

## Ultrastructural Variation of Cuticular Layers in Cephalobinae (Nemata: Rhabditida)

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**Abstract:** The ultrastructure of the body wall cuticle in *Acrobeles complexus*, *Cervidellus alutus*, and *Zeldia punctata* was studied as a step toward understanding biological diversity within Cephalobinae, and to discover new characters for phylogeny-based classification of the suborder. In each species the cuticle consists of cortical, median, and basal layers. The cortical layer includes an external trilaminar and internal granular zone; the basal layer is striated. In *Z. punctata* the median layer is electron-lucent, vacuolar, and penetrates the cortical layer; it also includes periodically dense columns that apparently correspond to punctuations visible with light microscopy. In contrast, the median layer of the body wall cuticle in *A. complexus* and *C. alutus* is bisected by a zone that undulates parallel to the nematode surface and with periodicity corresponding to annuli. Phylogenetic analysis, using derived cuticle patterns of Cephalobinae, requires an understanding of ecological pressures that could result in convergent evolution of cuticle characters.

**Key words:** *Acrobeles complexus*, Cephalobidae, *Cervidellus alutus*, cuticle, nematode, phylogeny, ultrastructure, *Zeldia punctata*.

The suborder Cephalobina (Rhabditida) is significant to basic research on eukaryotic model organisms (e.g., *Caenorhabditis elegans*) because it includes evolutionary progenitors of a range of plant- and animal-parasitic taxa (Blaxter et al., 1998). Cephalobina, however, is underdeveloped in taxonomic and phylogenetic resolution. For example, the subfamily Cephalobinae Filipjev, 1934, including *Acrobeles complexus* Thorne, 1925, *Cervidellus alutus* (Siddiqi, 1993) Shahina and De Ley, 1997, and *Zeldia punctata* (Thorne, 1925) Thorne, 1937, is problematic because genera often have been defined by characters later demonstrated to be unreliable (Anderson, 1965, 1968; Anderson and Hooper, 1970, 1971; De Ley et al., 1993a, 1993b; Rashid et al., 1985). Phylogenetic taxonomy requires reliable characters that are testable by congruence with independent character sets, such as

those provided by robust molecular frameworks (Baldwin et al., 1997a, 1997b). Previous investigations of Heteroderinae have demonstrated that layering of the body wall cuticle, as shown with transmission electron microscopy (TEM), can be a source of phylogenetically informative characters (Baldwin, 1992).

During studies on the stoma ultrastructure in several species of Cephalobidae (Baldwin and Eddleman, 1995; De Ley et al., 1995; Van de Velde et al., 1994), the body wall cuticle also was observed with TEM. Light microscope descriptions suggest distinctive cuticle in these nematodes including a “double cuticle” in *A. complexus* and *C. alutus*, and punctuations in the cuticle of *Z. punctata* (Shahina and De Ley, 1997; Thomas, 1965; Thorne, 1925, 1937). Our objective was to test the hypothesis that cuticle layering is variable among Cephalobinae, by first comparing a small sample of species within the subfamily. This paper presents the ultrastructure of the body wall cuticle at the level of the esophagus in species of three genera, *A. complexus*, *C. alutus*, and *Z. punctata*.

### MATERIALS AND METHODS

*Acrobeles complexus*, *C. alutus*, and *Z. punctata* were collected from Nebe in the semi-arid zone of Senegal (km 5, road Diourbel to Kaolack). A second population of *A. com-*

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*plexus* was collected from Landegem, Belgium. Senegalese populations of the three species were cultivated in the laboratory on YMA medium at 34 °C (Vincent, 1970). The second population of *A. complexus* was cultivated at the University of Gent, Belgium (Van de Velde et al., 1994). Fixation, embedding, and sectioning were performed as previously described (Van de Velde et al., 1994). At least five female specimens of each species were examined through the region corresponding with the level of the esophagus. Ultrathin sections were photographed with a Siemens Elmiskop 1A or a Jeol 100CXII electron microscope.

