Fine structure of body wall cuticle of females of Meloidodera charis, Atalodera lonicerae, and Sarisodera hydrophila (Heteroderidae)

J. G. BALDWIN

Abstract: The body wall cuticle of adult females of *Meloidodera charis, Atalodera lonicerae,* and *Sarisodera hydrophila* is examined by transmission electron and light microscopy for comparison with *Heterodera schachtii* and previous observations of additional species of *Heterodera, Globodera,* and *Punctodera.* The cuticle of *M. charis* is least complex, consisting of layers A, B, C (with A outermost), and varies in overall thickness from 3 to 8 μm. As in other species, the cuticle is thickest in mature specimens. The cuticle of *A. lonicerae* is 6–9 μm thick; unlike *M. charis* it has an innermost layer, D, in addition to A, B, and C. The cuticle of *S. hydrophila* varies from 14 to 30 μm thick and includes a D layer similar to *A. lonicerae;* layer C is subdivided into additional zones relative to other heteroderids, and the external portion of the cuticle is infused with an electron-dense material. The presence of a D layer in *A. lonicerae* and *S. hydrophila* is a character state which is shared with *Globodera* spp. and *Punctodera* sp. The electron-dense material in the outer layers of *S. hydrophila* also occurs in *Globodera* spp. and *Punctodera* sp. On the other hand, *H. schachtii* resembles other *Heterodera* spp. as well as *M. charis* by the absence of a D layer and lack of electron-dense material in the outer layers. The pattern of occurrence of shared character states, including those of the cuticle, may be useful for phylogenetic analysis of Heteroderidae. *Key words:* cyst, electron microscopy, *Globodera, Heterodera, Punctodera,* Heteroderoidea, Tylenchida, phylogeny.

Journal of Nematology 15(3):370-381. 1983.

The cuticle of adult females of Heteroderidae has morphological characters which may be diagnostic among genera. For example, in some groups the cuticle surface is striated similarly to vermiform types, or it may be modified into various "lace-like" or rugose patterns; in addition, the body wall develops into a cyst in some genera but not in others. Ferris (4) indicated polarities (primitive versus derived expressions) for such character states and demonstrated their application to a phylogenetic analysis of Heteroderidae. Shepherd et al. (11) observed differences in layering of the female cuticle among species of *Heterodera sensu lato* and broader differences among groups of species which supported subsequent separation into *Heterodera* Schmidt, 1871 and *Globodera* (Skarbilovich, 1959) Behrens, 1975. In contrast, layers of cuticle of vermiform males and juveniles are apparently consistent among species (1,11,15).

In spite of interspecific differences and certain variations with age of specimens, Shepherd et al. (11) identified fundamental layers of the cuticle and designated them as A, B, C, D; zones of layers A, B, C were indicated with subscripts. I have adapted this terminology to the present study. The outermost layers, A and B, were considered by Shepherd et al. (11) to be at least partially homologous with the cuticle of males: A_1 was described as homogeneous, thin (about 30 nm), and moderately to very electron-dense; A_2 was composed of fine fibrils; A_3 was often vacuolated with electron-dense globules among fibrous strands. The B layer was interrupted into "patches" in females and was striated, having dark and light lines with a periodicity of about 18 nm in transverse section. Generally the thickest layer was C, which was characterized by fibers. Fibers of C_1 were randomly arranged. Fibers of C_2 were similar to C_1, but as individual females matured the fibers became embedded in an electron-dense matrix. Fibers of C_3 were fine-textured with random orientation. The D layer was composed of clearly defined fibers arranged in a repeating helicoidal pattern. Shepherd et al. (11) further characterized the layers and zones on the basis of specific stains and optical properties.

Detailed structure of the female cuticle has been previously reported for species of only 3 of the 13 genera of Heteroderidae. Additional information would be useful for a more complete understanding of character states and homologies among species groups

Received for publication 30 August 1982.

[1]Assistant Professor of Nematology, University of California, Riverside, CA 92521.

The author is grateful to Mr. A. H. Bell for technical assistance in preparing specimens, and to Dr. A. M. Shepherd for helpful suggestions on the manuscript.

and may ultimately be useful for phylogenetic inference for Heteroderidae.

Detailed structure of the body wall cuticle of females of three species representing the morphologically diverse genera of *Meloidodera* Chitwood et al., 1956, *Atalodera* Wouts and Sher, 1971, and *Sarisodera* Wouts and Sher, 1971 is examined in this paper. In addition, *Heterodera schachtii* Schmidt, 1871, although previously examined (11), is included as a reference for comparison.

MATERIALS AND METHODS

Females of four genera of Heteroderidae were collected and processed for examination of the body wall culticle by transmission electron (TEM) and light (LM) microscopy. *Meloidodera charis* Hopper, 1960 was collected from native peony (*Paeonia californica,* Nutt) at Badger Canyon, California; topotypes of *Atalodera lonicerae* (Wouts, 1973) Luc et al., 1978 and *Sarisodera hydrophila* Wouts and Sher, 1971 were isolated from honeysuckle (*Lonicera involucrata* [Richards] Banks ex Spreng) and willow (*Salix lasiolepis* Benth.), respectively, at Dripping Springs, California. Living females at varying degrees of maturity from white to yellow, or the early stages of tanning were selected; maturity was judged and recorded on the basis of overall size, color, and the proportion of the body occupied by eggs. Approximately 10 females of each species were examined.

Whole females were fixed for 12 h in cold (4C) 3.5% glutaraldehyde prepared in 0.05 M phosphate buffer at pH 7.2, subsequently pierced with an occulist's knife, and transferred to fresh glutaraldehyde solution for an additional 4 h. After thorough rinsing in buffer the material was postfixed for 2 h in 1% osmium tetroxide (OsO_4) in phosphate buffer and again rinsed. Dehydration was in a graduated acetone series followed by infiltration with Spurr's epoxy during 72 h. The specimens were embedded in Beem® flat embedding plates. Sectioning was with a Porter Blum MT-2B ultramicrotome; a glass knife was used to accommodate thick as well as thin sections and to avoid possible damage to a diamond knife from minute sand particles which occasionally remained attached to the surface of the specimens.

Sections were generally transverse from the midregion of the specimen, although in some cases specific layers or regions were further investigated with oblique and longitudinal sections. Sections with silver to gold interference colors were mounted on fomvar and carbon-coated 100 mesh grids, and adjacent thick (about 0.25 μm) sections for LM were mounted on glass slides previously coated with Haupt's adhesive. Staining for TEM was with a saturated solution of uranyl acetate in 50% ethanol followed by lead citrate (13). Staining for LM included the optically dense osmium tetroxide, as well as toluidine blue (8). Thin sections were examined with a Hitachi H-600 TEM, and thick sections were examined with bright field as well as Nomarski interference LM optics. Calibrations for TEM measurements were made using a carbon grating replica grid.

