Fine structure of the posterior cone of females of *Cactodera cacti* Filip'ev & Schuurmans Stekhoven (Nemata : Heteroderinae)

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SUMMARY

Detailed development of the posterior cone of females of *Cactodera cacti* gives insight into phylogenetic characters which differ from previously described *Heterodera schachtii*. Cone growth continues after the final molt in *H. schachtii*, but not in *C. cacti*. Although a gelatinous matrix is produced in both species, *H. schachtii* deposits eggs whereas *C. cacti* does not. Contrary to *H. schachtii*, the body wall cuticle of the cone in *C. cacti* includes a D layer but lacks and E layer and bullae. The fenestral region of *C. cacti* lacks the cuticular fibers present in the same region of *H. schachtii*. Unlike *H. schachtii*, the cone of *C. cacti* has a short vagina with no underbridge, and the vaginal musculature is greatly reduced. These vestigial vaginal muscles are closely associated with the cyst wall as denticles.

RÉSUMÉ

Structure du cône postérieur des femelles de Cactodera cacti *Filip'ev & Schuurmans Stekhoven (Nemata : Heteroderinae)*

L'étude détaillée du développement du cône postérieur des femelles de *Cactodera cacti* apporte des éléments sur des caractères phylogéniques différant de ceux décrits précédemment chez *Heterodera schachtii*. La croissance du cône se poursuit après la dernière mue chez *H. schachtii*, mais non chez *C. cacti*. Encore qu'une gelée soit produite par les deux espèces, *H. schachtii* pond des œufs à l'extérieur, ce qui n'est pas le cas de *C. cacti*. A l'inverse de *H. schachtii*, la cuticule du cône de *C. cacti* possède une couche D, mais il n'y existe ni couche E, ni bullae. La région des fenêtres de *C. cacti* est dépourvue des fibres cuticulaires que l'on observe dans la région homologue de *H. schachtii*. A l'inverse de *H. schachtii*, *C. cacti* présente un vagin court, dépourvu de sous-pont, et la musculature vaginale est très réduite. Ces muscles vaginaux vestigiaux sont étroitement associés à la paroi du kyste, en tant que denticules.

Cactodera cacti (Filip'ev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978, first described from ornemental cacti in Holland, is distributed worldwide and considered indigenous to Mexico (Adam, 1932; Cooper, 1955*a;* Minz, 1956; Southey, 1957; Kumar, 1964; Golden & Raski, 1977; Scognamiglio, Caputi & Ciancio, 1985; Ebsary, 1986). *Cactodera* damages certain cacti grown as food crops in Mexico (Mundo-Ocampo, unpubl.) as well as ornamental cacti (Langdon & Esser, 1969; O'Bannon & Esser, 1970; Hamlen, 1975; Mitchell, 1985). Since its host range also includes families other than Cactaceae, *C. cacti* may damage a variety of other crops (Scognamiglio, Caputi & Ciancio, 1985).

Despite the parasitic potential of *C. cacti*, little attention has been given to its biology. The life cycle of *C. cacti* has not been documented, and the relative abundance of males is unclear. Adam (1932) regarded the six males of the original description as inadequate for obtaining reliable measurements. Males continued to be reported as rare in *C. cacti*, consequently, measurements of Adam (1932) have been repeated, apparently without considering additional specimens (Filip'ev & Schuurmans Stekhoven, 1941; Franklin, 1951; Mulvey & Golden, 1983). There is concern that putative *Cactodera* males have been misidentified because mixed populations of *Cactodera* and *Punctodera* Mulvey & Stone, 1976, or *Meloidogyne* (Goeldi, 1887) Chitwood, 1949, are frequently found in the field (Spears, 1956; Southey, 1957; Schneider, 1961). Cooper (1955*a*), noting cyst variability, considered his collections of putative *C. cacti* to be mixed, and therefore, excluded the species from the key to British *Heterodera* Schmidt, 1871.

The genus *Cactodera* was erected by Krall and Krall (1978) to accommodate cyst-forming, circumfenestrate, lemon-shaped Heteroderinae with *C. cacti* as the type species. Diagnosis of *Cactodera* is based primarily on vulval-cone related structures; an emendation adds presence of vulval denticles, but excludes presence of

bullae and underbridge (Krall & Krall, 1978; Mulvey & Golden, 1983). More recently emendations include presence of a D layer in the female cuticle (Luc, Maggenti & Fortuner, 1988). Many proposed diagnostic characters of *Cactodera* also occur either in *Heterodera* (e.g. vulval cone) or *Globodera* Skarbilovich, 1959 (Behrens, 1975) (e.g. vulval denticles, circumfenestration, D layer, absence of bullae and underbridge). Thorough understanding of traditional and new characters is required, so that the phylogenetic relationships of *Cactodera* to other cyst nematodes can be established as a basis for an increasingly meaningful classification (Baldwin & Schouest, 1990).

Recently, Cordero and Baldwin (1990, 1991) described detailed developmental morphology of the cone of *Heterodera schachtii* Schmidt, 1871, and elucidated characters useful in taxonomy and phylogeny of Heteroderinae. Findings in *H. schachtii* will be of greater phylogenetic significance when they can be interpreted in the context of knowledge of the cone and posterior terminus in additional Heteroderinae. Presently, we report on the detailed structure of the cone of *C. cacti*. Unlike *H. schachtii*, the cyst of *C. cacti* has a reduced cone with one circumfenestra, all eggs are retained and the female has a pronounced D layer (Cliff & Baldwin, 1985). Development of the cone of *C. cacti* is investigated for comparison with *H. schachtii* using a combination of light (LM), scanning (SEM) and transmission (TEM) electron microscopy.

