

Ultrastructure of *Gonionchus australis* (Xyalidae, Nematoda)

WARWICK L. NICHOLAS AND AIMORN C. STEWART¹

Abstract: Observations are reported on the ultrastructure of the buccal cavity, body cuticle, spermatozoa, male genitalia, and caudal glands of *Gonionchus australis*. The buccal cuticle is a continuation of the pharyngeal cuticle. Anteriorly it is secreted by arcade tissue and overlaps the mouth rim; laterally it forms longitudinal tooth ridges. The non-annulated cephalic cuticle differs sharply from the remainder of the body wall cuticle. The cortical and basal zones become much thinner, while a largely structureless, lucent median zone expands to fill the bulk of the lips and lip flaps. Spermatozoa possess fibrous bodies, multimembrane organelles, mitochondria, and compact chromatin. The spermatozoa of *G. australis* resemble those of most other nematodes by the absence of the nuclear envelope and presence of fibrous bodies, mitochondria, and compact chromatin. The ejaculatory duct possesses microvilli. Two ejaculatory glands lie beside the duct. Two neurons are located within each spicule and each part of the paired gubernaculum. Caudal gland nuclei are large, with dispersed chromatin. The ducts of all three caudal glands are filled with secretory vesicles.

Key words: caudal gland, cuticle, *Gonionchus australis*, marine nematode, spermatozoa, ultrastructure, Xyalidae.

Ultrastructural studies are vital for clarifying nematode evolutionary relationships. The objectives of this study were to describe in detail organ systems that are important in this respect within Adenophorea. The buccal cavity, cuticle, spermatozoa, male genitalia, and caudal glands are of particular interest. *Gonionchus australis* Stewart and Nicholas, 1994 (Xyalidae; Monhysterida; Adenophorea), a nematode from Australian ocean beaches belonging to a genus with world-wide distribution, was chosen for the study.

The ultrastructure of Adenophorea is more diverse than that of Secernentea, but perhaps less well known apart from Dorylaimida and Trichurida, which have received more attention because of their agricultural or medical significance. Historically, the nomenclature and presumed homologies of

the secernentean buccal cavity were based on light microscope observations of cuticular structure in various free-living Rhabditida (reviewed by Baldwin and Eddleman, 1995; De Ley et al., 1995). This interpretation recently has been revised following comparative ultrastructural studies of several Rhabditida (Van de Velde and Coomans, 1991; Van de Velde et al., 1994; Baldwin and Eddleman, 1995; De Ley et al., 1995). Homologies depend on cell structure as much as on cuticular structure. No such basic plan has been proposed for the buccal cavities of Adenophorea. Although comparatively few ultrastructural studies have been made of the buccal cavities of nematodes belonging to the subclass Chromadoria (Stewart and Nicholas, 1994; Tchesunov, 1990a, 1990b, 1991), they clearly are quite unlike those of Rhabditida, Dorylaimida, or Trichurida.

Several different nomenclatures are in use for nematode cuticular structure. We have followed the nomenclature proposed by Bird and Bird (1991). A survey of the ultrastructure of the cuticles of many marine Ad-

Received for publication 15 May 1996.

¹ Division of Botany and Zoology, Australian National University, Canberra 0200, ACT, Australia.

E-mail: warwick.nicholas@anu.edu.au

The authors thank S. Stowe and the electron microscope unit of the Australian National University for their help and facilities, and Ruo Lan Shen for technical assistance.

enophorea shows diverse ultrastructure (Yushin and Malakhov 1994b).

Nematode spermatozoa differ from those of other animals in several respects (Baccetti et al., 1983; Bird and Bird, 1991). Nuclear structure appears more primitive in two of the three marine species of Adenophorea in which it has been described (Baccetti et al., 1983; Lippens, 1974; Noury-Srairi et al., 1993). The ultrastructural study of the caudal glands has been described in *Chromadorina germanica* (Lippens, 1974), *Theristus caudaliensis*, and *Perepsilononema* sp. (Adams and Tyler, 1980). *Theristus* and *Gonionchus* spp. are both in the family Xyalidae.

MATERIALS AND METHODS

Specimens of *G. australis* were collected from sand at low tide on Moruya beach, New South Wales, Australia. Sand was resuspended in sea water, and the water was filtered through a nylon mesh sieve. The residue was back-washed into sea water and allowed to settle. Sea water was replaced with 2.5% glutaraldehyde in phosphate-buffered saline, pH 7.2, containing 3% sucrose. After washing with 3% sucrose in phosphate-buffered saline, several specimens of *G. australis* were picked up under the microscope and transferred to 5% aqueous glycerol for light microscopy. These were then mounted on slides in anhydrous glycerol.