OBSERVATIONS

The cuticle of females of *M. charis, A. lonicerae, S. hydrophila,* and *H. schachtii* have in common A, B, C layers, but each is distinctive by thickness of layers, specific morphology of sublayers (zones), and in some cases the presence of additional layers (Figs. 1, 2, 3, 4, 5, 12, 19).

The cuticle of *M. charis* is the least complex of the four species examined (Fig. 1). It varies in thickness from 3 μm in young adult females to 8 μm in more mature individuals. The cuticle is transversely striated so that the surface is relatively smooth in transverse sections which are perfectly aligned with the plane of striations. More typically, sections only approximate transverse so that the surface shown is scalloped (Figs. 5, 6, 7). With staining for LM, the A layer is moderately dense (Fig. 2; with TEM, distinct A_1, A_2, A_3 zones are resolved (Figs. 5, 6). A very thin fringed material or deposit generally occurs at the surface of A_1 (Figs. 6, 7), and in some cases a portion of previously molted cuticle also occurs near the surface.

The A_1 zone is about 35 nm thick, is highly electron-dense, and can sometimes be resolved into finer bands (Fig. 7). The A_2 zone is about 0.5 μm thick and

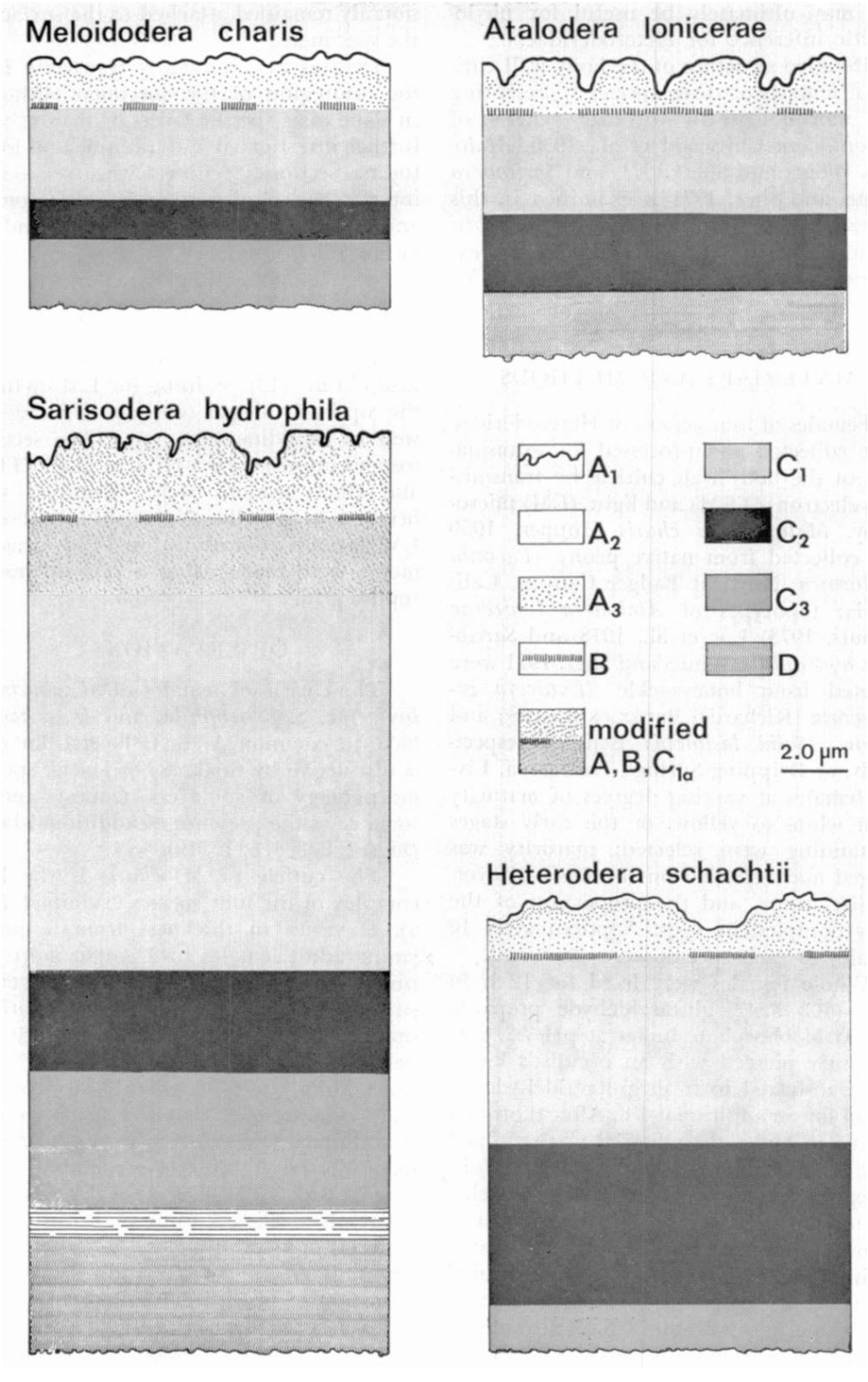
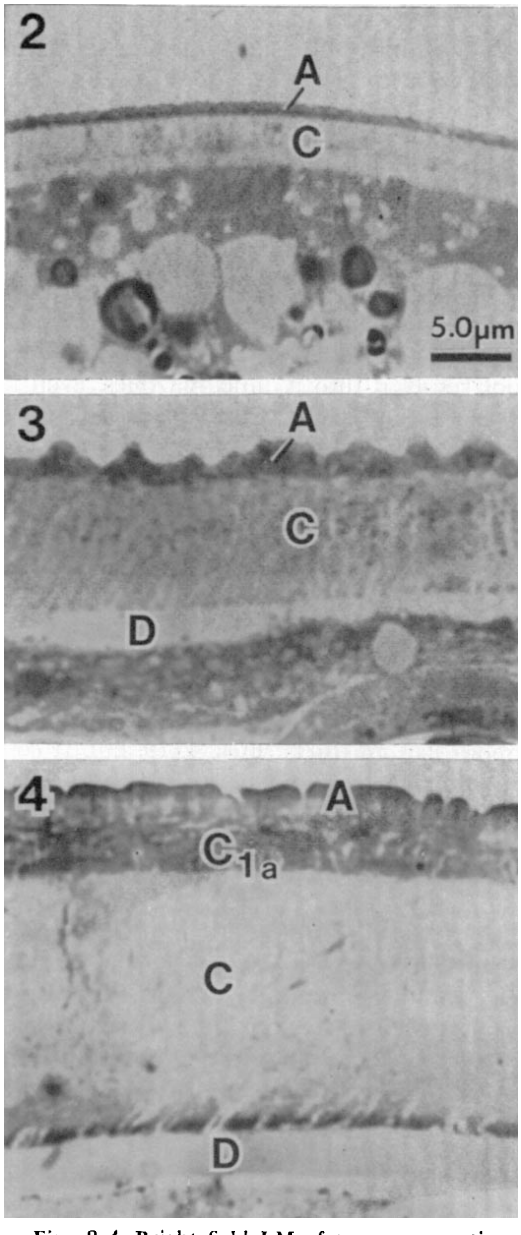


Fig. 1. Diagram illustrating layering of cuticle in midregion of females of four species of Heteroderidae.