Materials and methods

Cactodera cacti was established on *Schlumbergera* sp. Lem, 1858 (Christmas cactus) by *in vitro* culture utilizing sterile techniques. Christmas cactus cladodes (leaflike modified stems) were washed briefly in commercial detergent and disinfested in 10 % commercial bleach for 4 min. After rinsing in sterile water, cladodes were transferred to Petri dishes containing Gamborg's B-5 medium, pH 6.5 plus White's organics (Gamborg *et al.*, 1976; White, 1943). Gelrite® (0.25 %) was used as a gelling agent.

Rooting of Christmas cactus cladodes was induced by incubating cultures at 25 °C under 16 h illumination per day (3 000 lux, GroLux® light) for a month. Subcultures of axenically produced cladodes produced roots 10 days after incubation.

Schlumbergera cladodes were inoculated with 25 second stage juveniles (J2) or one monoxenically produced cyst. Second stage juveniles were disinfested by four rinses with sterile water followed by incubation for 10 min with saturated aqueous Rifampicin® solution. A final rinse with sterile water was given to J2s prior to inoculation. Incubation of cultures proceeded as described above.

Cultures with monoxenically produced cysts were stored at 4 °C for a period of 2 months to 3 years. To induce hatching, cysts were incubated for 10 days at 28 °C in a sterile BPI dish (containing four to five drops of sterile water) in a sterile Petri dish sealed with Parafilm®. Second stage juveniles and cysts were added close to roots, using a Pasteur pipette.

Second stage juvenile infection was monitored using a Nikon inverted microscope equipped with Hoffman interference optics and a Garr time-lapse high resolution video recorder as previously described (Cordero & Baldwin, 1990). Development of a specimen was recorded and studied or stopped at particular points and the specimen was excised for detailed examination with LM, SEM, or TEM.

Transmission electron microscopy and LM were used to study internal cone structures at the following points of development : immediately after the fourth molt, two and four weeks after the final molt, and in the young cyst. The same stages were examined with SEM to follow surface pattern development of the posterior end.

Specimen preparation for LM and TEM was as previously reported (Cordero & Baldwin, 1990). Excised cones were fixed for 8 h in modified Karnovsky's (1965) fixative, 8 h in glutaraldehyde-hydrogen peroxide solution (Byard, Sigurdson & Woods, 1986), and 3 h in osmium tetroxide fumes. Dehydration was done in a graduated ethanol series and infiltration in Spurr's epoxy. Thin (silver grey) sections for TEM and semithick (0.28 μm) sections for LM were stained as reported by Baldwin (1983). Specimens were prepared for SEM by glycerin infiltration (Sher & Bell, 1975).

To visualize actin, and thus the presence of muscles in the cone, females were treated with fluorescence phalloidin (FITC labeled) following the protocol outlined by Wulf *et al.* (1979). Cones labeled with FITC were prepared as described by Cooper (1955b) and observed with a Zeiss photomicroscope III equipped with epifluorescence optics.

Results

Monoxenic culture of *C. cacti* is simple, allowing ample reproduction, and permitting continuous observation of the life history. Postembryogenesis of *C. cacti* from J2 to adult female is completed 12 days after establishment of a feeding site and resembles that of *H. schachtii* (Raski, 1950), except that no males were observed in *C. cacti*.