About 20 specimens of *G. australis* were post-fixed in phosphate-buffered 1% OsO₄ for 24 hours. Thirteen of the specimens were used for scanning electron microscopy (SEM), and five males and two females were sectioned for transmission electron microscopy (TEM). All the electron micrographs in this paper come from three males. SEM specimens were briefly sonicated prior to post-fixation to remove adherent mucus. After post-fixation, they were freeze-dried, mounted on stubs, and coated with gold-palladium. TEM specimens were progressively dehydrated through graded ethanols and then transferred through propylene oxide-ethanol mixtures and 100% propylene oxide to Spurr epoxy resin (Spurr, 1969) for embedding. Sections were cut with a dia-

mond knife and stained on slot grids with uranyl acetate and lead citrate (Reynolds, 1963).

RESULTS

General morphology: Adult *G. australis* are cylindrical with a long, tapering tail (Fig. 1A). The body is strongly annulated, except for the smooth cephalic region (Fig. 1B,D). The large, conical buccal cavity is armed with two lateral tooth ridges (Fig. 1B) and surrounded by six prominent, thin, leaf-shaped lips (Fig. 1D). Inner labial setae arise from the base of the lips, while outer labial and cephalic setae arise farther posteriorly from the non-annulated cephalic region. Large amphid apertures are located at the anterior of the annulated body cuticle (Fig. 1D).

Buccal cavity: Longitudinal sections of the buccal cavity in TEM (Fig. 2A-E) show a deep, conical cavity largely enclosed by pharyngeal muscle cells. The buccal cavity is lined with an amorphous, electron-dense cuticle identical with that lining the pharynx. The pharyngeal cuticle is greatly thickened to form the tooth ridges, which have no cytoplasmic core (Fig. 2B). The buccal cuticle extends beyond the rim of the mouth and is reflexed over the arcade cells to form an electron-dense collar beneath the lips (Fig. 2B,C). Three tiers of pharyngeal muscle cells enclose the posterior three fourths of the buccal cavity (Fig. 2A). Close to the anterior limit of the muscles, marginal cell fibrils cross the muscle cell to insert on the pharyngeal basal lamina (Fig. 2C). The dorsal pharyngeal gland contains vesicular secretions (Fig. 2E). The cuticle of the anterior one-third of the cavity is secreted by arcade cells (Fig. 2C).

Body annulation ceases, and the smooth cephalic region begins at about the anterior limit of the pharyngeal muscles. Cuticular ultrastructure changes abruptly from that of the post-cephalic body. The epicuticle continues as the outermost layer extending over the thin lip flaps (Fig. 2D) to become confluent with the pharyngeal cuticle just within the rim of the mouth. A thin, electron-dense