Figs. 2–4. Bright field LM of transverse sections of female cuticle stained with toluidine blue. 2) *Meloidodera charis* with layers A, C. 3) *Atalodera lonicerae* with layers A, C, D. 4) *Sarisodera hydrophila* with layers A, C, D. A portion of C is distinct as C_{1a}. All figures are in same scale as Fig. 2.

is basically electron lucent in TEM, sometimes with patches of moderate density (Fig. 6). The inner surface of A_2 is highly convoluted, and is continuous with the walls of a labyrinth of chambers which compose A_3; the chambers are filled with a highly electron-dense material (Figs. 5, 6, 8). The walls of the chambers are penetrated by fine electron-dense channels or fibers (Fig. 8). The A_3 zone is up to 1.5 μm thick. Indentations of the surface pattern of *M. charis,* as in other species, are primarily accommodated by varying thickness of A_2 and A_3 (Fig. 5).

The B layer occurs as periodic patches at the base of A_3, but its typical striations were less consistently resolved in *M. charis* than in other species. Patches of B are about 0.2 μm thick and generally less than 1 μm long (Figs. 5, 9).

The C layer of *M. charis* stains lightly in LM, and zones are not clearly resolved (Fig. 2). The presence of C_1, C_2, and C_3 in the cuticle of mature females is confirmed with TEM; the thickness of the zones may extend to about 3.5 μm, 1.5 μm, and 2 μm, respectively (Fig. 5). Zones of C have a fibrous texture in which faint strands predominately are parallel to the surface as viewed in transverse section (Figs. 5, 6, 10). In older specimens, dense material occurs in the hypodermis (Fig. 11) and apparently migrates through radial channels to accumulate and form the C_2 zone, thus separating C_1 and C_3. Additional radial channels extend through the C_1 zone and terminate at A_3 (Fig. 5).

The cuticle of *A. lonicerae* varies in thickness from 6 to 9 μm, with the thicker cuticle occurring in mature females and particularly in the region of the posterior prominence (Figs. 1, 3, 12, 13, 18). The surface is deeply convoluted in transverse or longitudinal sections, especially in young specimens, and is frequently associated with a fringe of parallel columns up to 2 μm high (Figs. 13, 14). Sometimes a portion of molted cuticle of the fourth stage also persists, generally associated with a similar fringe (Fig. 13). With LM, the A layer is densely stained (Fig. 3). Zones A_1 (35 nm thick), A_2 (0.5 μm thick), and A_3 are distinct with TEM and similar to those of *M. charis* (Figs. 12, 13, 15, 18). However, the A_2 zone in *A. lonicerae* is more granular (fibrous ?) than in *M. charis,* and A_3 is particularly variable in thickness (1–3 μm) to accommodate the deeply invaginated surface pattern (Fig. 13). Broad portions of the matrix of A_2 extend into A_3, sometimes contacting B or C (Fig. 13). The electron

lucent walls of the chambers of A_2 contain fine electron-dense channels or fibers, as in *M. charis* (Figs. 8, 15). The B layer of *A. lonicerae* is well developed with striated patches 0.1–0.3 μm thick and extends up to 10 μm long. The B layer frequently permeates A_3 (Figs. 12, 13, 15, 18).

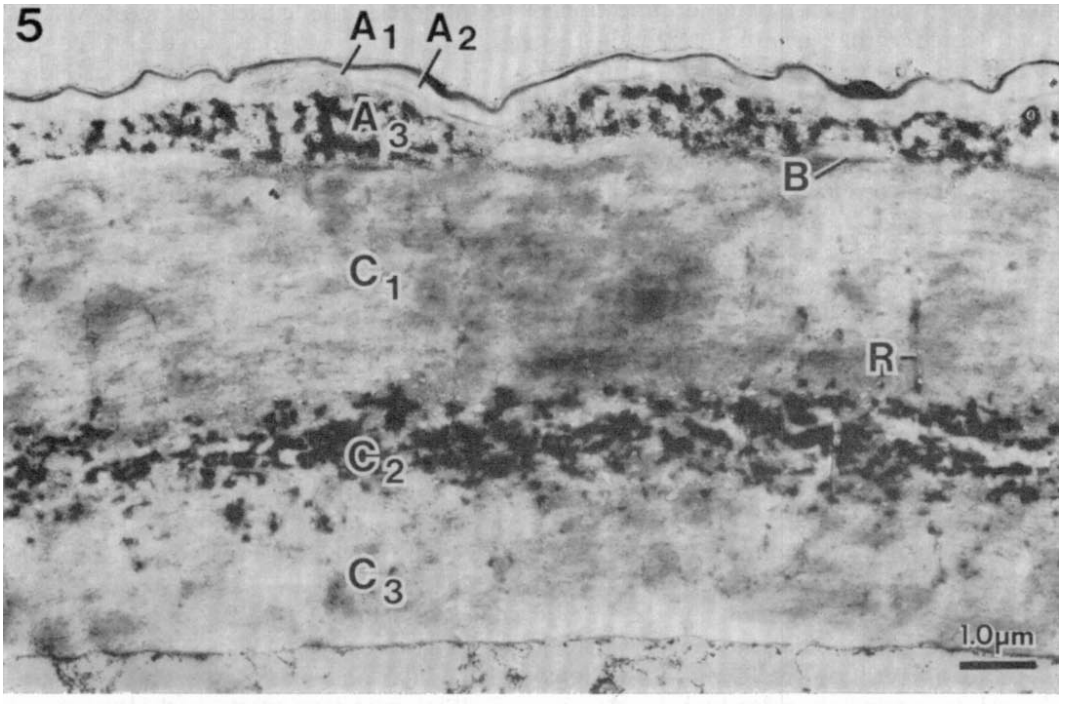
The C layer of mature *A. lonicerae* is up to 6 μm thick and is moderately dense in LM and lucent in TEM (Figs. 3, 12, 13). Zones C_1 and C_2 generally are not clearly resolved, even with TEM, although they may be indicated by slight variation in texture. The outermost portion of C is characterized by fibers which are associated with lengths of electron-dense material and aligned parallel to the surface in transverse section (Figs. 13, 16). In the posterior prominence near the point of attachment of vulval musculature, C_1 and C_2 are clearly delimited (Fig. 18). The C_2 zone is highly electron dense, and radial channels extend through C_2 to A_3. In mature females the innermost layer of the cuticle, D, is characterized by a helicoidal pattern of fibers 20–30 nm thick (Figs. 12, 13, 17). The helicoidal pattern frequently results in a single lamella about 1 μm thick or less, but sometimes the layer is thickened to about 3 μm and includes several lamellae. The D layer stains very lightly with toluidine blue (Fig. 3).