The final molt begins three to four days after shedding the third stage cuticle. The newly molted adult has a shallow broad cone (Figs 1 A, 2 A). The vagina moves sinuously in conjunction with contraction of the entire cone. A clear gelatinous matrix is secreted through the vulva. Body volume of the nematode increases rapidly after molting, particularly at the midbody as eggs develop. Thus, the previously flask-shaped body becomes
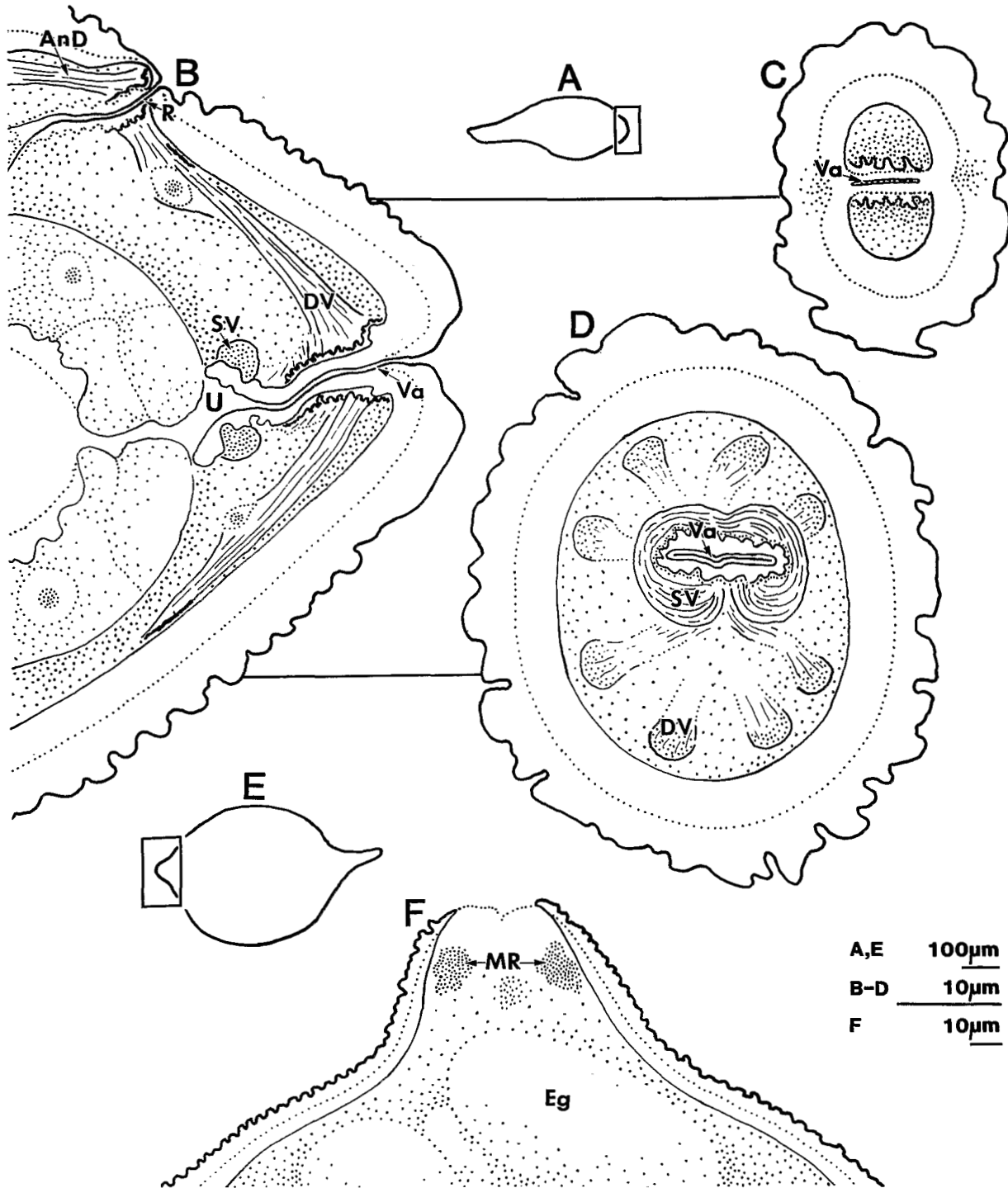
Fig. 1. Schematic representation of cone of newly emerged female and cyst of *Cactodera cacti*. A : Outline of newly molted female. Box area corresponds to area depicted in B; B : Longitudinal section through vagina of newly emerged cone in lateral orientation; C : Cross section near cone terminus of young female; D : Cross section at level of sphincter vaginae (SV) muscles of young female; E : Outline of cyst. Box area corresponds to area depicted in F; F : Longitudinal section through posterior terminus of cyst in dorsoventral orientation. (AnD = anal depressor muscle, DV = *dilatores vaginae* muscles, Eg = egg, MR = muscle remnant, R = rectum, SV = *sphincter vaginae* muscle, U = uterus, Va = vagina).
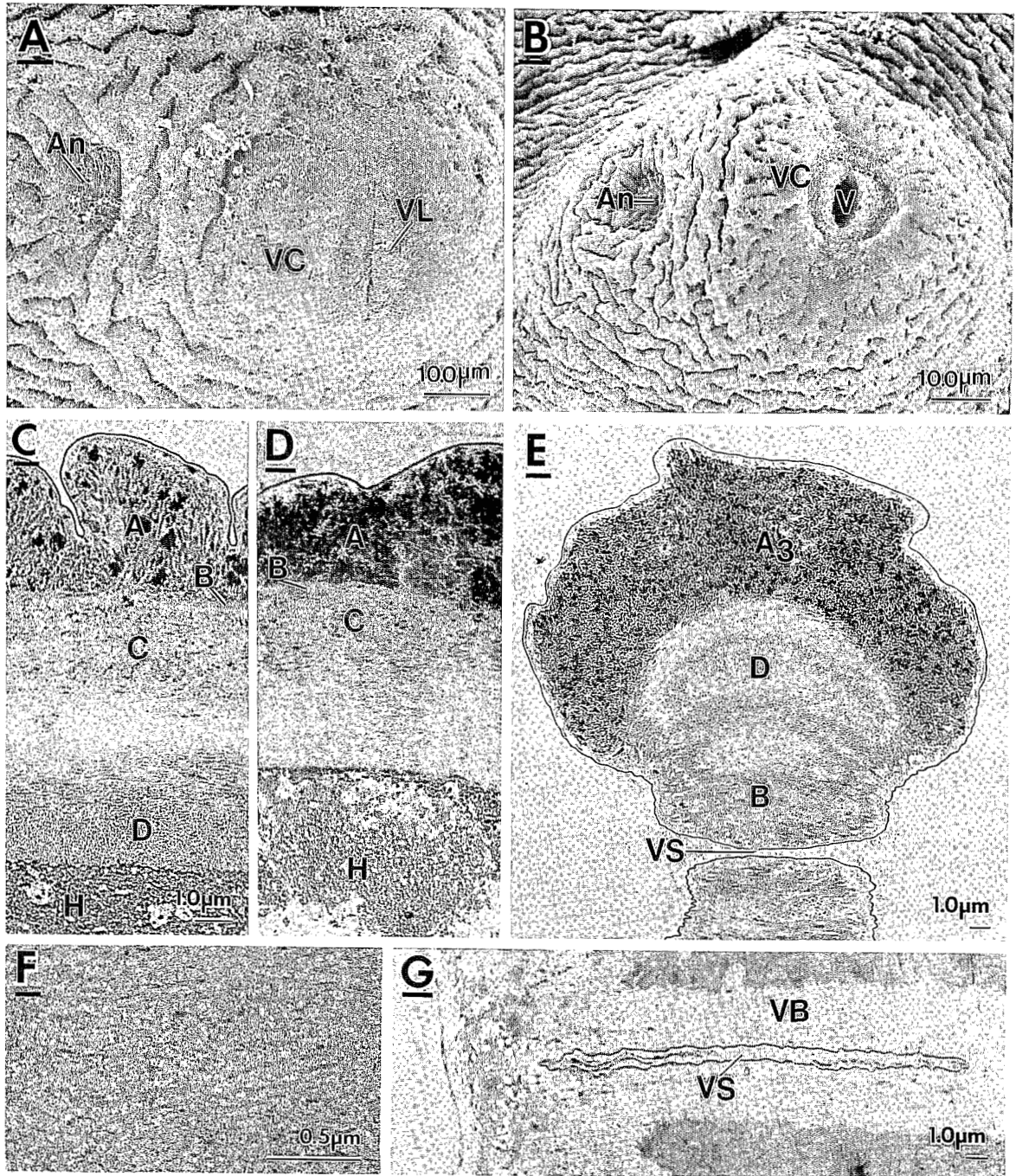
