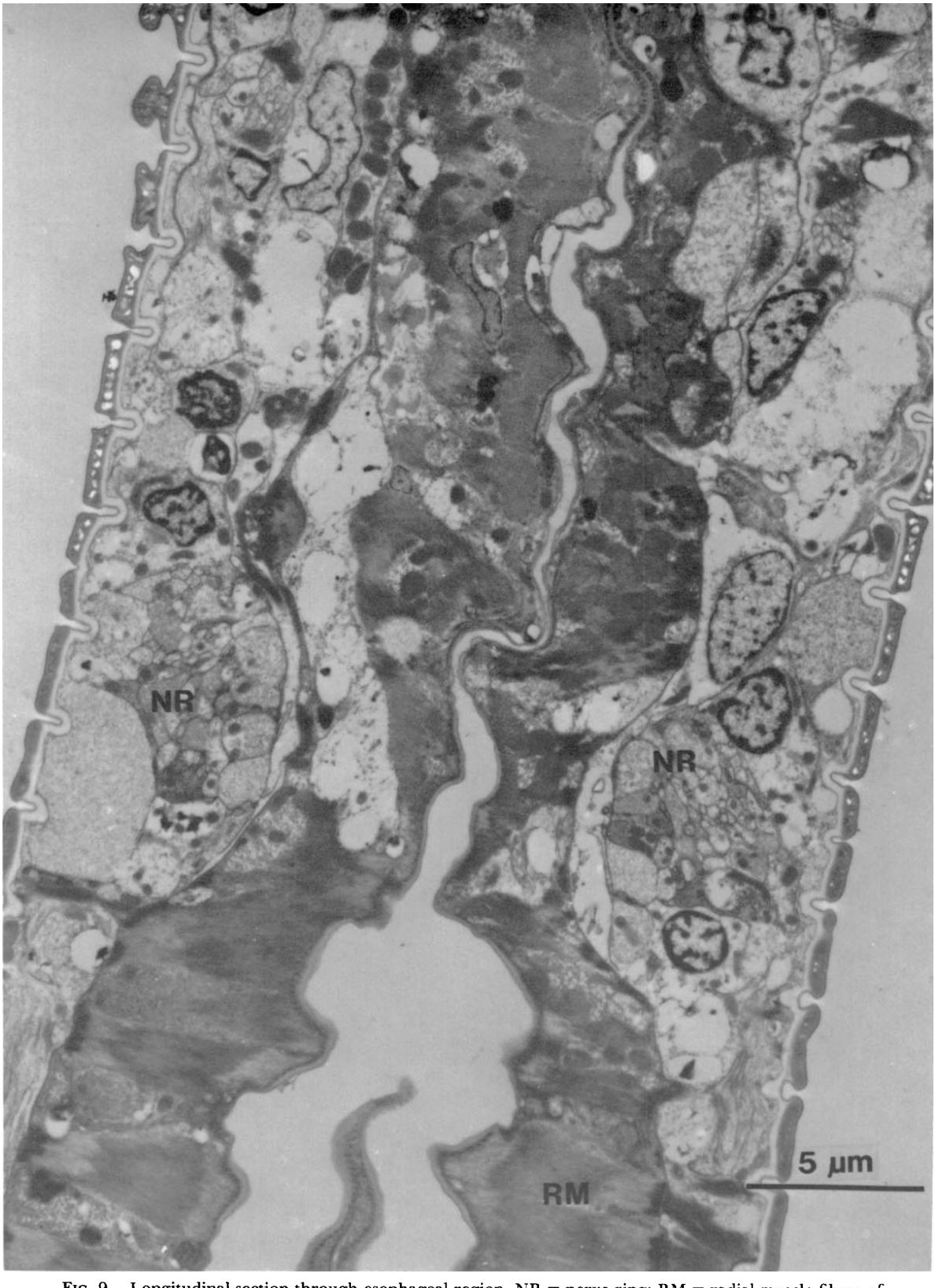


FIG. 9. Longitudinal section through esophageal region. NR = nerve ring; RM = radial muscle fibers of esophagus.