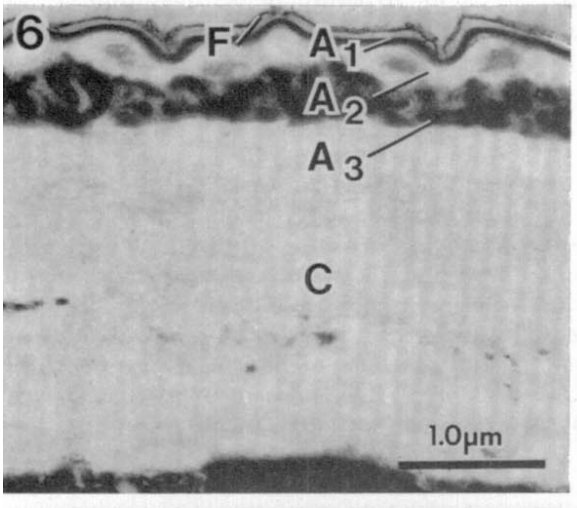
The female cuticle of *S. hydrophila* is strikingly distinctive among the Heteroderidae examined. Its thickness varies from 14 to 30 μm, with the thickest cuticle usually occurring in the most mature individuals (Figs. 1, 4, 19, 20). The surface is rough textured and irregular, including narrow (about 0.15 μm thick) invaginations which penetrate up to 2 μm into the external layers (Fig. 21). No fringe layer was seen on the surface, although frequently a remnant of the A layer of the fourth-stage cuticle persists outside the adult cuticle. The A layer stains uniformly densely in LM with toluidine blue and osmium tetroxide and is also relatively homogeneous and electron dense, as observed with TEM (Figs. 4, 19, 20). Generally, A is obscured by the density of adjacent tissue, but in very thin sections additional bands are resolved (Fig. 22). Surface invaginations are lined with A_1 (Fig. 21).

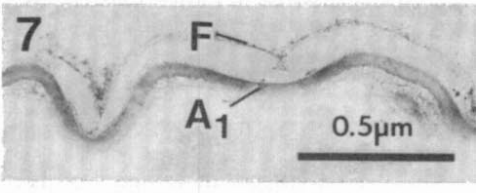
The A layer, excluding A_1, is composed of fine granules among a flocculent, slightly more electron-lucent background; A was not resolved into A_2 and A_3 zones, even in very young adults. The characteristic B layer was occasionally noted in young specimens and generally occurred in small patches about 0.2–0.3 μm thick and 1 μm long (Fig. 23).

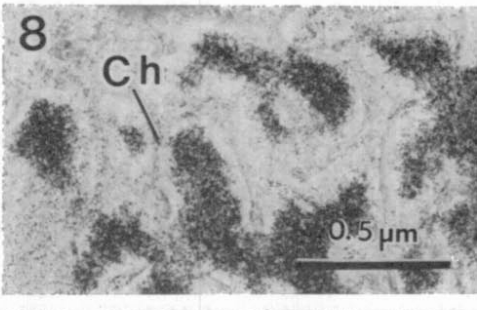
Zones of C were best resolved with TEM in more mature specimens. However, regardless of age, the zones stain uniformly lightly with toluidine blue, with the exception of the outer portion of C_1 which stains densely. This portion, designated C_{1a}, is about 2.5 μm thick (Figs. 4, 19, 20). It consists of the course fibers present throughout C. However, in tangential section the fibers are oriented more nearly parallel to the surface than those of C_{1b}, C_2, and C_3 (Fig. 19). Zones C_{1b} and C_3 are electron lucent and up to 12 and 6 μm thick, respectively, in contrast to C_2 which includes electron-dense granules and is 5 μm thick or less (Figs. 19, 20). The electron-dense material of C_2 apparently differs from that of A and the matrix of C_{1a} because with LM, the same material of C_2 is relatively optically lucent (Figs. 4, 19). In young adult females the dense material of C_2 is randomly distributed within C, but in more mature individuals it is often associated with radial channels (Figs. 19, 20) extending throughout C. Zone C_3 consists of course fibers oriented similarly to C_{1a} (Fig. 19). Internal to C is a narrow

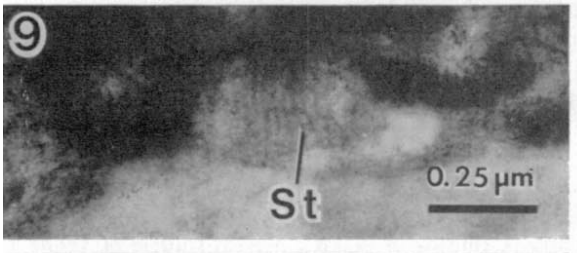
Figs. 5–11. TEM of transverse sections of female cuticle of *Meloidodera charis*. 5) Cuticle of mature female, composed of layers A, B, C. Layer A consists of A_1, A_2, A_3; layer C has fibrous texture and includes C_1, C_2, C_3. Radial channels (R) extend through C. 6) Cuticle of young female. Layer A includes A_1, A_2, and A_3; C has fibrous texture and is relatively homogenous. F = fringed material. 7) Layer A, and adjacent fringed material (F). 8) Layer A_3 with fine electron-dense channels (Ch) penetrating electron-lucent walls of chambers. 9) Layer B showing periodic striae (St). 10) Portion of C of young female showing fibrous texture. 11) Hypodermis with dense material (DeM). Radial channels (R) penetrate cuticle (Cu).