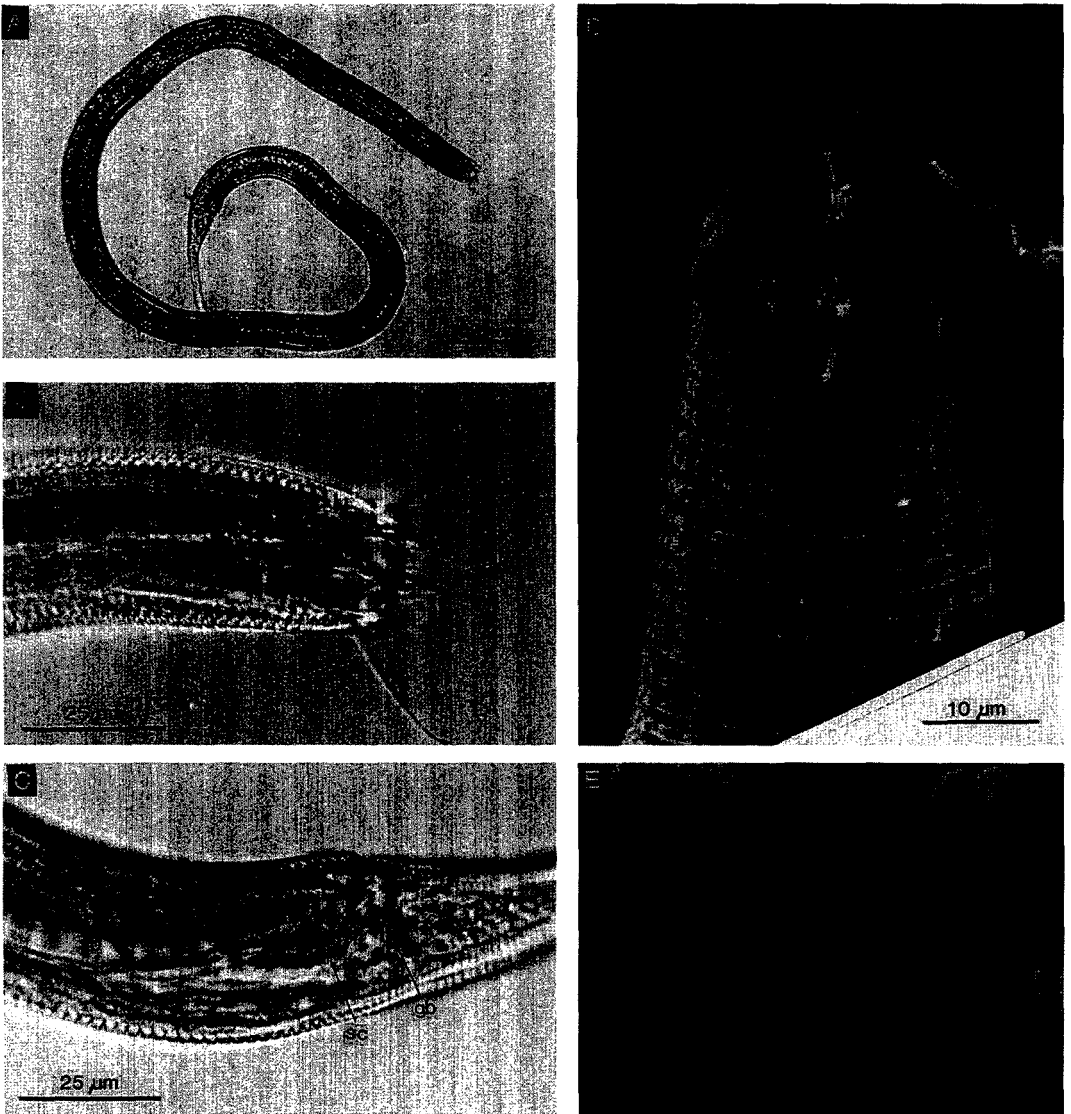


FIG. 1. A–C) Light microscopy of *Gonionchus australis*. A) Entire male. B) Head of male. C) Posterior region of male. D,E) Scanning electron microscopy. D) Head of male. E) Spicules and gubernaculum. am = amphid, an = annulation, bc = buccal cavity, cg = caudal glands, gb = gubernaculum, lp = lip, sc = spicules, se = cephalic seta, sp = spermatozoa, tr = tooth ridge.

cortical zone covers the cephalic region and the lips. An electron-lucent median zone widens toward the mouth rim (Fig. 2C) and expands to fill the lips (Fig. 2B,D). Discontinuous, homogeneous electron-dense material lies within the otherwise lucent median zone in the cephalic region. The lips arising from the mouth rim are relatively large and without a cytoplasmic core, but contain some apparently structureless denser inclusions (Fig. 2D). They collapse on freeze-

drying for SEM as in Fig. 1D, and must have a liquid or semi-liquid median zone. The basal zone of the cuticle becomes separated from the cortical zone by a widening median zone where annulation ceases, and extends as far as the mouth rim (Fig. 2C).

Amorphous electron-dense buccal cuticle, continuous with the pharyngeal cuticle, thickens at the mouth rim and is reflexed over the rim to extend posteriorly within the median zone of the cephalic cuticle (Fig.