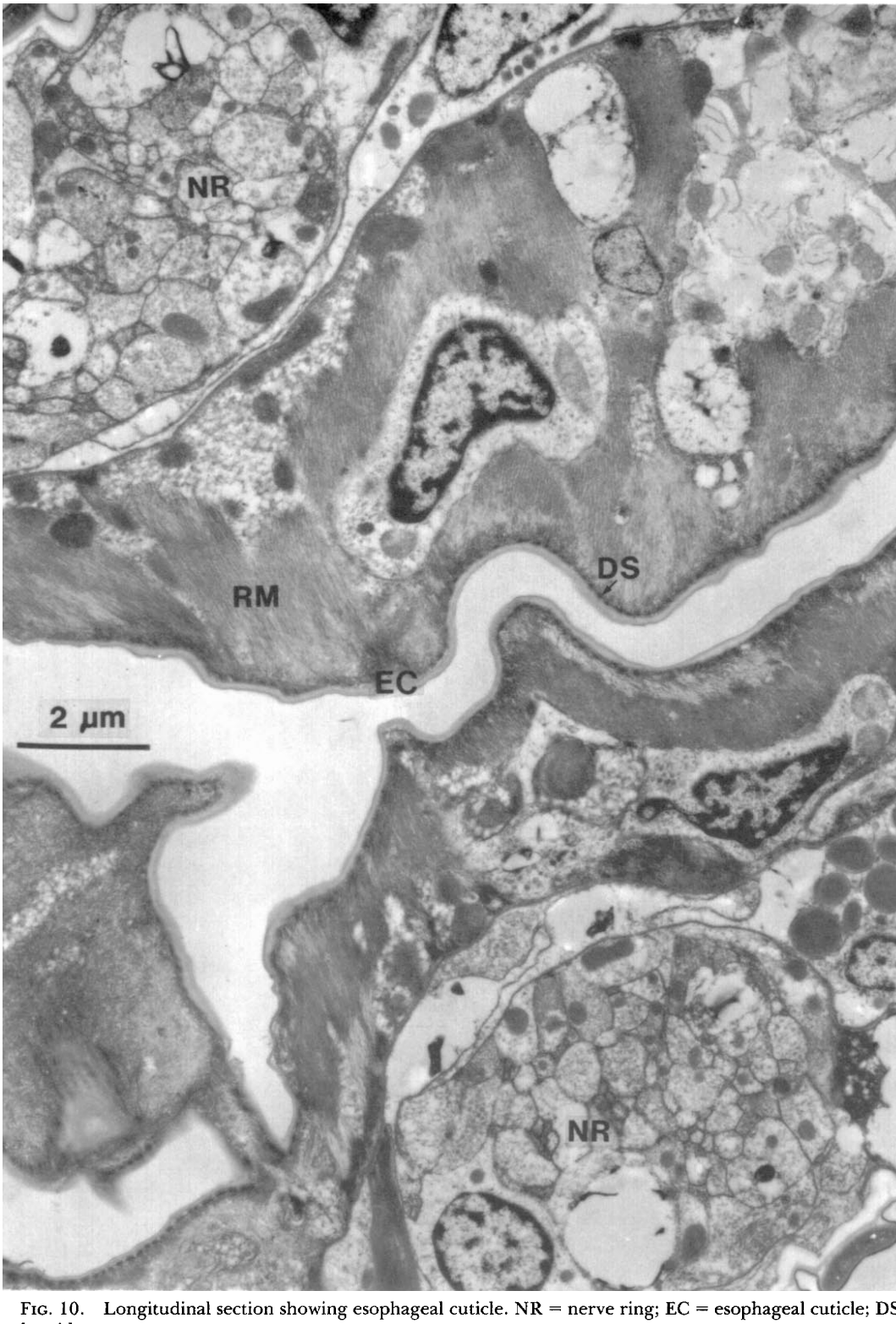


FIG. 10. Longitudinal section showing esophageal cuticle. NR = nerve ring; EC = esophageal cuticle; DS = hemidesmosomes.