5
A1
A2
A3
B
C1
R
C2
C3
1.0μm

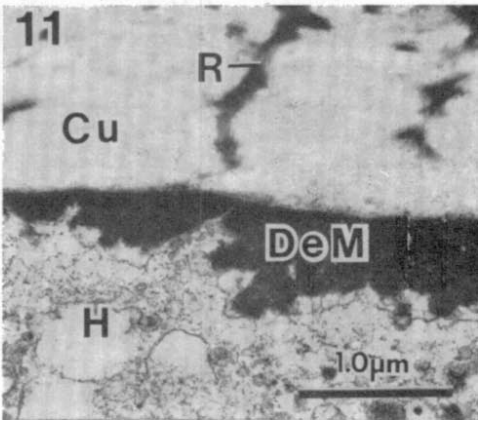

6
F
A1
A2
A3
C
1.0μm


7
F
A1
0.5μm


8
Ch
0.5 μm


9
St
0.25 μm


10
0.25μm


11
R
Cu
DeM
H
1.0μm

(about 0.75 μm thick) band of fine fibers from which radial channels extend; this band stains densely with toluidine blue and is also electron dense (Figs. 1, 4, 19, 20). It is subtended by D, which is 3–8 μm thick and stains very lightly with toluidine blue (Fig. 4). The layer is composed of fine fibers (20–30 nm d), but these are frequently obscured by a granular matrix; however, in some cases, a pattern of lamellae similar to that of D in *A. lonicerae* is observed in both longitudinal and transverse sections (Fig. 24). Generally 2–4 lamellae occur within the layer. The plasmalemma of hypodermal tissue adjacent to D is deeply infolded, suggesting vesicle formation and an active role in transport of materials (Fig. 25).

The female cuticle of the California population of *H. schachtii,* although thin (7-15 μm) relative to that of the previously described population of this species (11), did not otherwise differ morphologically. Mature white females had A_1, A_2, A_3, B, C_1, C_2, C_3 layers and zones but lacked a D layer (Fig. 1).

DISCUSSION

Body wall cuticle of females of Heteroderidae is characterized by the addition of internal layers to a basic pattern which occurs among most vermiform Tylenchida including heteroderid males and juveniles (1, 11, 15). Cuticular layers in heteroderid females which are homologous with basic nomenclature proposed by Bird (2) are A_1 = epicuticle, A_2 = cortical zone, A_3 = medial zone, and B = basal zone. Layer A_3 has a fibrillar matrix which is continuous with A_2, as in males of *Meloidogyne hapla* Chitwood, 1949 (5), and the dense contents of chambers may be modified from the "fluid" attributed to this layer in other tylenchids (2).

Whereas the cuticle of most veriform Tylenchida is limited to the three basic layers, it is noteworthy that, as in Heteroderidae, the cuticle of certain Hoplolaiminae is characterized by the occurrence of additional layers internal to the basal zone (6,14, S. A. Lewis, personal communication). This possibility of a shared derived trait may strengthen the hypothesis of Wouts and Sher (17) that heteroderids evolved from a form "equal or close to Hoplolaiminae."

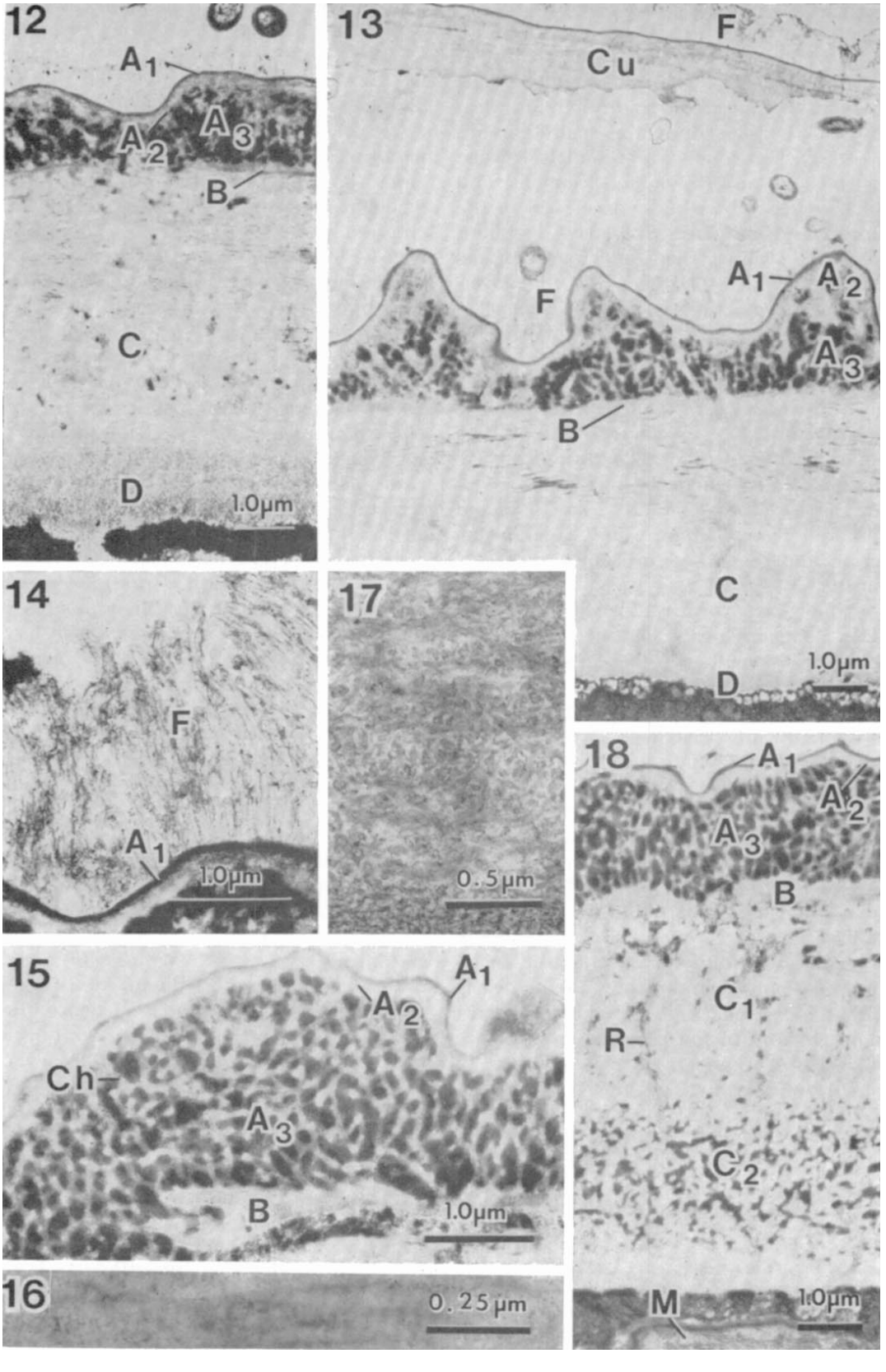
Homologies of layers of cuticle among females of *M. charis, A. lonicerae, S. hydrophila,* and *H. schachtii* generally can be proposed, as well as among previously described *Heterodera* spp., *Globodera* spp., and *Punctodera* sp. (11). Furthermore, layers could be identified with LM using toluidine-blue-stained thick sections; results of staining were similar to those reported by Shepherd et al. (11) regardless of differences in fixatives and embedding media.