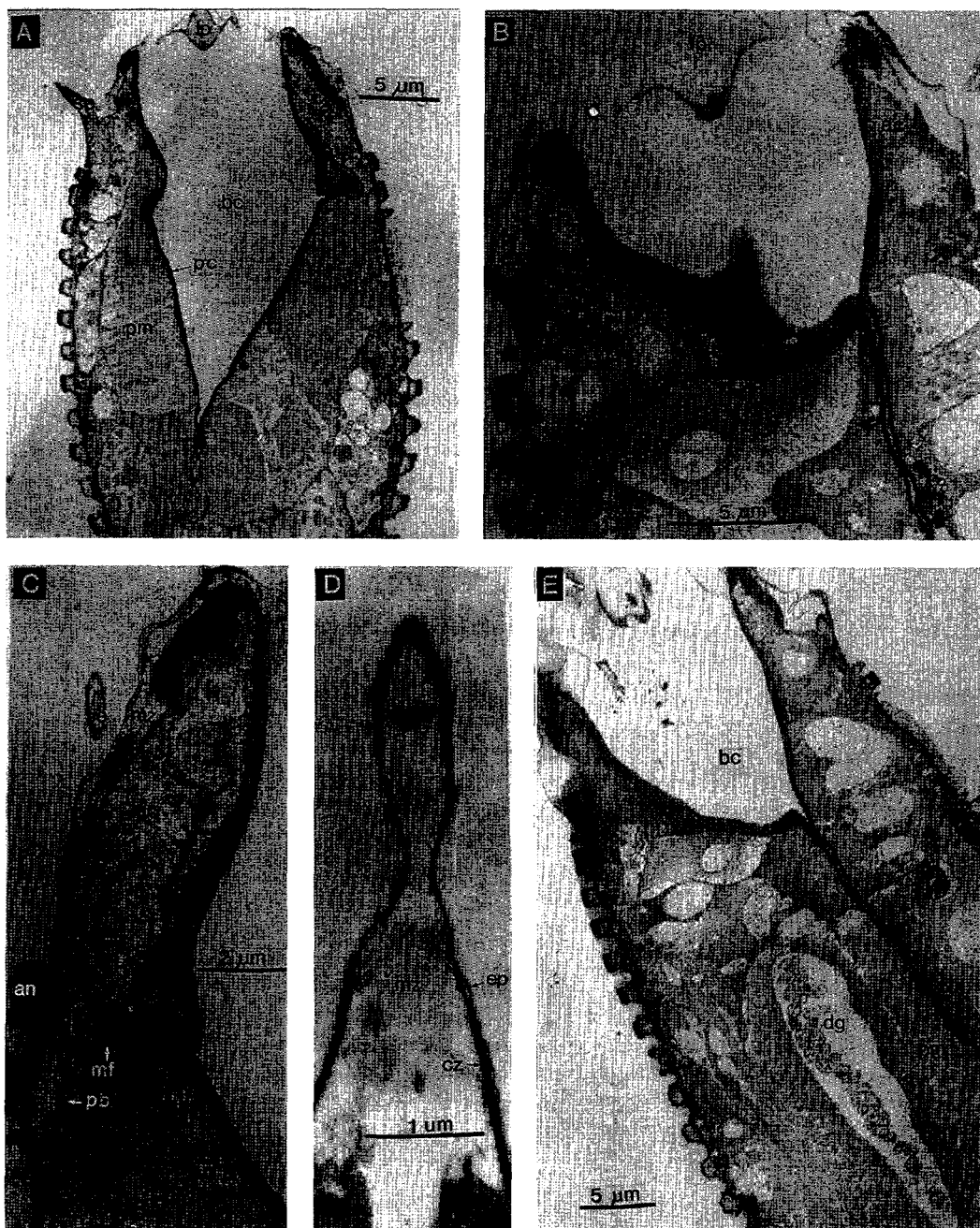


FIG. 2. Longitudinal sections through buccal cavity and pharynx. A) Low magnification section. B) Through tooth ridge. C) Cuticle and arcade cells. D) Tangential section through lip flap. E) Through pharyngeal gland. ac = arcade cell, an = annulation, bc = buccal cavity, bz = basal zone, cf = connective fibers, cz = cortical zone, dg = dorsal pharyngeal gland, ep = epicuticle, lp = lip, mc = mucus, mz = median zone, ol = pharyngeal cuticle reflexed over mouth rim, pc = pharyngeal cuticle, pm = pharyngeal muscles, se = seta, tr = tooth ridge.

2C,E). Arcade tissue fills the space between the anterior third of the buccal cavity and the external cephalic cuticle, lying anterior to the pharyngeal muscle cells (Fig. 2B,C).

Cuticle and body wall: The borders of the raised annules are serrated and separated by deep grooves (Fig. 1D,E). A thin epicuticle, about 8 nm thick, forms the outer layer (Fig.