In accordance with the recommendations of the *Caenorhabditis* Genetics Center, the three isolates were given the following strain codes: *A. complexus* PDL0002, *Z. punctata* PDL0003, and *C. alutus* PDL0004. Cultures of all three strains are available from the third author.

## RESULTS

The body wall cuticle was similar among isolates of *A. complexus* and *C. alutus*, but that of *Z. punctata* was distinctive. In all species examined, the body wall cuticle consisted of cortical, median, and basal layers. The cortical layer included an external trilaminar zone and an internal granular zone, and the basal layer of all three species was striated (Figs. 1A,B; 2C–E; 3). The thickness of the body-wall cuticle averaged 1.1 µm in *Z. punctata* and 1.3 µm in the other two species.

The median layer of *Z. punctata* primarily was electron-lucent, vacuolar, and thickest in the middle of the annules (Fig. 1A). The electron-lucent vascular material extended radially into the internal granular zone of the cortical layer, resulting in an ambiguous demarcation between the layers. The electron-lucent radial extensions tapered and became narrower as they approached the external cortex (Fig. 1A). Their conical shape was confirmed in tangential section (Fig. 1B,C). Embedded within the electron-lucent, vacuolar substrate, the median layer of *Z. punctata* had electron-dense columns that extended from the granular zone of the

cortex to the striated basal layer (Fig. 1A). In tangential sections, these columns were roughly circular and seemed to be randomly distributed (Fig. 1B,C).

In contrast to the median zone of *Z. punctata*, the median zone of *A. complexus* and *C. alutus* consisted of an electron-dense vacuolar matrix bisected by a thick (>0.5 µm) undulating zone; periodicity of the undulations corresponded to annuli (Figs. 2A,C;3). Overall, the undulating zone was relatively electron-lucent, although at a finer level it consisted of striated lamini with alternating degrees of electron density (Fig. 2A,C). The undulating zone was clearly visible in the light microscope where it appeared as a second cuticle beneath the outer “cuticle” as depicted in the original descriptions. The outer part of the electron-dense vacuolar matrix of the median zone appeared continuous in longitudinal sections and thickest between the annules (Fig. 2A). The inner part of the electron-dense vacuolar matrix was restricted to the middle of the annules as viewed in longitudinal sections (Figs. 2A,C,D;3). Periodic radial canals spanned the undulating zone, linking the inner and outer vacuolar zones; radial canals occurred in the middle of each annule (Figs. 2B–D;3). In addition, periodic extensions of outer vacuolar zone of the medial layer projected into the granular inner cortical zone, and these extensions occurred between two annules as viewed in transverse section (Fig. 2E).

## DISCUSSION

We have shown that body wall cuticle layers vary among Cephalobinae. The potential for using variation in cuticle layers for insight into phylogeny requires an understanding of their broader distribution throughout the suborder, and their uniqueness relative to cuticle layers in other Nematoda. Body wall cuticle of Nematoda most often is defined within a basic structure including a cortical, median, and striated basal layer (Bird and Bird, 1991). The basic three-layer pattern is widely distributed within Secernentea, including Rhabditida

