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Structure of the Cuticle of *Ceramonema carinatum* (Chromadorida: Ceramonematidae)

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Abstract: The cuticle of *Ceramonema carinatum* (Chromadorida: Ceramonematidae) is described and illustrated from scanning and transmission electron microscopy. Each of ca. 200 annules is composed of a single ring with eight external flat faces (plates), which are divided by longitudinal ridges formed by pairs of parallel upstanding vanes. Vanes and plates overlap those of the adjacent annules. Longitudinal ridges extend from the cephalic capsule to the tail spike. On the cephalic capsule a simple ridge extends each of the eight ridges to a position just anterior to the amphid. Cuticular plates are formed from the electron-dense cortical layer and contain lacunae filled with fine fibrils. The vanes are denser, with laminations on a central core. In the annular grooves between the plates there is an electron-lucent layer, which it is suggested, by comparison with other nematodes, is the basal layer. An epicuticle overlies the cortical plates, the vanes, and the interannular lucent layer. Cuticular structure is compared with that of other Ceramonematidae and related nematodes.

Key words: annulation, *Ceramonema carinatum,* cuticle, marine nematode, nematode, scanning electron microscopy, transmission electron microscopy, ultrastructure.

Members of Ceramonematidae are small marine nematodes possessing strongly armored cuticles of complex structure. With a few exceptions, the morphology of the cuticles has been described from light microscopy by De Coninck (3) and Haspeslagh (4). Nicholas and Stewart (6) provided the first detailed description of the cuticle of one species, *Metadasynemoides cristatus,* based on light, scanning, and transmission electron microscopy. The cuticular plates and ridges that make up the cuticle can be resolved by light microscopy, though they are more easily interpreted from scanning electron micrographs, but their interconnections cannot be resolved without transmission electron microscopy. Our studies of the external body cuticle of *Ceramonema carinatum* Wieser, 1959 (8) show that there are broad similarities between the cuticles of these two taxonomically related species, but also interesting differences in their structure. It has been our aim to correlate the ultrastructure of these cuticles with the general structure of nematode cuticles (1) and to clarify some of the unresolved homologies that remain after the study of *M. cristatus* (6).

MATERIALS AND METHODS

Ceramonema carinatum was collected from sandy beaches near the low tide mark in the vicinity of Moruya, New South Wales, on the southeast coast of Australia. Specimens were washed from sand and picked up with a fine pipette while still alive in sea water, using a binocular microscope. For light microscopy (LM), specimens were fixed in 5% formalin in sea water, washed in distilled water, and transferred to anhydrous glycerol by evaporation. Permanent mounts were made in dehydrated glycerol. For electron microscopy, specimens were fixed overnight in 2.5% glutaraldehyde in phosphate buffer, pH 7.2, containing 3% sucrose, then postfixed in 2% aqueous osmium tetroxide. For scanning electron microscopy (SEM), specimens were washed in distilled water, freeze-dried, mounted on metal stubs using nail-varnish as glue, coated with gold palladium under vacuum, and finally viewed in the microscope. For transmission electron microscopy (TEM), postfixed specimens were progressively transferred through graded ethanols and epoxypropane to Spurr epoxy resin. After hardening the resin at 60 C for 48 hours, thin sections were cut with glass knives,

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mounted on formvar coated slot grids, and stained with 6% aqueous uranyl acetate and Reynolds lead citrate.

Results

Scanning electron microscopy: Surface cuticular structure is much clearer by SEM (Fig. 1C,D) than by LM (Fig. 1A,B), because the deeper cuticular layers with superimposed patterns do not interfere with the SEM image. The cuticle is strongly annulated from the head capsule to the tail spike (Fig. 1A–D). Anteriorly, the smooth surface of the head capsule bears short labial and cephalic setae and deep amphidial grooves, but no annulation (Fig. 2B). Longitudinal ridges are formed by thin vanes that project from each annule at eight equidistant positions (Figs. 1C,D;2A–D). The ridges divide each annule into eight plates, the abutting margins of which protrude as vanes, partially overlapping those of the neighboring annules (Figs. 1D;2A–D). The ridges extend forward to the nonannulated cephalic capsule to just in front of the amphids as simple ridges without overlapping vanes (Fig. 2B). The lateral pairs of ridges curve slightly around the front of the male amphid. Ridges also continue along the tail spike beyond the last annule, reaching almost to the tip of the tail (Fig. 1D).

Each annule is a complete ring, but between each ridge there appears superficially to be two square plates, the right-hand square set back from the left-hand square, with the two squares united where they meet (Fig. 2A). The offset, equivalent to two-thirds of a side, is the same all the way around each annule. Where the body bends, a gap appears between the annules (Figs. 1D;2D), revealing parts of the plates that are otherwise concealed with SEM. Each plate has an anterior flat platform "stepped down" from the right-hand square and underlying the backward-projecting square of the corresponding plate of the preceding annule, whereas the left-hand square has a posterior "stepped down" platform underlying the forward-projecting plate of the annule behind (Figs. 1D;2D). A fractured specimen also illustrates the underlying platforms and plate projections very clearly (Fig. 2C). The side of each square carries a longitudinal extension or vane, the right-hand vane extending forward and the left-hand vane extending backward beyond the square upper part of the plates, with the vanes from adjacent plates lying parallel and close together (Fig. 2A). The architecture and arrangement of plates are shown in Figure 3. No lateral line is evident.