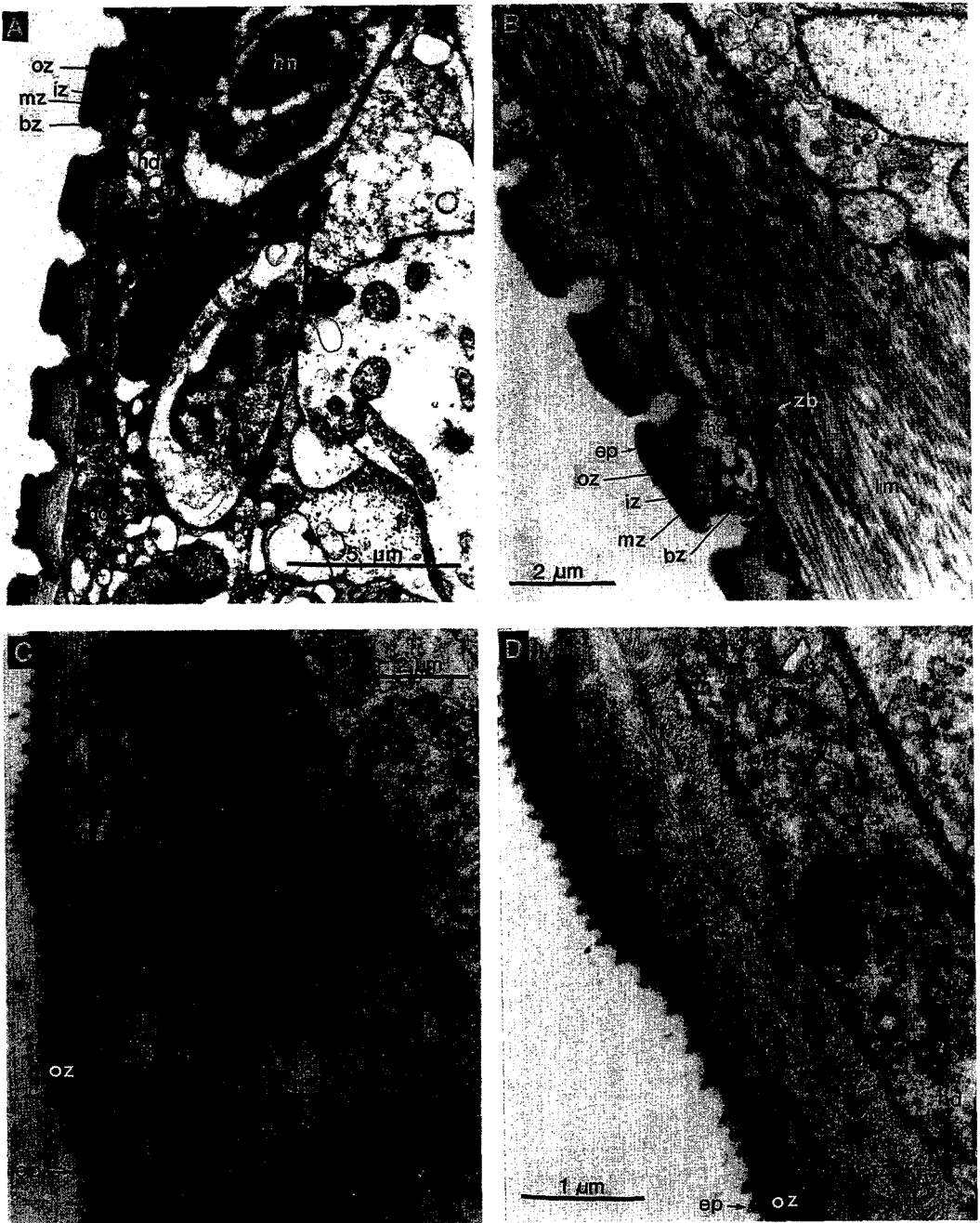


FIG. 3. A,B) Longitudinal sections through cuticle. C,D) Transverse sections through cuticle. bz = basal zone, ep = epicuticle, hd = hypodermis, hn = hypodermal nucleus, iz = inner cortical zone, lm = longitudinal muscle, mz = median zone, oz = outer cortical zone, zb = z-bodies.

3B,D). An electron-dense, finely granular cortical zone is divisible into an outer denser zone, about 400 nm thick, that caps the top of each annule, and an inner zone of uniform density, about 300 to 320 nm thick,

that is continuous across both the tops of and the grooves between annules (Fig. 3A,B). The tops of the annules are of uneven thickness, giving rise to weak longitudinal ridging, about 200 nm between crests

(Fig. 3C,D), which is not obvious in scanning electron micrographs (Fig. 1D,E). Closely set longitudinal ridges, 30 to 70 nm high, run along the tops of the annules, visible as triangular elevations between the outer cortical zone and the epicuticle in transverse sections (Fig. 3C,D) as uneven dark lines in longitudinal sections (Fig. 3B), and as serrations at anterior and posterior borders of annules in scanning electron micrographs (Fig. 1D,E).