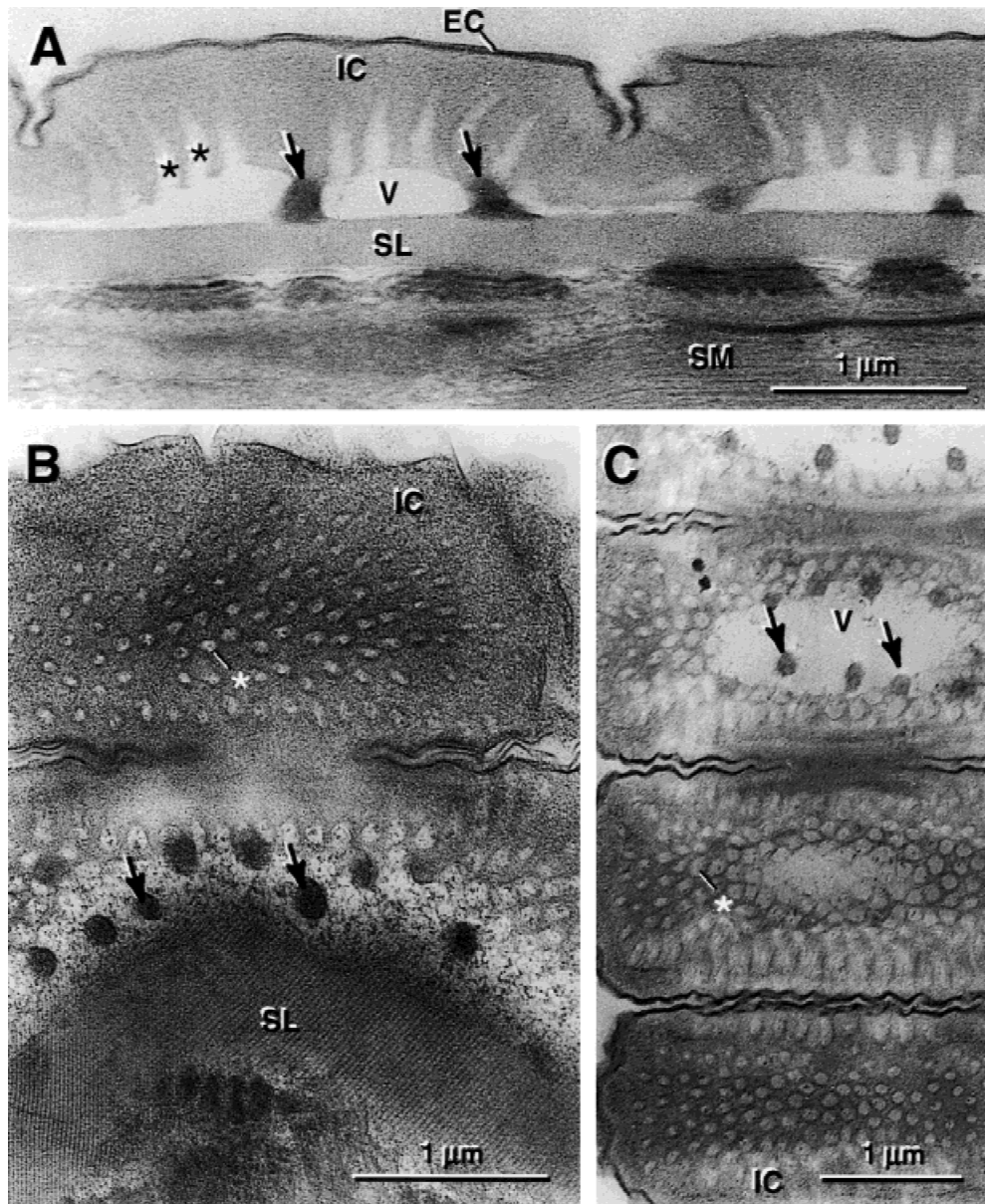


FIG. 1. TEM of the body wall cuticle of females of *Zeldia punctata*. A) Longitudinal section. B) Oblique tangential section through cortex median and basal layers. C) Oblique tangential section primarily through cortex. Arrows show column-like electron-dense structures linking the granular layer to the basal striated layer. Asterisks indicate tapered electron-lucent radial extensions. EC = external zone of cortex; IC = internal zone of cortex; SL = striated basal layer; SM = somatic muscles; V = vacuolar zone of median layer.