Transmission electron microscopy: In longitudinal section, the overlapping plates, i.e., annules, are separated by deep grooves (Figs. 4A,B;6B). The plates are formed from the amorphous electron-dense cortical cuticular layer, within which there are lacunae containing fine fibrils (Figs. 4B,C;7A). Between annules there is an electron-lucent cuticular basal layer, confluent with but not underlying the plates. The cortical plates and interplate lucent layers lie directly on the hypodermis (Fig. 4B,C). The epicuticle, a thin trilaminar membrane about 20 nm thick (Figs. 4C;7B,C) covers the plates, vanes, and basal layer between plates. The three laminae forming the epicuticle are each about 7 nm thick.

In the middle of the annule, the cortical plate extends beneath and between the ridges to enclose the circumference (Fig. 5A,B). In sections, the forward and backward overlapping projections of the cortical plates appear as discrete structures (Fig. 5A,B). The lacunae in the cortical layer are also evident in transverse sections (Figs. 5;6A;7A), but take up more space in some sections (Fig. 5B) than in others (Fig. 5A). Tangential sections (Fig. 6B) show that the lacunae are not distributed uniformly through the cortical plates, but are concentrated in a band at the center of each annule. The longitudinal body musculature is very poorly developed (Figs. 5;6A).

The vanes are very electron-dense (Figs. 5A;6A;7A,C) and clearly different from the underlying cortical plate, from which