The cuticular median zone, 400 to 600 nm thick, is finely fibrous, with the fibers arranged circumferentially and probably helically (Fig. 3D). The basal zone, about 160 nm thick, is also finely fibrous but with the fibers predominantly radial (Fig. 3D). The hypodermal layer between the muscle quadrants is wider, with nuclei, cytoplasmic organelles, and dense granules (Fig. 3A,D). Where the section cuts through the longitudinal muscles, the hypodermis is thin and irregular in thickness. Obliquely striated circomyarian muscles are evident below the hypodermis (Fig. 3A,C).

Spermatids and spermatozoa: Spermatids are recognizable within the vas deferens as rounded cells with numerous multimembrane bodies toward the periphery of the cell and many mitochondria more centrally placed (Fig. 4A,B). Chromatin is centrally located. Multimembrane bodies are packed with tubules and dense bodies. Spermatozoa are elongated cells containing a central rod of compact chromatin surrounded by four dense fibrous bodies (Fig. 4A–D). Several mitochondria with reduced cristae remain in spermatozoa (Fig. 4D). No nuclear membrane is apparent enclosing the chromatin. The gonoduct cells contain numerous dense inclusions and nuclei with well-dispersed chromatin (Fig. 4B,C).

Spicules, gubernaculum, ejaculatory duct, and rectum: The rectum, lined by homogeneous cuticle, lies adjacent to the ejaculatory duct (Fig. 5A,B). Many long microvilli partially fill the ejaculatory duct. Two ejaculatory glands completely filled with dense vesicles lie on either side of the posterior intestine (Fig. 5B) and open into the ejaculatory duct (opening not illustrated). Posterior to the

ejaculatory glands, two spicules lie beside the rectum and ejaculatory duct (Fig. 5B,D). Each spicule lies within a cuticular pouch. Two neurons with microtubules lie within each spicule (Fig. 5C). Hollow, thick, dense cuticle of the paired gubernaculum crura about the distal ends of the spicules. Each crura of the gubernaculum contains the two neurons within its hollow center (Fig. 5D). The spicule tips are recurved (Fig. 1E).

Caudal glands: Three caudal glands lie at slightly different levels within the tail (two visible in Fig. 1C). One of the caudal gland ducts contains few secretory vesicles (Fig. 6A), while the other two contain many vesicles (Fig. 6B). A canal at the center of the tail is the terminal extension of the pseudocoel. All three ducts are filled with secretory vesicles nearer to the tail tip and are flanked by three circomyarian muscle cells (Fig. 6B). Each caudal gland cell has a large, dense nucleus containing many discrete strands of chromatin and also possesses many dense secretory vesicles within its perinuclear cytoplasm (Fig. 6C). The three ducts and muscle cells extend to the tail tip (Fig. 6D).

DISCUSSION

The buccal cavity is divisible into two regions—an anterior cylindrical region enclosed by non-muscular cells and a posterior conical region enclosed by the myoepithelial cells of the anterior pharynx. Both regions secrete a homogeneous, electron-dense cuticle that is structurally similar to that of the rest of the pharynx. These two regions correspond to the second and third regions of the rhabditid buccal cavity as defined by Van de Velde et al. (1994) and De Ley et al. (1995) in their discussion of the homologies of secernentean buccal cavities. The first region, in which the buccal cavity is enclosed by cuticle continuous with the external body cuticle, is not represented in *G. australis*. This is also true of other Chromadoria that have been studied (Stewart and Nicholas, 1994; Tchesunov, 1990a, 1990b).