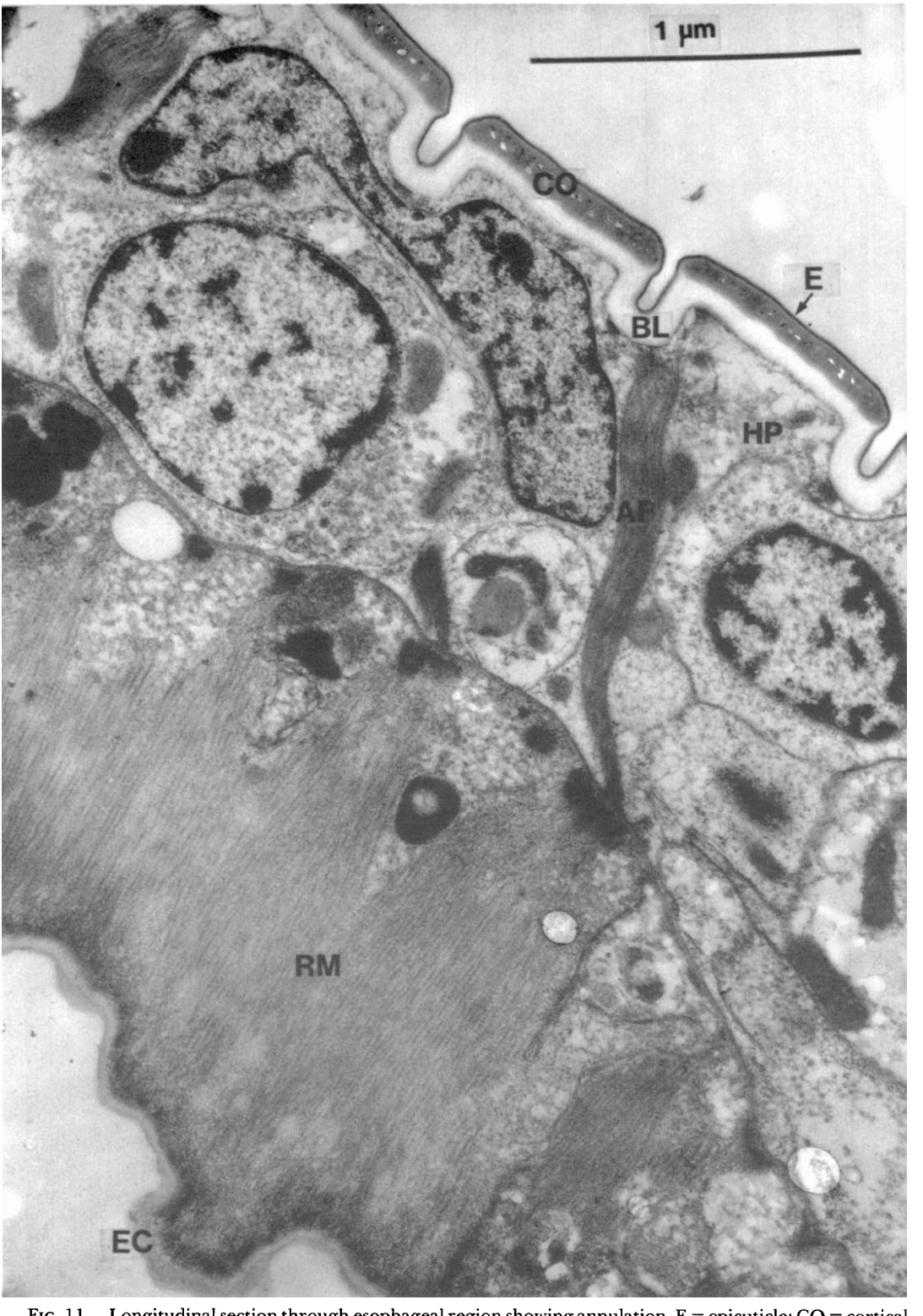
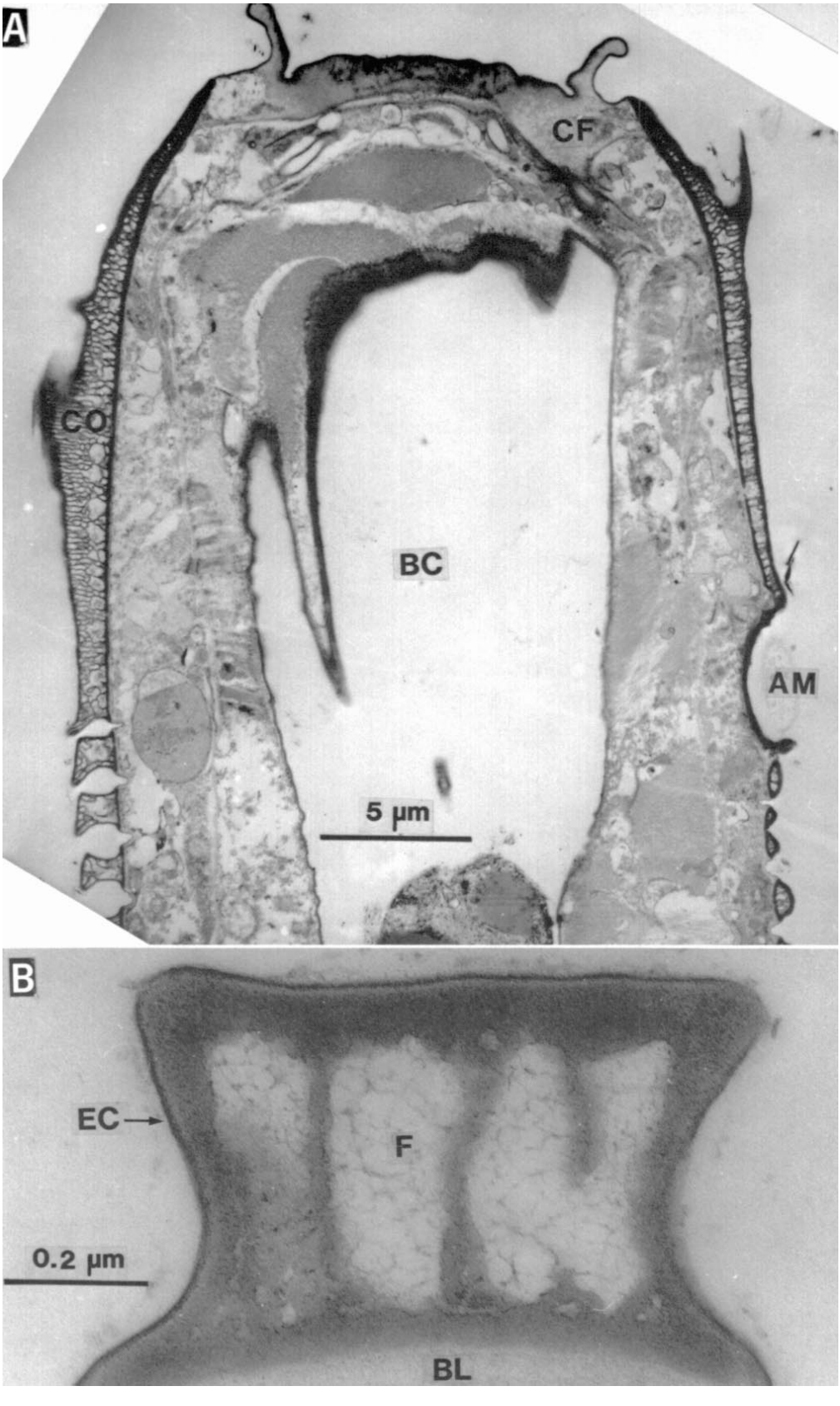