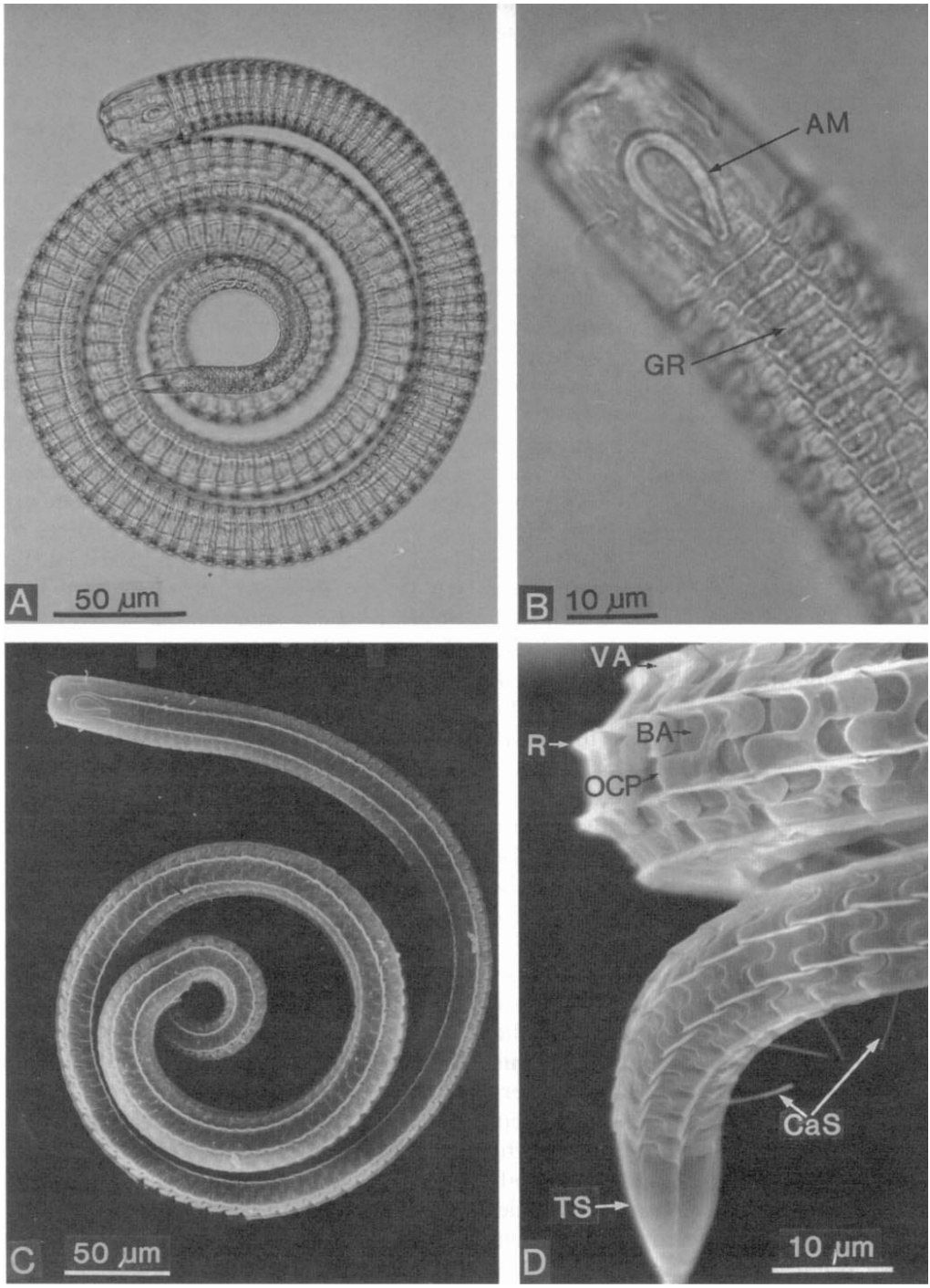

FIG. 1. *Ceramonema carinatum;* A,B) by light microscopy; C,D) by scanning electron microscopy. A) Entire female. B) Head of male. C) Entire male with large amphid and longitudinal ridges. D) Posterior region of male showing prominent longitudinal ridges. AM = amphid that demonstrates sexual dimorphism—compare female amphid in A) with male amphid in B); BA = basal platform of cortical plate; CaS = subventral caudal setae; GR = groove between annules; OCP = overlapping projections of cortical plates, wide apart on the outside of a bend in the body (above in D) and close together where the body is straight (below in D). R = ridge; TS = tail spike; VA = vane.

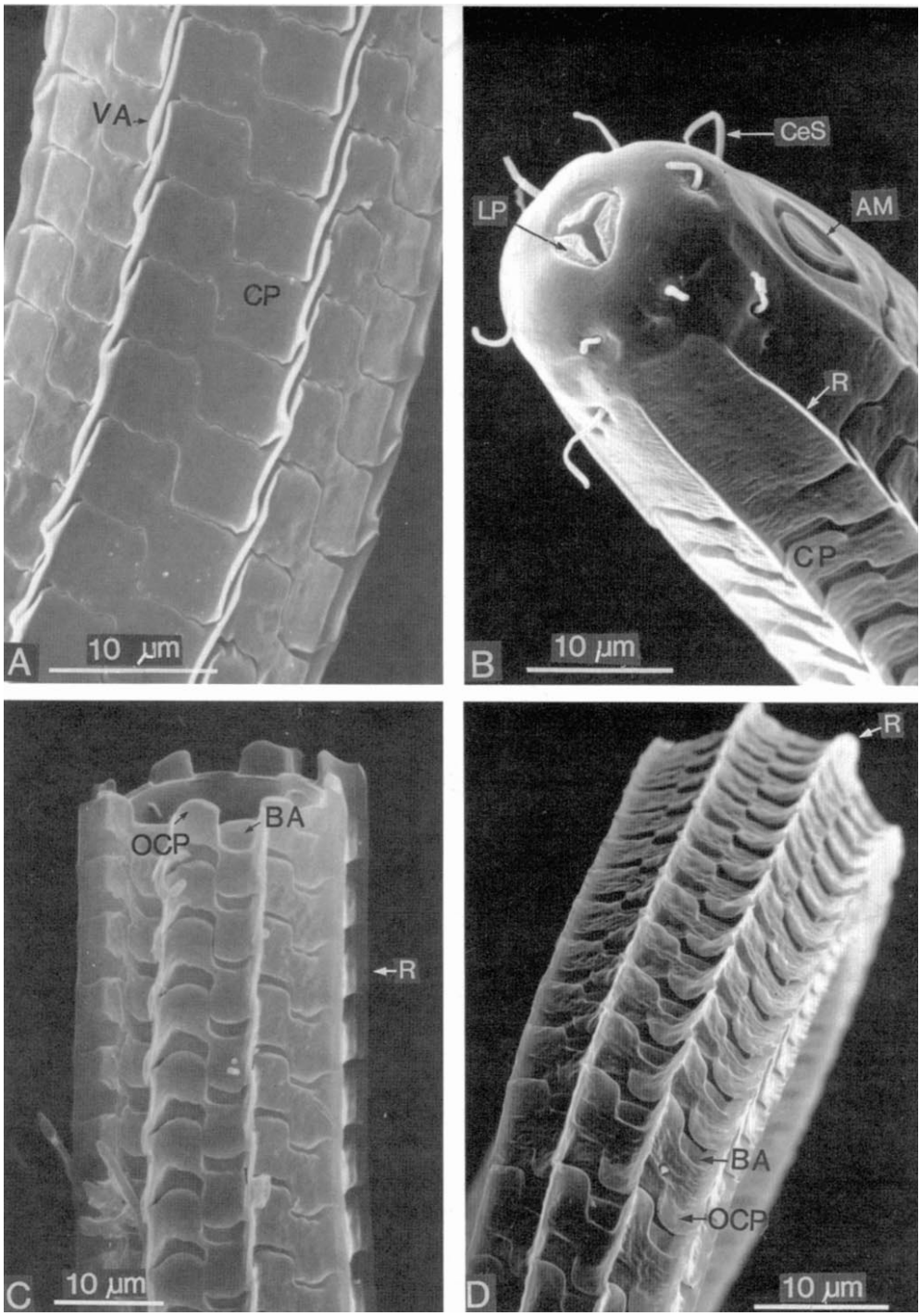

FIG. 2. *Ceramonema carinatum* by SEM. A) Anterior region of male showing structure of cortical plates. B) Cephalic capsule of male. C) Midbody of female broken to show the overlapping projections of the cortical plates. D) Posterior region of male showing cuticular plates wide apart on the outside of a bend. AM = amphid; BA = basal platform of cortical plate, normally concealed below preceding cortical plate (except partially when the body bends as in Fig. 1D); CeS = cephalic setae; CP = cortical plate; LP = lip region; OCP = overlapping projection of cortical plate; R = ridge (in profile in C); VA = vane.