(Cox et al., 1981; Popham and Webster, 1978; Zimmerman et al., 1973) and Tylenchida (Baldwin and Hirschmann, 1975; Byers and Anderson, 1972; Johnson et al., 1970; Kisiel et al., 1972; Mounport et al., 1990, 1991, 1993a, 1993b; Nicholas, 1972), as well as in the adenophorean outgroup

including some Monhysterida (Van de Velde and Coomans, 1991). This distribution suggests the basic pattern of three layers is pleiomorphic in Secernentea, and modifications such as those demonstrated for Cephalobinae are likely to be apomorphic and, therefore, useful for interpreting



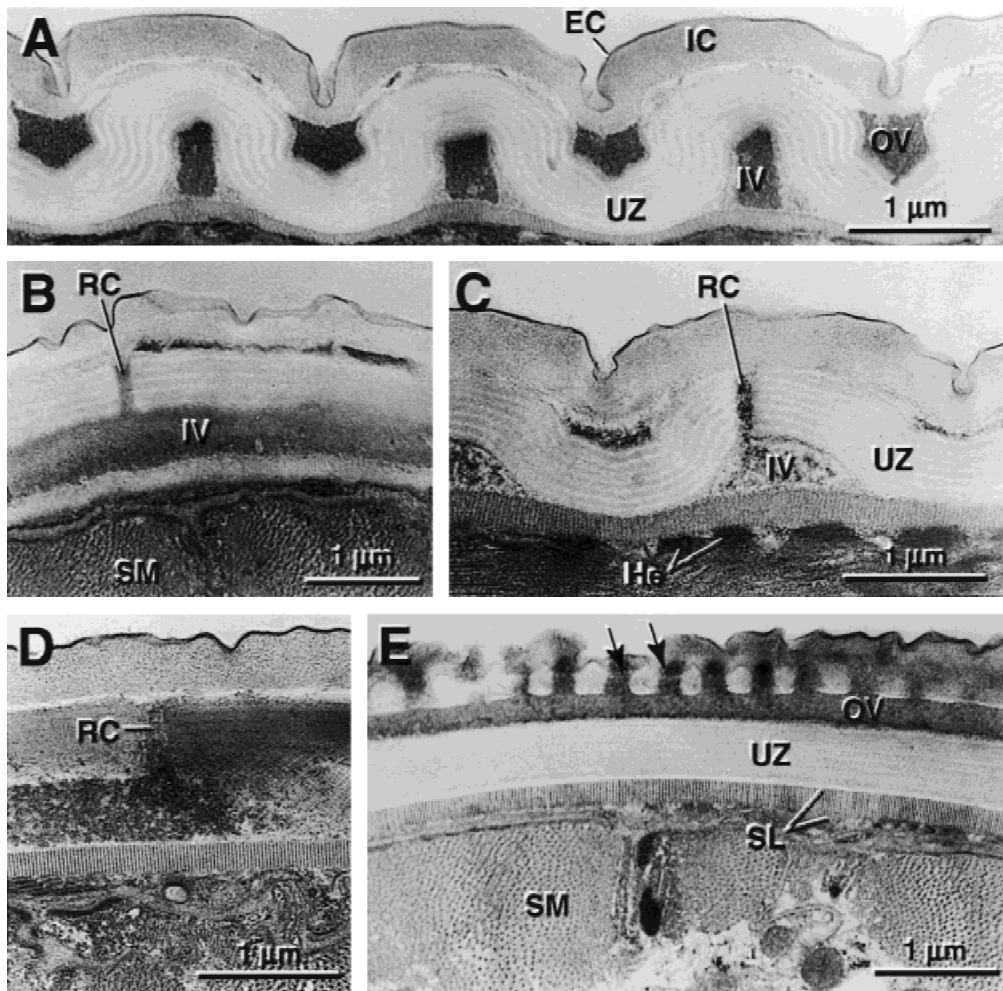


FIG. 2. TEM of the body wall cuticle of females of *C. alutus* (A, B) and *A. complexus* (C-E). A) Longitudinal section. B) Oblique transverse section showing radial canal (RC) in the undulating zone. C) Longitudinal section showing a radial canal (RC) in the undulating zone. D) Oblique transverse section in the middle of an annule. E) Oblique transverse section between two annules, showing periodic radial extensions (arrows) of the outer vacuolar zone of the median layer. EC = external zone of cortex; He = hemidesmosomes; IC = internal zone of cortex; IV = inner vacuolar region of the median layer; OV = outer vacuolar zone of the median layer; RC = radial canals; SM = somatic muscles; SL = striated basal layer; UZ = undulating zone of medial layer.