Fig. 2. *Cactodera cacti.* A : SEM of surface pattern of vulval cone of young female; An = anus, VC = vulval crescent, VL = vulval lip; B : SEM of surface pattern of vulval cone of cyst (An = anus, V = vulva, VC = vulval crescent); C : TEM of cross section of body wall cuticle near base (anterior end) of cone of young female (letters A, B, C, D indicate corresponding cuticular layers, H = hypodermis); D : TEM of cross section of body wall cuticle near base of cone of young female, labels as in C; E : TEM of cross section of body wall cuticle near cone terminus of mature female showing alternating bands of layers D and modified bands of B (A_3 = zone of A layer, VS = vulval slit); F : TEM of cross section of body wall cuticle showing enlargement of layer D; G : TEM of cross section near cone terminus showing vulval bridge (VB) and vulval slit (VS).
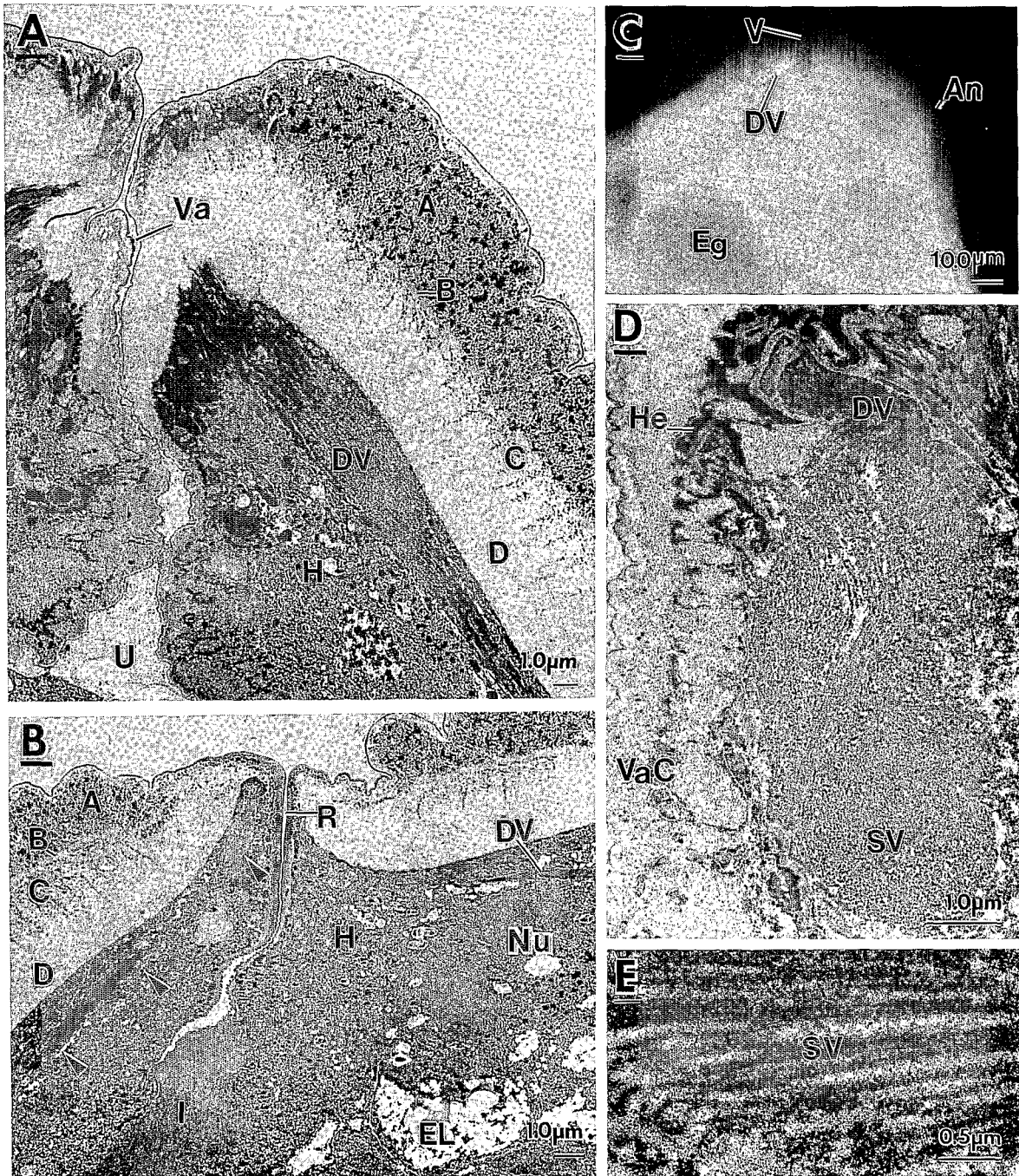
Fig. 3. *Cactodera cacti.* A : TEM of longitudinal section through vagina (Va) of newly emerged cone in lateral view (letters A, B, C, D indicate corresponding cuticular layers, DV = *dilatores vaginae* muscles, H = hypodermis, U = uterus); B : TEM of longitudinal section through rectum (R) of newly emerged cone in lateral view (arrowhead = anal depressor muscle, letters A, B, C, D indicate corresponding cuticular layers, DV = *dilatores vaginae* muscles, EL = electron lucent matrix, H = hypodermis, I = intestine, Nu = hypodermal nucleus); C : LM of female cone incubated in FITC labeled phalloidin; lateral orientation showing positive fluorescence as an indicator of actin (An = anus, DV = *dilatores vaginae* muscles, Eg = egg, V = vulva; D : TEM of longitudinal section of vaginal lining and musculature in lateral view, DV = *dilatores vaginae* muscles, He = hemidesmosomes, SV = *sphincter vaginae* muscles, VaC = vaginal cuticle); E : TEM of longitudinal section through *sphincter vaginae* muscles (SV) showing filaments.
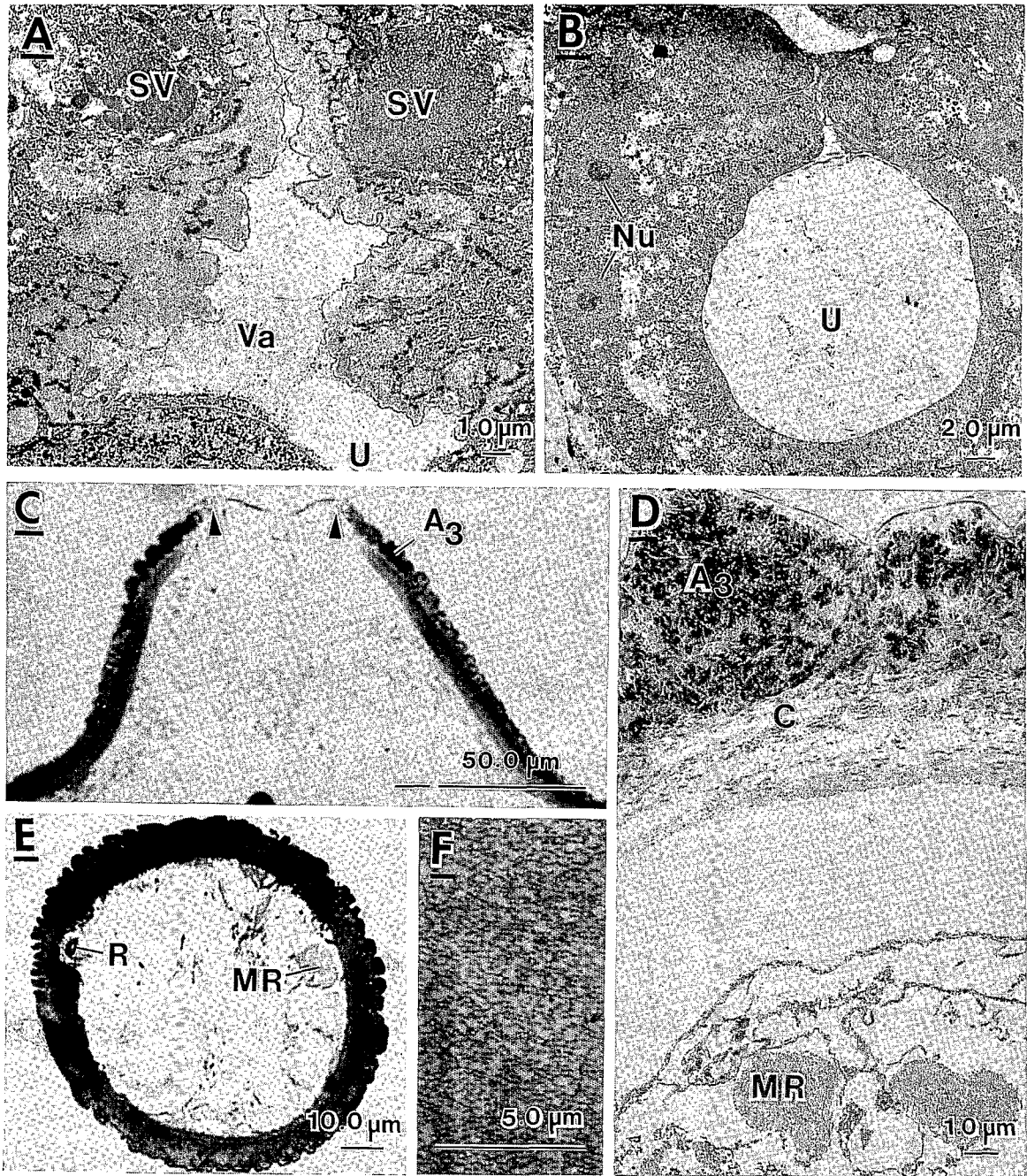
Fig. 4. *Cactodera cacti.* A : TEM of longitudinal section in lateral view through terminus at junction of vagina (Va) with uterus (U) in young female (SV = *sphincter vaginae* muscles); B : TEM of longitudinal section of female and cross section of uterus (U), lining of uterus composed of large cells with distinct nuclei (Nu); C : LM of longitudinal section of cyst cone terminus in lateral view (arrowheads correspond to modified cuticle in region of fenestrae) (A₃ = corresponding zone of cuticle); D : TEM of cross section at base of cyst cone (A₃ and C correspond to layers and zone of cuticle, MR = muscle remnant); E : LM of cross section at base of cyst cone (MR = muscle remnant, R = rectum); F : TEM of muscle remnant in cyst showing persistent filaments.