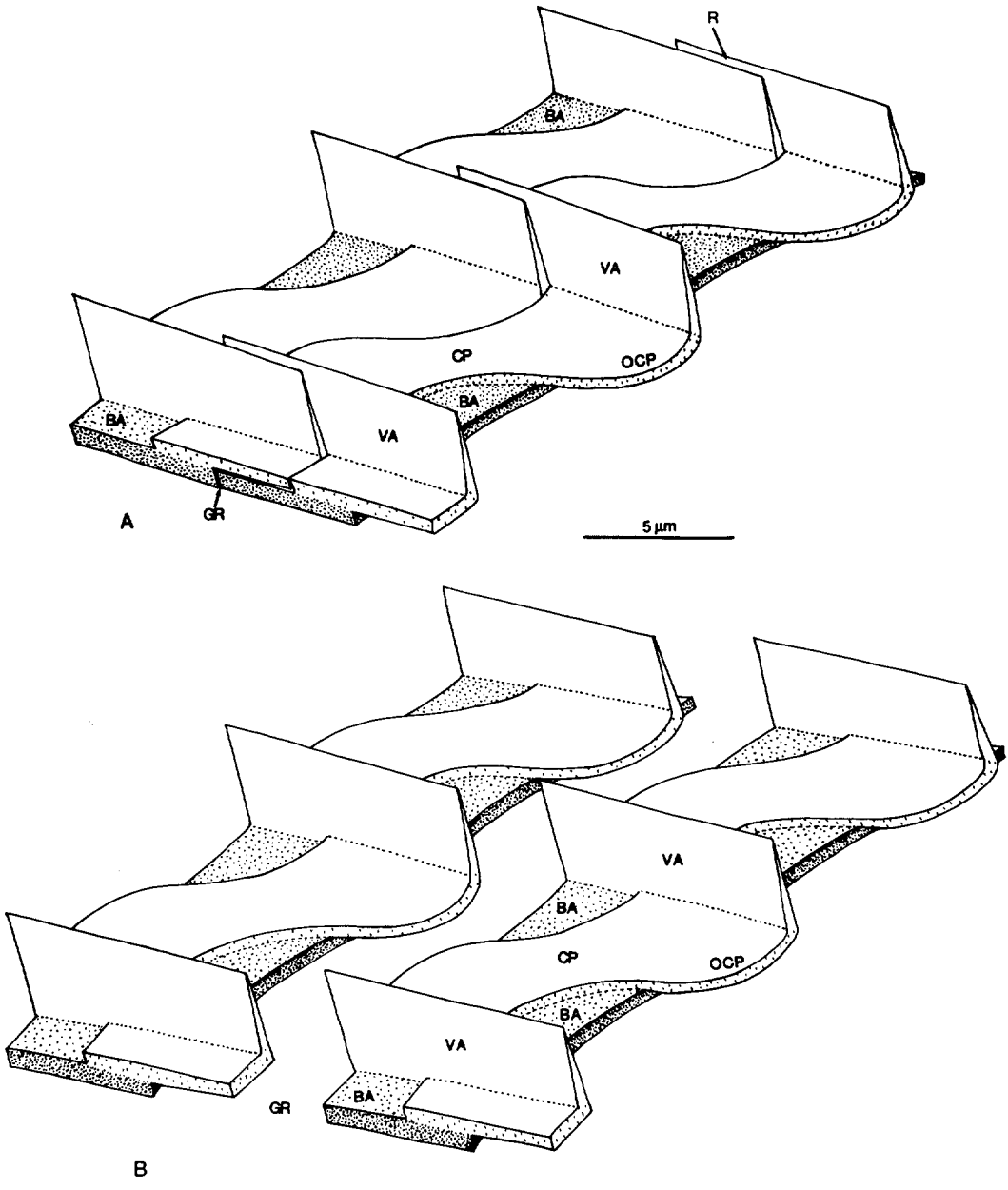

FIG. 3. Perspective sketches of parts of cuticular plates of *Ceramonema carinatum* based on SEM and TEM. A) Parts of two annules fitted together, showing overlapping vanes forming longitudinal ridges. B) Parts of two annules separated to show the basal platform, which is normally concealed by the overlapping projections of neighboring cortical plates. BA = basal platform of cortical plate; CP = cortical plate; GR = groove between annules; OCP = overlapping projection of cortical plate; R = ridge; VA = vane.

they arise. They have a central solid electron-dense core, successively mantled by a homogeneous less dense layer, a multilamellar layer, and then the trilaminar epicuticle (Fig. 7C).