The buccal cavity of *G. australis* resembles that of several Xyalidae and other closely re-

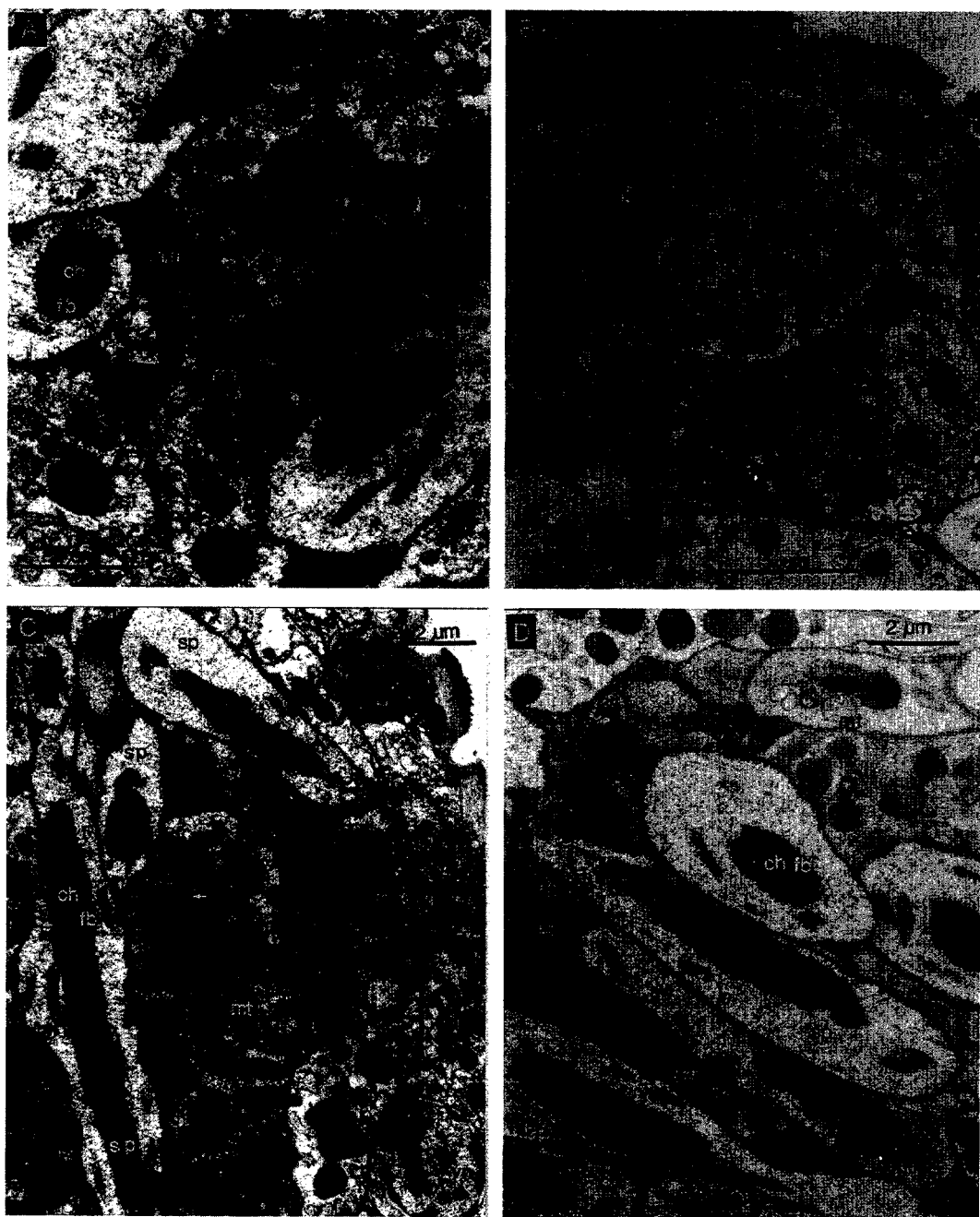


FIG. 4. A-D) Sections through vas deferens, spermatids, and spermatozoa. ch = chromatin, fb = fibrous body, mm = multimembrane organelle, mt = mitochondria, sp = spermatozoa, st = spermatid, vd = vas deferens, vn = vas deferens nucleus.

lated Monhysteroidea (Tchesunov, 1990a, 1990b). In these nematodes the external cephalic cuticle differs sharply from the post-cephalic cuticle. The median zone of the cuticle (mesocuticle of Tchesunov, 1990a)

expands to form the bulk of the lips. Tenuous lip flaps are not nearly as well-developed in the nematodes described by Tchesunov as they are in *G. australis*, but the bulkier convex lips in those species have supporting