lemon-shaped including a distinct small posterior cone (Figs 1 E, 2 B). After the final molt, there is little additional growth of the cone. The mature female is filled with eggs which are retained within the body. All eggs within cysts contain juveniles and the surface of the eggs has punctations. Young eggs within females that contain developing embryos lack punctations.

Four weeks after the final molt, growth at the middle of the body continues and the frequency of vaginal movement decreases, stopping about 9 weeks after the final molt. At this time the vulval area of the cone terminus collapses (Fig. 2 B). The resulting depression excludes the vulval " domed " crescents *sensu* Green (1975). The surface of the posterior terminus of the young cone, including the crescents, is relatively smooth (Fig. 2 A). As the female matures, small ridges on the crescents surround the sunken vulval lips; these ridges are retained in the cyst (Fig. 2 B). In aging females, the region surrounding the anus becomes progressively sunken, and, in the cysts, the anus is encircled by fine ridges (Fig. 2 B).

The body wall (BW) cuticle of the cone of females of *C. cacti* generally resembles that of the midbody with typical A, B, C and D layers and zones (Figs 2 C, D) *sensu* Cliff and Baldwin (1985). However, in the area corresponding to the circumfenestra, the BW cuticle is modified with zone A3 and layer D predominating; layer C is progressively reduced in thickness posteriorly and is absent at the terminus (Figs 2 E, 3 A). Zone A 3 includes large irregular electron dense inclusions and layer D is crossed by laterally oriented bridges of layer B. However, in the cyst, the BW cuticle of the terminus is further reduced. Layer D diminishes in the region from the outer boundary of the crescents to the cone terminus so that eventually an electron dense zone, A_3, predominates (Figs 2 D, 4 C, D).

The vagina is about 20 μm long. Near the vulva, the vagina is dorsoventrally flattened, and connected to the BW cuticle at its lateral ends (Figs 1 C, 2 G) forming a vulval bridge which persists until circumfenestration. At the cone terminus, the lumen of the vagina is narrow (Fig. 2 G), but closer to the uterus it doubles in width forming a broad chamber (Figs 1 B, 3 A, 4 A). The vagina is lined by cuticle layers A and D (Fig. 3 A), but zone A_3 is not resolved and layer D is modified near the uterus with disoriented fibers and irregular boundaries. The vaginal lining is thinnest in the middle and thicker at both ends, becoming particularly broad and diffuse near the uterus (Figs 3 A, 4 A). Near the uterus, the vagina branches dorsolaterally (Figs 3 A, 4 A). In young females, the uterus epithelium lacks muscular fibers and is composed of large cells with electron dense cytoplasm and large nuclei (Figs 1 B, 4 B).

Although somatic musculature is lacking in the cone, a diminutive musculature is associated with the vagina of *C. cacti* (Figs 1 B, D; 3; 4 D-F). A prominent set of *dilatores vaginae* (DV) muscles radiate obliquely, just

beneath the BW cuticle, from the vagina near the cone terminus anteriorly to the wall near the base of the cone (Figs 1 B, 3 A). A heart-shaped *sphincter vaginae* (SV) muscle encircles the vagina approximately 16 μm anterior to the vulva (Figs 1 B, D; 3 D; 4 A). Vaginal musculature is characterized by fine filaments and presence of actin was confirmed by staining with fluorescent phalloidin (Figs 3 C, E). Muscles attach across the hypodermis to the cuticle of the vagina and BW by distinct junctional complexes and hemidesmosomes (Fig. 3 D). In the cone of the cyst, muscle remnants with distinct fibers persist (Figs 1 F, 4 D-F).