DISCUSSION

The complicated arrangement of plates, ridges, and vanes that characterize the cuticle of *Ceramonema* are visible by light mi-

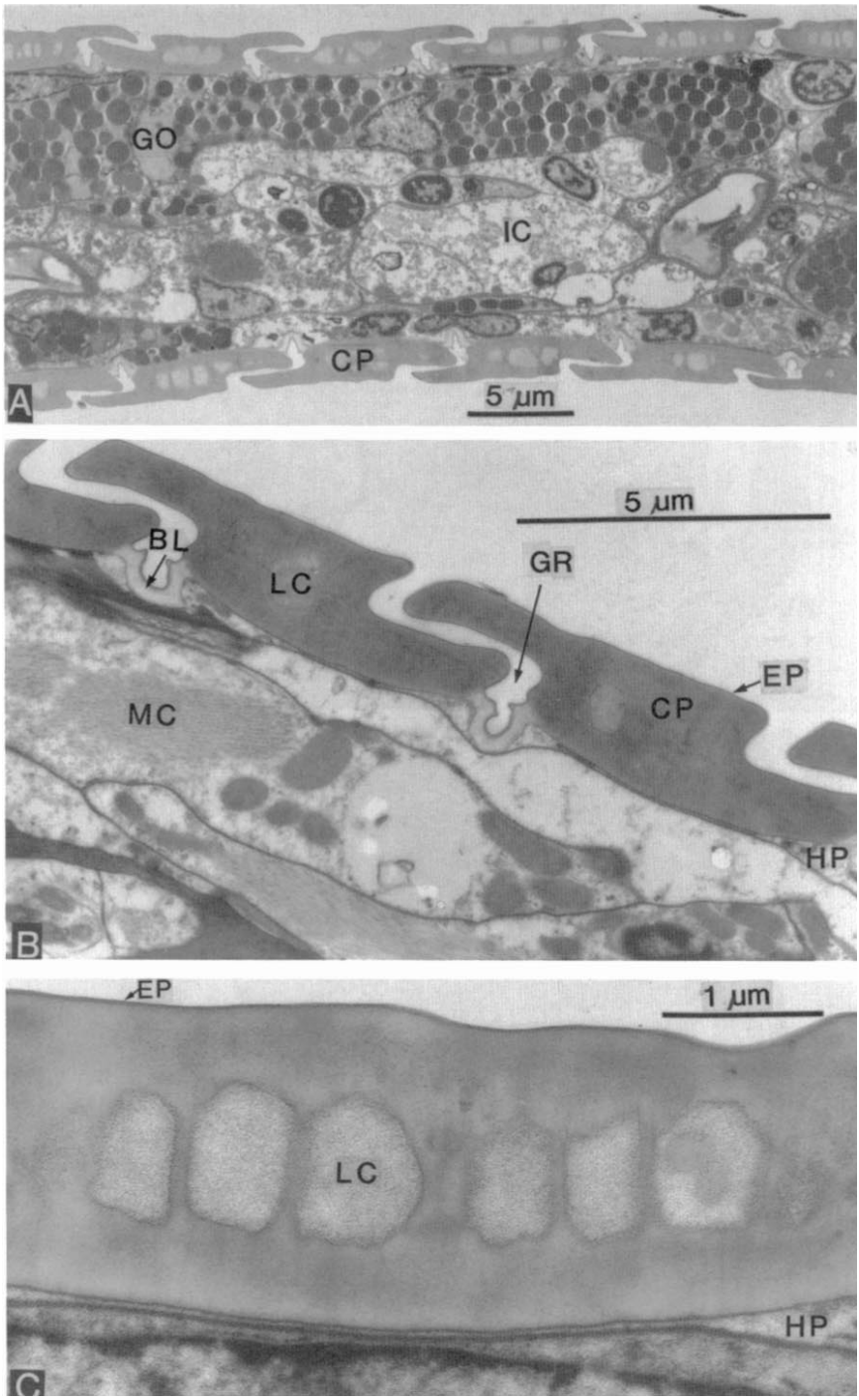

FIG. 4. Longitudinal sections of *Ceramonema carinatum* by TEM. A) Section through midbody of male showing overlapping cortical plates. B) Enlargement of cortical plates near anterior end of animal, showing paucity of lacunae compared with A) resulting from uneven distribution of lacunae in annules (see Fig. 6B). C) Enlargement of part of cortical plate. BL = basal layer of cuticle; CP = cortical plate; EP = epicuticle; GO = male gonad; GR = groove between annules; HP = hypodermis; IC = intestinal cell; LC = lacuna; MC = muscle.