The structure of A, including A_1, A_2, and A_3 is basically consistent among *M. charis, A. lonicerae,* and *Heterodera* spp. However, in *S. hydrophila, Globodera* spp., and *Punctodera* sp., layer A, together with B and at least a portion of C_1, is infused with a material which strains electron densely. The outer layers were described as less dense in young adult females of *Globodera* and *Punctodera* than in more mature specimens, revealing typical zones of A (11). However, in *S. hydrophila, Globodera* spp., homogeneously dense, regardless of age.

Layer B is present in all heteroderids examined, although in *M. charis* characteristic striae were rarely resolved. On the other hand, patches of B are particularly large and well defined in *A. lonicerae.*

The C layer was described by Shepherd et al. (11) as consisting of randomly oriented fibers. These are particularly coarse and occur in an electron-dense matrix in

Figs. 12–18. TEM of transverse sections of female cuticle of *Atalodera lonicerae.* 12) Cuticle of mature female composed of layers A, B, C, D. Layer A consists of A_1, A_2, A_3. 13) Cuticle of young female composed of A, B, C, and faint indication of D. Layer A consists of A_1, A_2, A_3. Cu = portion of cast-off fourth stage cuticle. F = fringed material. 14) Fringed material (F) extending from A_1. 15) Layer A including A_1, A_2, A_3. Fine electron-dense channels (Ch) penetrate electron-lucent walls of chambers in A_3. Patch of B partially extends into A_3. 16) Portion of C showing fibrous texture and lengths of electron-dense material. 17) Layer D showing helicoidal pattern of fibers. 18) Region of posterior prominence adjacent to attachment of vaginal musculature (M). Cuticle includes A, B, C. Layer A consists of A_1, A_2, A_3; layer C includes C_1, C_2, C_3. Radial channels (R) extend through C.


12
A1
A2
A3
B
C
D
1.0μm
13
F
Cu
F
A1
A2
A3
B
C
D
1.0μm
14
F
A1
1.0μm
17
0.5μm
15
A1
A2
Ch
A3
B
1.0μm
16
0.25μm
18
A1
A2
A3
B
C1
R
C2
M
1.0μm

C_2, whereas fibers are finer textured in C_3. Frequently channels of dense material radiate perpendicular to the surface through C. The C of *M. charis* and *A. lonicerae* is of relatively fine texture throughout, with individual fibers less apparent than in other species. Zones and channels became conspicuous only in older specimens, and in *A. lonicerae* C is most developed in the terminal prominence. Similarly, Shepherd et al. (11) noted that zones are best delimited in older specimens and that layering in the "neck" may vary from that in the "thorax" (12). In *S. hydrophila,* C is composed of coarse random fibers. Curiously, C_3 is well defined despite the presence of D. In *Globodera,* characterized by the presence of D, C_3 is apparently absent. The outer portion of C_1 of *S. hydrophila* is infused with dense material further subdividing this zone into C_{1a} and C_{1b}. In *Globodera* spp. and *Punctodera* sp. similar dense material apparently is uniformly dispersed throughout C_1 (11).

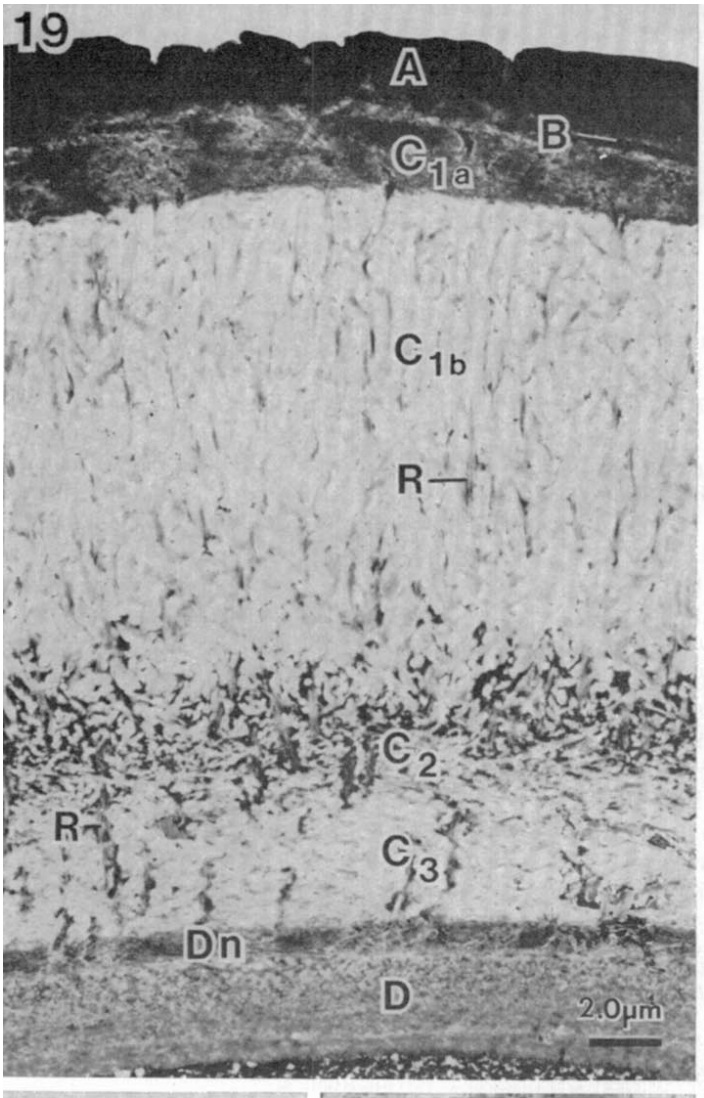
The D layer consists of 20–30-nm-d fibers (11), characteristically oriented in a helicoidal pattern that is repeated to form lamellae (11). Shepherd et al. (11) noted that clarity of the pattern varies among individuals and perhaps is correlated with the "phase of crystallization" of the component collagen-like protein. In addition, the fibers tend to be randomly oriented in the neck region (12). The D is absent in *M. charis* and *Heterodera* spp., but occurs in *A. lonicerae, S. hydrophila, Globodera* spp., and *Punctodera* sp. (11). The helicoidal pattern was most consistently observed in *A. lonicerae,* although it varied among specimens in thickness (being nearly absent in young adult individuals and in some portions of the posterior prominence of older specimens) and in number of lamellae. Generally, D is much narrower in *A. lonicerae* than in *Globodera* spp., and in this respect *Atalodera* resembles *Punctodera* (11).