In addition to the vaginal muscles, a set of anal depressor muscles attaches to the rectum and runs obliquely beneath the BW cuticle anteriorly to the cone base (Fig. 3 B). The BW cuticle is thin at the shallow depression surrounding the anus; layer C and zone A_3 are particularly reduced and D is predominant. The cuticle lining of the rectum is thin and continuous with layer A of the BW cuticle.

Discussion

Monoxenic culture of *Cactodera* has not been previously reported, and the method discussed herein is simpler than published approaches for other Heteroderinae (Widdowson *et al.*, 1958; Brown, 1974; Reversat, 1975; Lauritis, Rebois & Graney, 1982, 1983; Cordero & Baldwin, 1990, 1991). Monoxenic culture, combined with video recording, permits identifying particular points in development of the cone of *C. cacti* for more detailed SEM and TEM comparison with other Heteroderinae, and specifically with *H. schachtii* (Cordero & Baldwin, 1990, 1991).

Video recording revealed a gelatinous matrix previously reported to be absent in *C. cacti* by Southey (1954, 1957), and present but rudimentary by Cooper (1955a). Contrary to the gelatinous matrix present in *Heterodera* spp., that of *Cactodera* is clear, less copious, lacks eggs, and thus, is easily overlooked in field specimens. As in *H. schachtii*, cells of the uterus are dense with rough endoplasmic reticulum, and these cells are likely to be the source of the gelatinous matrix (Mackintosh, 1960).

Males have been reported as rare in *C. cacti* (Adam, 1932; Southey, 1954; Shmal'ko, 1959; Golden & Raski, 1977), and some other *Cactodera* spp. (Golden & Raski, 1977). The present study demonstrated a high rate of reproduction in the absence of males, further suggesting that *C. cacti* is parthenogenetic. It is not known if males develop in *C. cacti* under specific conditions. For example, temperature and/or CO_2 concentrations influence development of males in monoxenic cultures of *Aphelenchus avenae* Bastian, 1865 (Hansen, Buecher & Yarwood, 1972, 1973). Nevertheless, variation in mode or reproduction among Heteroderinae, and even within

Cactodera, suggests that the presence or absence of males may be a useful character for phylogenetic analysis (Triantaphyllou, 1970, 1983; Triantaphyllou & Hirschmann, 1980).

Interspecific differences and taxonomic value of surface patterns at the posterior terminus of Heteroderinae have been documented (Mulvey, 1972, 1973, 1974; Green, 1975; Othman, 1985). However, Othman (1985), and Othman, Baldwin and Mundo-Ocampo (1988) suggested that some intraspecific morphological variation may be attributed to ontogeny, and that these changes must be identified for reliable phylogenetic interpretation. Surface ornamentations of the vulval cone of *C. cacti* vary with age. In young females, vulval lips protrude and the surface of the lips and vulval crescents is smooth. However, as the female ages and the cyst forms, the vulva becomes sunken, the vulval lips become indistinct, and the vulval crescents become patterned with short ridges. Green (1975) reported domed vulval crescents which were finely or coarsely tuberculated in *C. cacti*, but he did not report differences between young females and cysts.

Homology of crescents in semifenestrate and circumfenestrate Heteroderinae is not clearly established. Green (1975) considered the two semifenestral regions of *Heterodera* as " crescents " whereas Mulvey (1973) and Green (1975) used " crescents " to describe the tuberculate areas on either side of the circumfenestra of *Globodera*. However, the region ruptured with fenestrae in *C. cacti* and *Globodera* does not include the crescents *sensu* Green (1975). Conversely, in *H. schachtii*, nearly the entire region may be ruptured. In other *Heterodera* species, the outer portion of the " crescent " persists after fenestration. Further comparison is needed to establish homology of these structures, to identify a possible transformation series in patterns of fenestration, and to verify that terminology is applied consistently.

The sunken vulva with inconspicuous vulval lips in cysts of *Cactodera* is also typical of *Globodera* and *Punctodera*, in which similar vulval circumfenestrae develop. Although the three genera share several unique characters, *Cactodera* shares other characters, such as the cone, with *Heterodera*. *Cactodera* is interpreted as phylogenetically intermediate between the round and lemon-shaped cyst nematodes (Baldwin & Schouest, 1990).

The anal region in *C. cacti* is similar to the vulval region in that it also varies with age. In young females, the anus opens into a shallow cavity, but in cysts the cavity becomes a deep depression. Green (1975) noted that the size and shape of the anal area differed within and between species of *Heterodera*, but species of *Cactodera* were not considered. However, Golden and Raski (1977) reported that the anal region of *C. cacti* lacks a specific pattern.

Differences between the anal region of *Cactodera* and *Heterodera* are suggested by our investigations (Cordero

& Baldwin, 1990, 1991). The BW. cuticle of the anal region in *C. cacti*, in contrast to *H. schachtii*, is very thin with only a narrow portion of layer A persisting in the cyst. We suggest that a transformation series exists in which the thin perineal region of *Cactodera* is intermediate between the thick perineal cuticle of *Heterodera* and the perineal fenestrae of *Punctodera*.