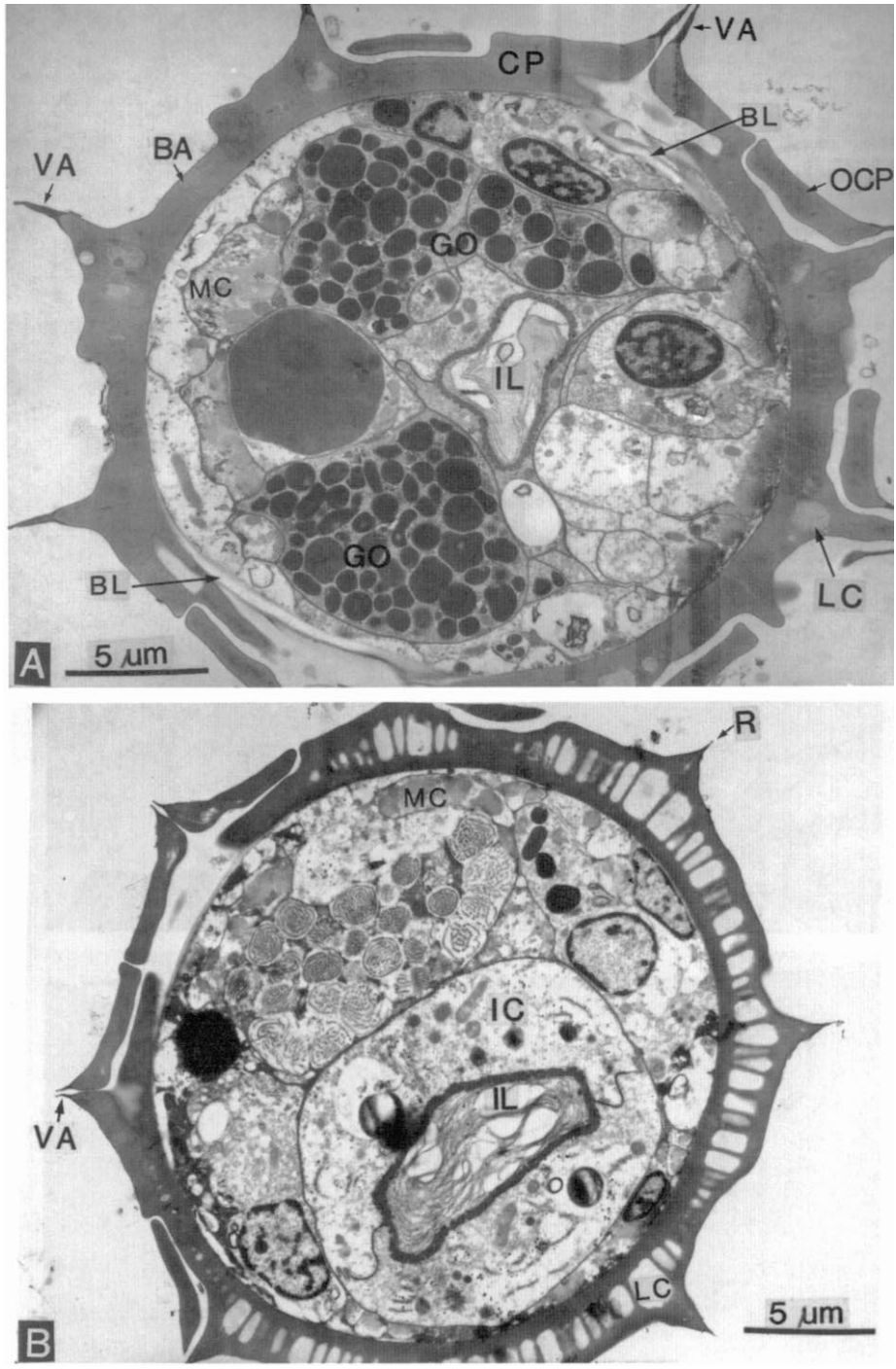

FIG. 5. Transverse sections of male *Ceramonema carinatum* by TEM. A) Section not quite at right angles to the body, intersecting two annules. B) More posterior than A) and cutting through the band of lacunae. BA = basal platform of cortical plate; BL = basal layer of cuticle; CP = cortical layer of cuticle; GO = gonad; IC = intestinal cell; IL = intestinal lumen; LC = lacuna; MC = muscle cell; OCP = overlapping projection of cortical plate; R = ridge; VA = vane.