phylogenetic relationships. However, it remains to be determined if similar modifications of cuticle in certain Cephalobinae are convergent and linked to ecological pressures, rather than a shared evolution.

Subjection to desiccation is an ecological pressure shared by many Cephalobinae that inhabit deserts and other stressful environments; one might anticipate that some specializations of the cuticle are adaptations to survive desiccation and are associated with anhydrobiosis. The undulating layer in *A.*

*complexus* and *C. alutus* might be related to such a function, since a high proportion of individuals of both species survive slow desiccation (Baujard and De Ley, unpub.). The strain of *C. alutus* used in the present study was collected from desert soil, whereas that of *A. complexus* was found in sand with sparse grasses; this cosmopolitan species is quite common in dune and desert sands throughout the world. Furthermore, Shahina and De Ley (1997) described *Nothacrobeles lunensis* from a desert valley, a new species having

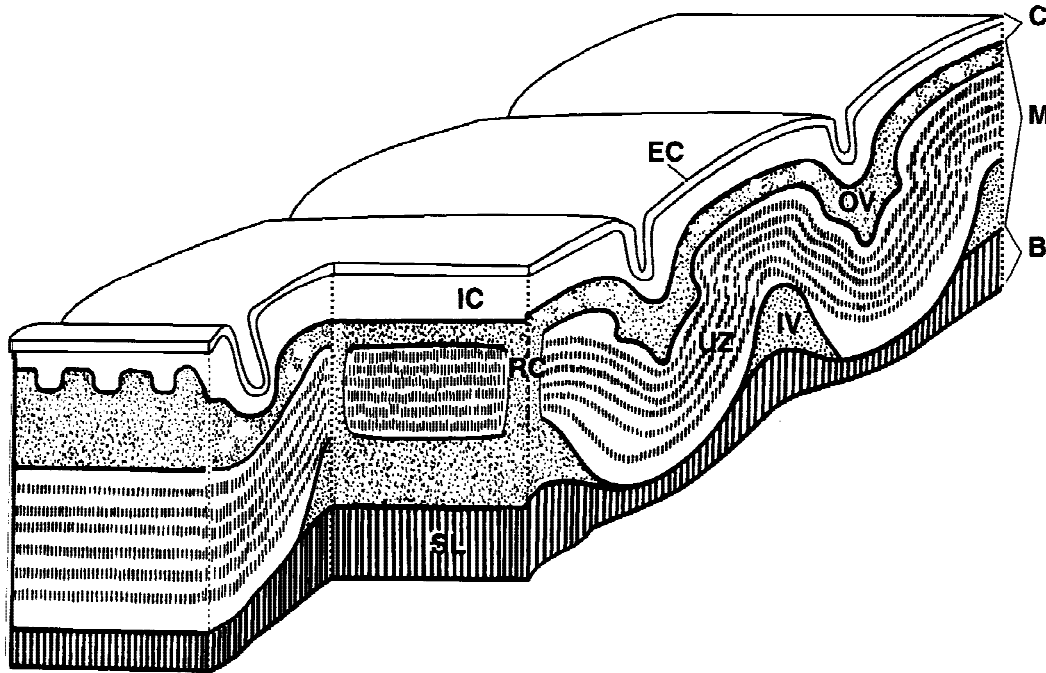


FIG. 3. Generalized representation of the cortex (C), median (M), and basal (B) layers of cuticle, as interpreted with TEM, in *A. complexus* and *C. alutus*. EC = external zone of cortex; IC = internal zone of cortex; IV = inner vacuolar region of the median layer; OV = outer vacuolar region of the medial layer; RC = radiant canal; SL = striated basal layer; UZ = undulating zone of median layer.

a “two-layered” appearance of the cuticle similar to that of *A. complexus* and *C. alutus*. Conversely, *Z. punctata*, which lacks the undulating layer, is widespread throughout deserts of California, and also survives slow desiccation. A broad survey of cuticle-layering throughout Cephalobinae would be useful to determine the range of variation and possible congruence of such layers with other characters that reflect phylogeny, or associations with particular environments.