FIG. 11. Longitudinal section through esophageal region showing annulation. E = epicuticle; CO = cortical layer; BL = basal layer; EC = esophageal cuticle; RM = radial muscle fibrils of esophagus; HP = hypodermis; AF = fibrils anchoring esophagus to cuticle.


A
CF
CO
BC
AM
5 µm
B
EC
F
0.2 µm
BL

had to decide whether to call it the median or the basal layer. We concluded it was the basal layer, which means the median layer was absent. Our reasoning for this is that the median layer is the most variable cuticle structure (2), often consisting of a soft semi-solid region of low electron density, which contains rigid struts, plates, or other supporting structures that are more electron dense. No such structure was observed. The basal layer is usually a more uniform layer, although it often contains fiber layers or periodically arranged striations, which can also be found in the other layers in some other nematodes. Neither fibers nor striations were observed in any of the layers in *Metadasynemoides.*

The esophageal cuticle lining is different from that of the external cuticle and has a simpler construction, as is generally true in nematodes. Bundles of fine fibers run from the basal lamina of the esophagus to the cuticle.

The cuticle of *Metadasynemoides* is rather different from that of other nematodes whose cuticle has been studied with electron microscopy. It shows some resemblance to that of *Euchromadora vulgaris* (Bastian, 1865) de Man, 1886, also in the order Chromadorida. In the description of *E. vulgaris,* the epicuticle was not resolved into a trilaminar membrane; the cortical layer showed denser regions within the annuli, and was lacking beneath the interannular grooves; and a homogeneous basal layer (7). In *E. vulgaris,* however, there is a wide median layer containing overlapping plates and there are canals within the cuticle, neither of which was evident in our specimens.

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Fig. 12. Longitudinal sections of *M. cristatus.* A) Cephalic region. CF = cuticle within circum-oral ridge; CO = cortical layer of cephalic region; BC = buccal cavity; AM = amphid external groove. B) Annulus at high magnification showing "vacuoles." EC = epicuticle; F = fibrils within "vacuole" in cortical layer; BL = basal layer.