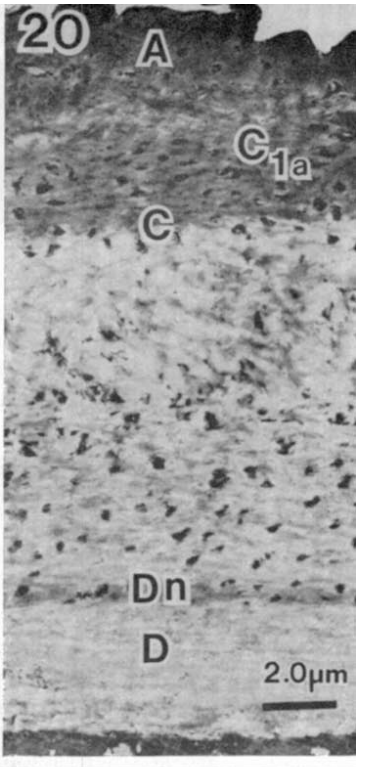
The D of *S. hydrophila* includes a matrix of electron-dense particles, so that individual fibers are particularly difficult to discern. Nevertheless, a repeating helicoidal pattern can be inferred from periodic regions of longitudinally oriented fibers. These regions occur regardless of the orientation of sections. The D is separated from C by a dense zone similar to that described in *Globodera* spp. (11), but the zone was not observed in *A. lonicerae.*

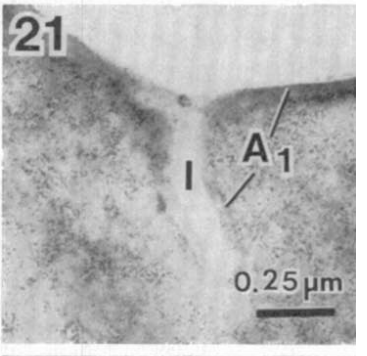
Shepherd et al. (11) suggests that electron-dense deposits in surface layers of *Globodera* spp. and *Punctodera* sp., as well as the presence of D, (both absent from *Heterodera* spp.) may be adaptations to survive desiccation. This explanation is plausible, since *S. hydrophila* and *A. lonicerae* are apparently well adapted to semiarid regions and their distribution may be restricted to such environments. Specimens of *S. hydrophila* and *A. lonicerae* are collected live in all but the driest months of the year. Conversely, *M. charis,* which lacks similar adaptations in the cuticle, can be collected in this region only during the rainy season of February and March. Other species of *Meloidodera* are widely distributed, and some (e.g., *M. floridensis* Chitwood et al., 1956) occur in areas of relatively consistent moisture.

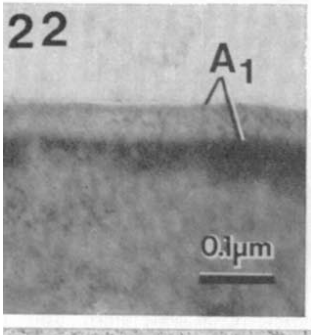
In addition to layers of the female cuticle per se, layers or substances occur external to A_1 in some heteroderids. The fringed material, associated with *A. lonicerae* as observed with TEM, is probably the subcrystalline layer, which Wouts (16) noted to be distinct and brittle in this species. Brown et al. (3) presented evidence that in *Heterodera* spp. the layer is a fatty acid which may be produced from a sugary waste secreted through the nematode cuticle; the transformation may occur through

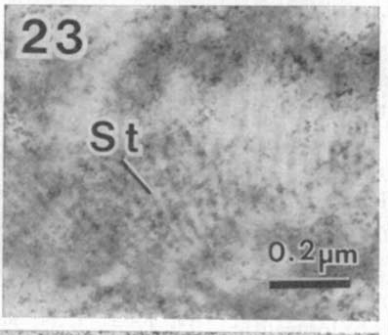
Figs. 19–24. TEM of female cuticle of *Sarisodera hydrophila.* 19) Transverse section of cuticle of mature female, composed of layers A, B, C, D. Layer C includes C_{1a}, C_{1b}, C_2, C_3. Layer D is separated from C by a dense band (Dn). R = radial channels. 20) Transverse section of cuticle of young female composed of A, C (including C_{1a}), and D. Layer D is separated from C by a dense band (Dn). 21) Transverse section of surface of cuticle with invagination (I) lined by A_1. 22) Transverse section of A_1. 23) Transverse section of patch of B showing periodic striae (St). 24) Longitudinal section of D showing dense matrix and faint indication of helicoidal pattern of fibers. 25) Transverse section showing invaginated plasmalemma (P) and vesicles (V) at junction of hypodermis (H) with cuticle (Cu).

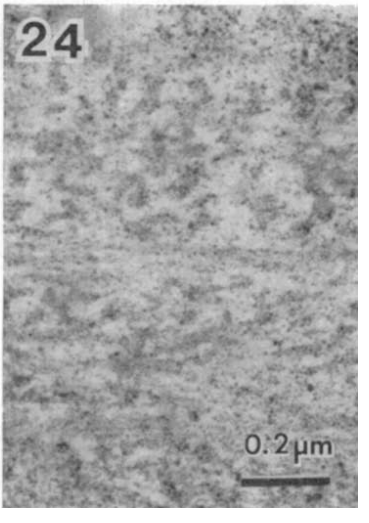

19
A
B
C1a
C1b
R
C2
R
C3
Dn
D
2.0μm

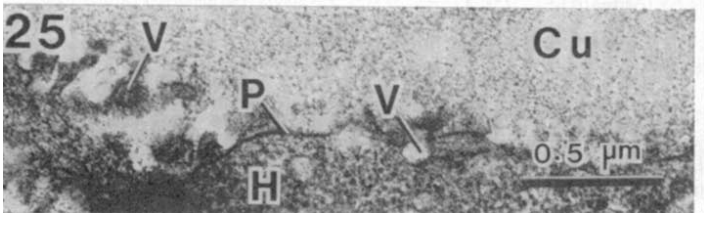

20
A
C1a
C
Dn
D
2.0μm


21
I
A1
0.25μm


22
A1
0.1μm


23
St
0.2μm


24
0.2μm


25
V
Cu
P
V
H
0.5 μm

activity of a fungal associate. A subcrystalline layer is also associated with *M. charis* and *H. schachtii* (9,10,16), but in the present study only a very thin fringe was observed on the surface. Apparently the subcrystalline layer of these species was lost in processing; the fringe may be a remnant of such a layer, or it may be a material secreted through the cuticle. No fringe was observed on *S. hydrophila,* which lacks a subcrystalline layer. However, in *S. hydrophila* as well as other species examined, remnants of cuticle, persistent from the fourth stage, frequently occurred on or near the surface of the female.