The use of cuticle characters for interpreting phylogeny requires precise recognition of homologous layers and structures across taxa. However, throughout Nematoda several nomenclatures have been used in the description of the layers, sublayers, and zones based on their number, position, and ultrastructure (Bird and Bird, 1991; Cliff and Baldwin, 1985; Johnson et al., 1970; Maggenti, 1979). In some cases it is difficult to recognize even the boundaries of particular cuticle layers. In *Z. punctata*, precise definition and measurement of some layers are

ambiguous due, for example, to penetration of the vacuolar median layer into the granular internal cortical zone. Referring to nematodes in general, Bird and Bird (1991) noted that there was no clear line of demarcation separating the inner cortical layer and the median (homogenous or matrix) layer. One interpretation is that the two layers, in fact, are differentiations of a single layer as suggested by Shepherd et al. (1972). In *Z. punctata*, penetration of the vacuolar median layer into the granular internal cortical zone confounds precise differentiation and measurement of the two layers.

In *A. complexus* and *C. alutus*, the median zone is distinctive, consisting of two components that differ ultrastructurally. To our knowledge, among taxa with a three-layered cuticle, the undulating region observed in the two species has never been described for the median layer of the cuticle of any other nematode. An undulating zone occurs, however, in the cortex of females of Criconematidae (Tylenchida) in the genera *Cricone-*

*mella* (De Grisse, 1972; Mounport et al., 1991) and *Criconema* (Decraemer et al., 1996). In these species a differentiated inner cortical layer is absent so that the median layer lies nearly adjacent to the external cortex (= epicuticle). In *A. complexus*, *C. alutus*, *Criconemella*, and *Criconema*, the basal striated layer is very thin (De Grisse, 1972; Mounport et al., 1991), whereas in most other nematodes with the basic three layers in the cuticle, the striated layer averages about half of the total thickness of the cuticle.

Similar to difficulty in recognizing homologous layers, precise definition of punctations also is difficult, since the term is used to describe dissimilar structures in disparate taxa (Sasser and Carter, 1985; Yushin and Malakhov, 1994). Under the light microscope, in *Z. punctata*, two irregular rows of punctations typically are resolved in each annule; these punctations probably correspond to the electron-dense structures, visible with TEM, that extend through the vacuolar median layer to the inner cortical layer (Fig. 1C). In Hoplolaimidae (Tylenchida) a similar pattern occurs in *Aphasmatylenchus variabilis*, in which radial extensions of the vacuolar median layer project into the granular zone of the cortex (Mounport et al., 1993b). This distribution outside Cephalobina, rather than supporting a shared evolution, suggests convergence of the character within Secernentea.

We have demonstrated that cuticle layers vary within Cephalobinae, and that an expanded study of additional taxa throughout the suborder and outgroups could provide valuable new characters for interpreting phylogeny. However, such future investigations must also consider the need to recognize homologous regions of cuticle among taxa in order to apply consistent terminology. Use of cuticle characters in phylogeny also requires that convergent similarities due to shared environmental factors be recognized relative to similarities that are the result of a shared evolution. These issues can be addressed not only by additional ultrastructural investigations of a wider range of Cephalobinae and outgroups but also by

investigation of the comparative development and biochemistry of cuticle layers, as well as by considering cuticle layers in relation to a broader set of characters, including those provided by comparative biology and molecular biology.

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