In both the vulval and anal region of *C. cacti*, layers of the BW cuticle are lost as the female ages and the cyst develops. This loss contrasts sharply to *H. schachtii* in which additional layers are laid down in the aging female. A D layer occurs in the BW cuticle of both *C. cacti* and *H. schachtii* (Cordero & Baldwin, 1990). However, in the former, the D layer occurs at both the midbody and cone in young females (Cliff & Baldwin, 1985), whereas in the latter, the D layer is relatively thin. It occurs only in aging females and it does not occur in the cone. In *H. schachtii* there is a distinct E layer and associated bullae in aging female and cysts, but in *C. cacti* there is no E layer or bullae. It is important to investigate additional Heteroderinae to determine to what extent the persistence or loss of BW layers is associated with circumfenestrae or semifenestrae. Such information could help to determine homologies of cone characters throughout Heteroderinae for more reliable phylogenetic analysis.

In females, zone A_3 is widest at the cone terminus in the region which corresponds to the vulval crescents. In addition, laterally oriented bands of layer B bridge the circumfenestra parallel with the vulval slit, alternating with bands of layer D. This pattern corresponds to the optical patterns on the cone top of *Globodera* cysts designated by Hesling and Ellis (1974) as " fenestral shelves ". Similar bridges of layer B were observed alternating with layer C in the cone of *H. schachtii* (Cordero & Baldwin, 1990). Future fine structural investigations of the terminal region of *Globodera* may substantiate homology of these structures.

In the cyst of *C. cacti*, the circumfenestral area is electron-lucent and composed only of the outer portion of the A layer. Contrary to the semifenestral region of *H. schachtii* there is no mesh-like network of fibers (Cordero & Baldwin, 1990, 1991). Furthermore, the vaginal lining and vulval bridge are thin and do not persist in cysts of *C. cacti* (Kiryanova & Terenteva, 1961; Green, 1975; Krall & Krall, 1978; Cordero & Baldwin, 1990, 1991). Comparisons of the fenestral region of additional Heteroderinae will provide insight into factors contributing to loss of the vulval bridge and thus formation of circumfenestrae. From comparative development and from arguments of parsimony, we can test hypotheses of homology of types of fenestrae for greater insight into phylogeny of Heteroderinae. Specifically, it is important to determine if circumfenestrae are homologous in *Cactodera*, *Globodera*, *Punctodera* and *Dolichodera* Mulvey & Ebsary, 1980.

The cuticle of the fenestral region in young females of

C. cacti is continuous with the lining of the apparently nonfunctional vagina. Layer D is prominent proximal to the cone terminus, however, a modification of this layer extends from approximately the midregion of the vagina to the uterus. A similar modification in the lining of the vagina, involving the C layer, was reported for *H. schachtii* (Cordero & Baldwin, 1991). Contrary to *H. schachtii*, the cuticle lining of the vagina of *C. cacti* is not greatly expanded near the uterus, however, the surface adjacent to the hypodermis is highly irregular with numerous projections. These projections might correspond to a vestigial underbridge (Green, 1975). Since the underbridge varies greatly in length among species (Mulvey, 1957, 1972, 1974), a vestigial underbridge could define the limits of a phylogenetically important transformation series.

Diminutive DV musculature attaches to the vagina in the cone of *C. cacti*. In contrast to the extensive DV musculature with four muscle sets in *H. schachtii*, there is only one prominent DV muscle set in *C. cacti*. These DV muscles attach to the posterior region of the vagina and extend parallel to the inner surface of the BW cuticle. The SV musculature is also reduced in *C. cacti* relative to *H. schachtii*. Although vaginal musculature presumably is associated with egg laying, it is interesting that muscles persist in a species of *Cactodera* in which eggs are not laid. Time lapse video recording of *C. cacti* suggest that muscles rhythmically contract the vagina during extrusion of the gelatinous matrix, but we have noted that the function of this matrix is unknown. Differences between *H. schachtii* and *C. cacti* with respect to size, number, and points of attachment of vaginal musculature suggest that investigation of these characters throughout Heteroderinae will provide important new characters for phylogenetic analysis.

Light microscopy and TEM of the cyst cone in *C. cacti* revealed remnants of muscles including bundles of filaments near the inner surface of the BW cuticle about 20 μm below the circumfenestra. The muscle remnants occur singly and in clusters and apparently correspond to previously described vulval denticles (Golden & Raski, 1977). Our investigations support the hypothesis that denticles are not homologous with bullae since bullae are of cuticular origin (Cordero & Baldwin, 1990, 1991). Muscle remnants are also retained in the cyst of *H. schachtii*. However, due to the position of vaginal muscles and persistence of the vagina in this genus, they are more closely associated with the vagina than the BW cuticle. Furthermore, bullae, the vagina, and the underbridge visually obscure the vestigial musculature in whole cone mounts of *H. schachtii*, whereas muscle remnants persist alone in *C. cacti*.

Comparison of the structure and development of the cone of *H. schachtii* and *C. cacti* with other Heteroderinae will provide new insight into cone characters and their phylogenetic significance. Specifically, comparison with *Globodera* and *Punctodera* will elucidate the range

of variation of cone characters. In addition, examination of noncyst-forming Heteroderinae such as *Atalodera* and *Sarisodera*, which have cones but lack fenestrae, will allow one to recognize homologies and the full range of cone character states throughout the subfamily.

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