Many Heteroderidae, including *Meloidodera* and *Atalodera,* do not form cysts, whereas others, including *Heterodera, Globodera, Punctodera,* and reportedly *Sarisodera,* are characterized by cysts. In the present study there is no morphological evidence, such as the presence or absence of a specific layer of cuticle, which is correlated with the capacity to form cysts. Shepherd et al. (11) noted that tanning is not limited to one zone, but rather the entire cuticle becomes infused with polyphenols. Cyst formation is apparently a function of phenol produced in the hypodermis and occurs in species which are otherwise quite variable in cuticular structure. For example, *Dolichodera fluvialis* Mulvey and Ebsary, 1980 has a thin (3–4 μm) striated cuticle as in *Meloidodera* and *Cryphodera* Colbran, 1966; yet it becomes tanned and forms a cyst (7). On the other hand, *S. hydrophila,* which has a very thick and complexly layered cuticle, is reported to tan (17), but the observations of Baldwin and Bell (unpublished) suggest that a typical cyst does not form. Our examination of numerous soil samples from the type locality, using standard cyst collection techniques, have never resulted in recovery of a single cyst.

Comparative morphology of the cuticle of females may contribute to phylogenetic inference for Heteroderidae. The cuticle of *Meloidodera* may be interpreted as basic, or relatively primitive. It has a narrow C layer, D is absent, its surface is striated, and it does not form a cyst; in these respects it may resemble vermiform adults of outgroups as well as heteroderid juveniles. *Meloidodera* is distinct from other heteroderids by having a broad spectrum of primitive character states (4). The cuticle of very young females of *A. lonicerae* and *H. schachtii,* as well as *Heterodera* spp. and *Globodera* spp. (11), most nearly resembles *Meloidodera,* although mature individuals acquire additional specific distinctive traits. Perhaps the most significant distinction is the presence of D in *Atalodera* and *Sarisodera,* an apparently derived character state which is shared with *Globodera* and *Punctodera,* but not with *Heterodera.* The occurrence of D among heteroderids suggests at least two hypotheses: 1) D is secondarily lost in *Heterodera,* 2) *Atalodera, Sarisodera* and *Globodera* share a common ancestor which is not shared by *Heterodera;* therefore, the cyst has arisen independently in *Heterodera* and *Globodera.* Furthemore, the occurrence of electron-dense deposits in *Sarisodera, Globodera,* and *Punctodera* may indicate a common ancestor among these groups. These hypotheses can be tested through identification of primitive and derived states of additional characters to be considered together to determine the most parsimonious pattern of phylogeny of Heteroderidae. Elucidation of the detailed morphology of the cuticle of additional heteroderids including *Verutus* Esser, 1981, *Cryphodera Thecavermiculatus* Robbins, 1978, *Hylonema* Luc et al., 1978, and *Dolichodera* will be useful toward further refinement of a hypothesis of phylogeny for Heteroderidae.

LITERATURE CITED

1. Baldwin, J. G., and H. Hirschmann. 1975. Body wall fine structure of the anterior region of Meloidogyne incognita and Heterodera glycines males. J. Nematol. 7:175-193.

2. Bird, A. F. 1980. The nematode cuticle and its surface. Pages 213-235 *in* B. M. Zuckerman, ed. Nematodes as biological models. Aging and other model systems. Vol. 2, New York: Academic Press.

3. Brown, G., R. K. Callow, C. D. Green, F. G. W. Jones, J. H. Rayner, A. M. Shepherd, and T. D. Williams. 1971. The structure, composition and origin of the subcrystalline layer in some species of the genus Heterodera. Nematologica 17:591-599.

4. Ferris, V. R. 1979. Cladistic approaches in the study of soil and plant parasitic nematodes. Amer. Zool. 19:1195-1215.

5. Johnson, P. W. 1981. Observations on the cuticle ultrastructure of Meloidogyne hapla males. J. Nematol. 13:231-233.

6. Lewis, S. A., and T. F. Huff. 1976. Cuticle anatomy of Hoplolaimus columbus. J. Nematol. 8:293 (Abstr.).

7. Mulvey, R. H., and B. A. Ebsary. 1980. Dolichodera fluvialis n. gen., n. sp. (Nematoda: Heteroderidae) from Quebec, Canada. Can. J. Zool. 58:1697-1702.

8. Mundo-Ocampo, M., and J. G. Baldwin. 1983. Host response to Sarisodera hydrophila Wouts and Sher, 1971. J. Nematol. 15:259-268.

9. Schmidt, A. 1871. Ueber den Rüben-Nematoden (Heterodera schachtii A.S.). Z. Ver Rüben-Zucker Ind. in Zollverein 21:1-19.

10. Schmidt, A. 1872. Sweiter Bericht über die Rüben-Nematoden, Z. Ver. RübenZucker Ind. in Zollverein 22:67-75.

11. Shepherd, A. M., S. A. Clark, and P. J. Dart. 1972. Cuticle structure in the genus Heterodera. Nematologica 18:1-17.

12. Shepherd, A. M., and S. A. Clark. 1978. Cuticle structure and 'cement' formation at the anterior end of female cyst-nematodes of the genera Heterodera and Globodera (Heteroderidae: Tylenchida). Nematologica 24:201-208.

13. Venable, J. H., and R. E. Coggeshall. 1965. A simplified lead citrate stain for electron microscopy. J. Cell Biol. 25:407-408.

14. Wen, G. Y., and T. A. Chen. 1972. Fine structures of the cuticle of Hoplolaimus galeatus. J. Nematol. 4:236-237. (Abstr.).

15. Wisse, E., and W. T. Daems. 1968. Electron microscope observations on second-stage larvae of the potato root eelworm Heterodera rostochiensis. J. Ultrastruct. Res. 24:210-231.

16. Wouts, W. M. 1973. A revision of the family Heteroderidae (Nematoda: Tylenchoidea) III. The subfamily Ataloderinae. Nematologica 19:279-284.

17. Wouts, W. M., and S. A. Sher. 1971. The genera of the subfamily Heteroderinae (Nematoda: Tylenchoidea) with a description of two new genera. J. Nematol. 3:129-144.