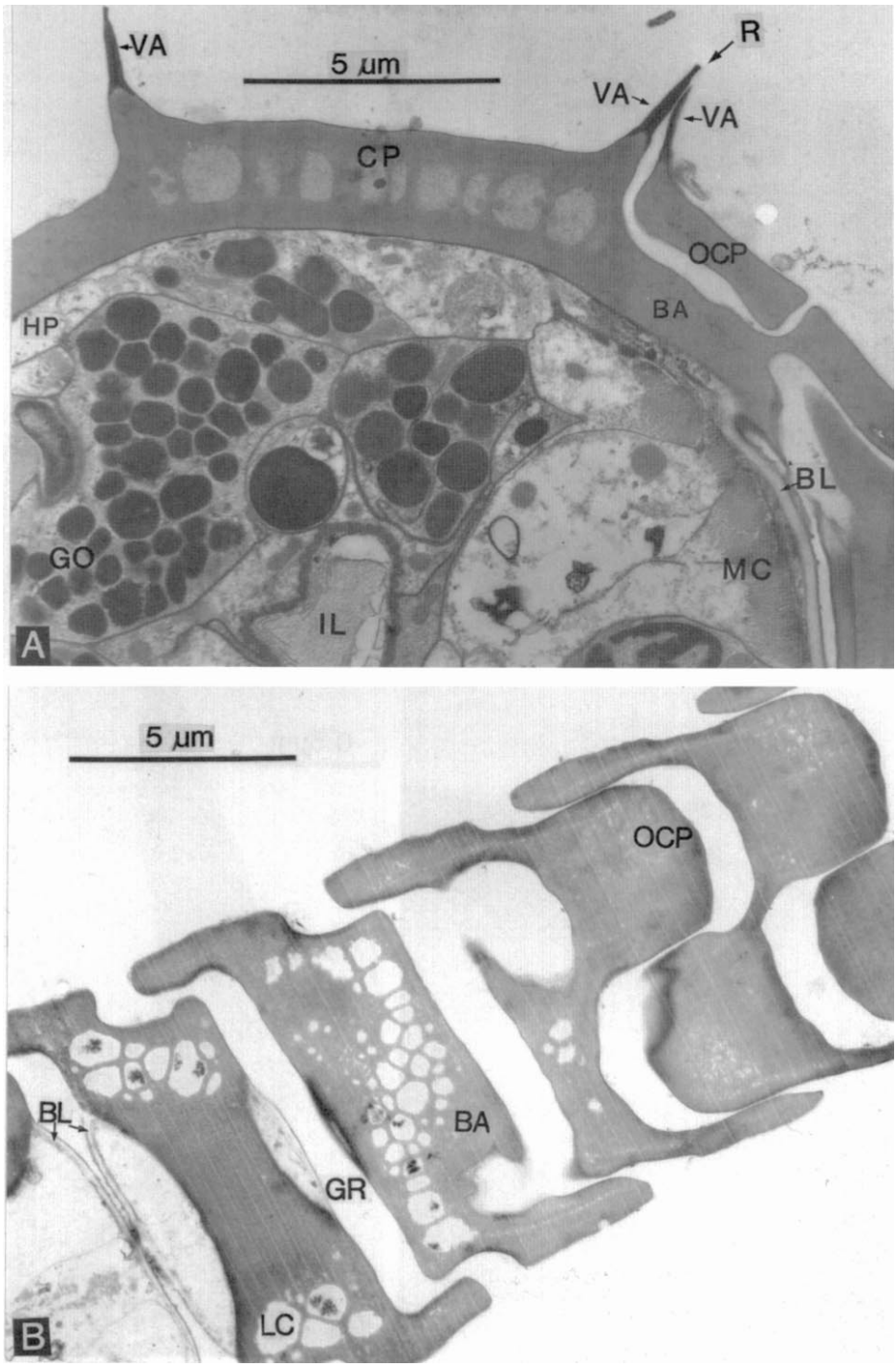

FIG. 6. A) Transverse section of *Ceramonema carinatum* by TEM through overlapping vanes forming longitudinal ridge. B) Slightly oblique tangential section through cuticle. BA = basal platform; BL = basal layer of cuticle; CP = cortical plate; GO = male gonad; GR = groove; HP = hypodermis; IL = intestinal lumen; LC = lacuna; MC = muscle; OCP = overlapping projection of cortical plate; R = ridge; VA = vane.

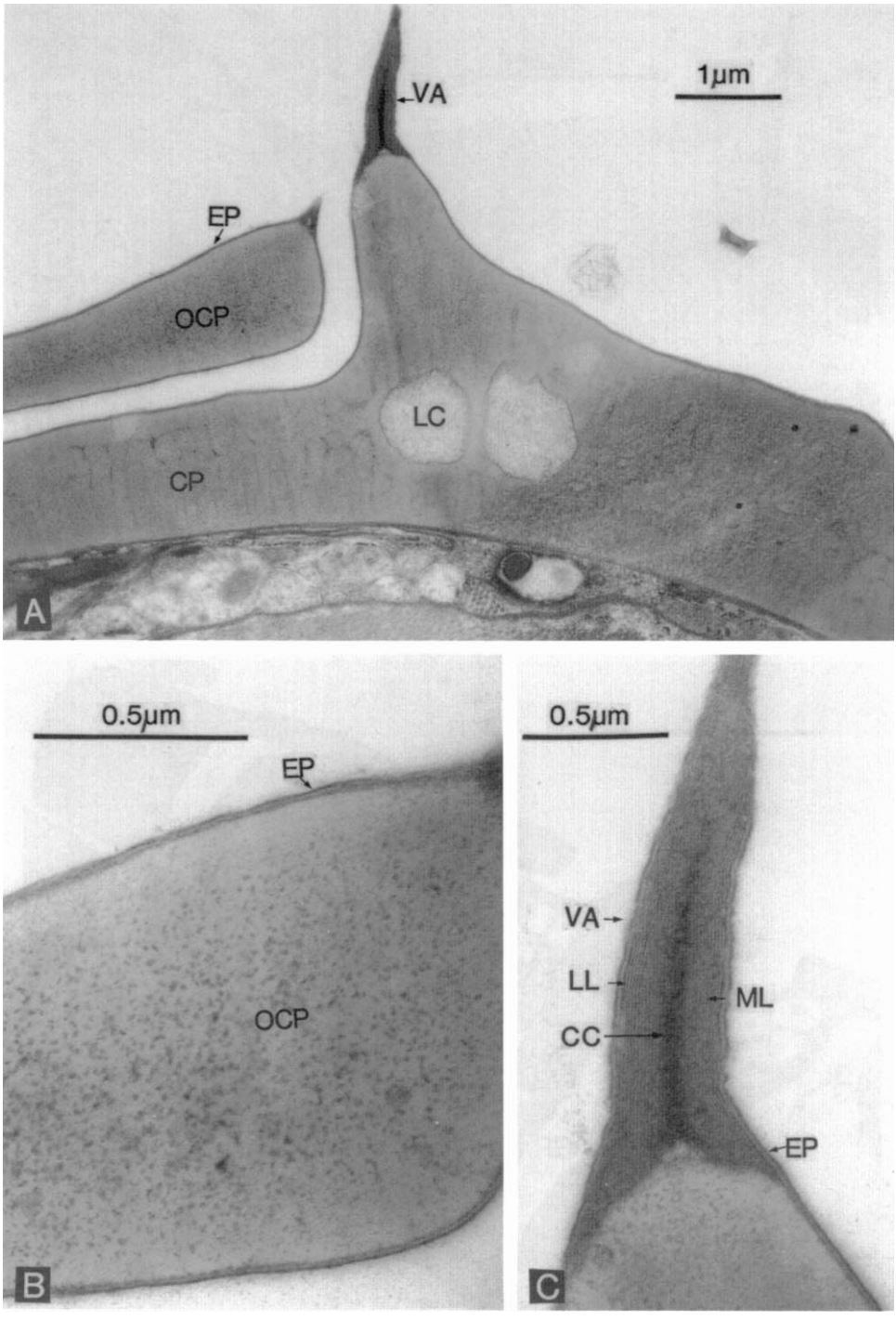

FIG. 7. A) Transverse section of *Ceramonema carinatum* by TEM near tail showing a cortical plate, overlapping projection of neighboring cortical plate, vane, lacunae, and hypodermis. B) Enlargement of overlapping projection of cortical plate of A), showing trilaminar epicuticle. C) Enlargement of vane of A) showing details of structure. CC = central core; CP = cortical plate; EP = trilaminar epicuticle; ML = middle layer; LC = lacuna; LL = lamellar region; OCP = overlapping projection of cortical plate; VA = vane.

croscopy using an oil immersion objective and were described by De Coninck (3) and Chitwood and Chitwood (2). They are customarily figured diagrammatically in taxonomic descriptions of species (7). However, light microscope images are difficult to interpret because successive levels are seen superimposed within the same depth of focus. Some fine details are beyond the resolving power of the light microscope. Electron microscopy clarifies the structure.

Homologies between the cuticular layers in *C. carinatum* and those of other nematodes at the ultrastructural level present difficulties. The cuticle resembles that of *Metadasynemoides cristatus* (6), belonging to the same family. *M. cristatus* has similarly strong annulation, with eight longitudinal ridges supporting vanes, dividing each annule into eight cortical plates. In *M. cristatus,* however, the ridges and vanes are more prominent, and the interridge plates less massive. An important difference is that in *Ceramonema* the electron-lucent layer (basal layer in *M. cristatus* [6]) is apparent in longitudinal sections only in the annular grooves and, in transverse sections, only between the plates, whereas in *M. cristatus* it is also evident beneath the cortical plates and encircles the whole circumference of the body. Nicholas and Stewart (6) concluded that the lucent layer was the basal layer, the plates the cortical layer, and that no median layer was present. In *Monoposthia* (from the related family Monoposthiidae), described by Malakov (5), the cuticle is also strongly annulated with longitudinal ridges. Electron microscopy of sections shows similar cortical plates, with lacunae, reminiscent of those in the two species of Ceramonematidae, and a continuous underlying lucent basal layer. Malakov equates the fine fibrilar material within the lacunae to the median layer of other nematodes. This seems to us a reasonable conclusion. We therefore suggest that the cortical layer within the annules has been greatly expanded, in comparison with other nematodes, enveloping the median layer, represented by the lacunae. The lucent layer represents the basal layer, complete in *M. cristatus* but discontinuous in *Ceramonema.* Unlike *M. cristatus,* we have been able to resolve the epicuticle into a trilaminar membrane. We have not, unlike *M. cristatus,* been able to recognise lateral lines.

Functional significance of the massive cuticle remains a problem. We speculate that it may be related to the habitat in which both *Ceramonema* and *Metadasynemoides* occur, i.e., within the surf zone of sandy beaches with medium to fine sand. Perhaps its function is to protect the nematode from the impact of sand grains, but if so the vanes and not the plates would take most of the impact, and we have seen no evidence of damage to the vanes or plates in the specimens we have examined. Alternatively, the vanes may aid locomotion (which may explain why the muscles are so small) as well as maintaining strength while still allowing the cuticle to bend. Still another possibility is that the vanes help to anchor the animal in the sand. So far we have commonly found *Ceramonema* where the waves are strong, and we have observed that heavily armored cuticles are a common characteristic of nematode genera collected from these habitats on Australian beaches. Another feature of the morphology of both *Ceramonema* and *M. cristatus* that may be related to the massive, complicated, articulated cuticle is the very poor development of the body wall musculature compared with that in other nematodes. Perhaps the strongly articulated cuticle lessens the role of muscle in maintaining pseudocoel pressure.

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