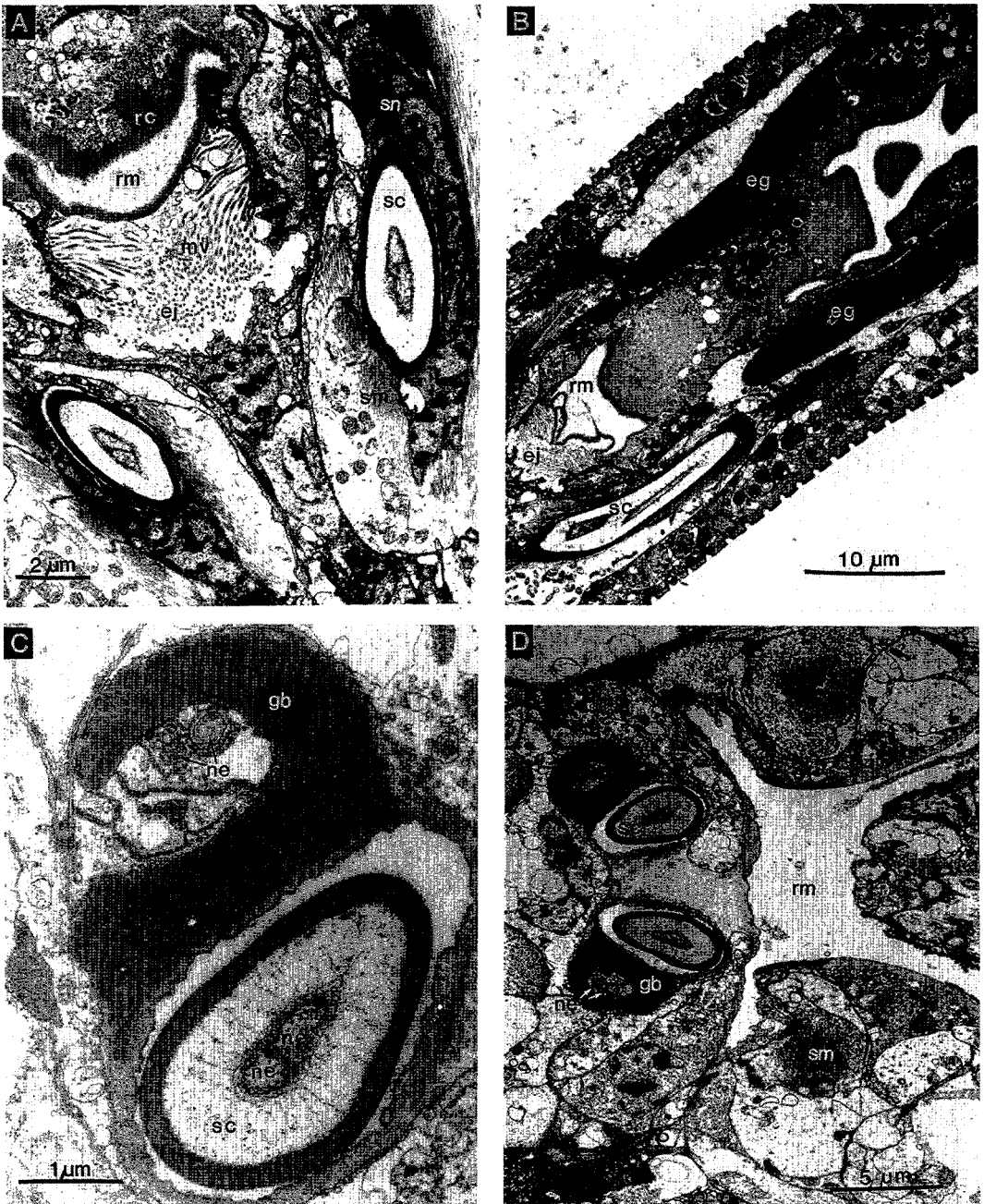


FIG. 5. Longitudinal sections through cloacal region. A) Section through rectum and ejaculatory duct. B) Section through ejaculatory glands. C) Cross-section of gubernaculum and spicule. D) Section through spicules, gubernaculum, and rectum. eg = ejaculatory gland, ej = ejaculatory duct, gb = gubernaculum, mv = microvilli, ne = neurons, rc = rectum cuticle, rm = rectum, sc = spicule, sm = spicule muscle, sn = spicule pouch nucleus, so = spicule pouch cuticle.

septa and trabeculae within the median zone of the lips that are not present in *G. australis*. In two Monhysteroidea described by Van de Velde and Coomans (1991), *Geo-*

monhystera disjuncta and *Diplolaimella dieven-gatensis*, lip flaps are not present, and the anterior of the buccal cavity is enclosed by a continuation of the body wall cuticle and is

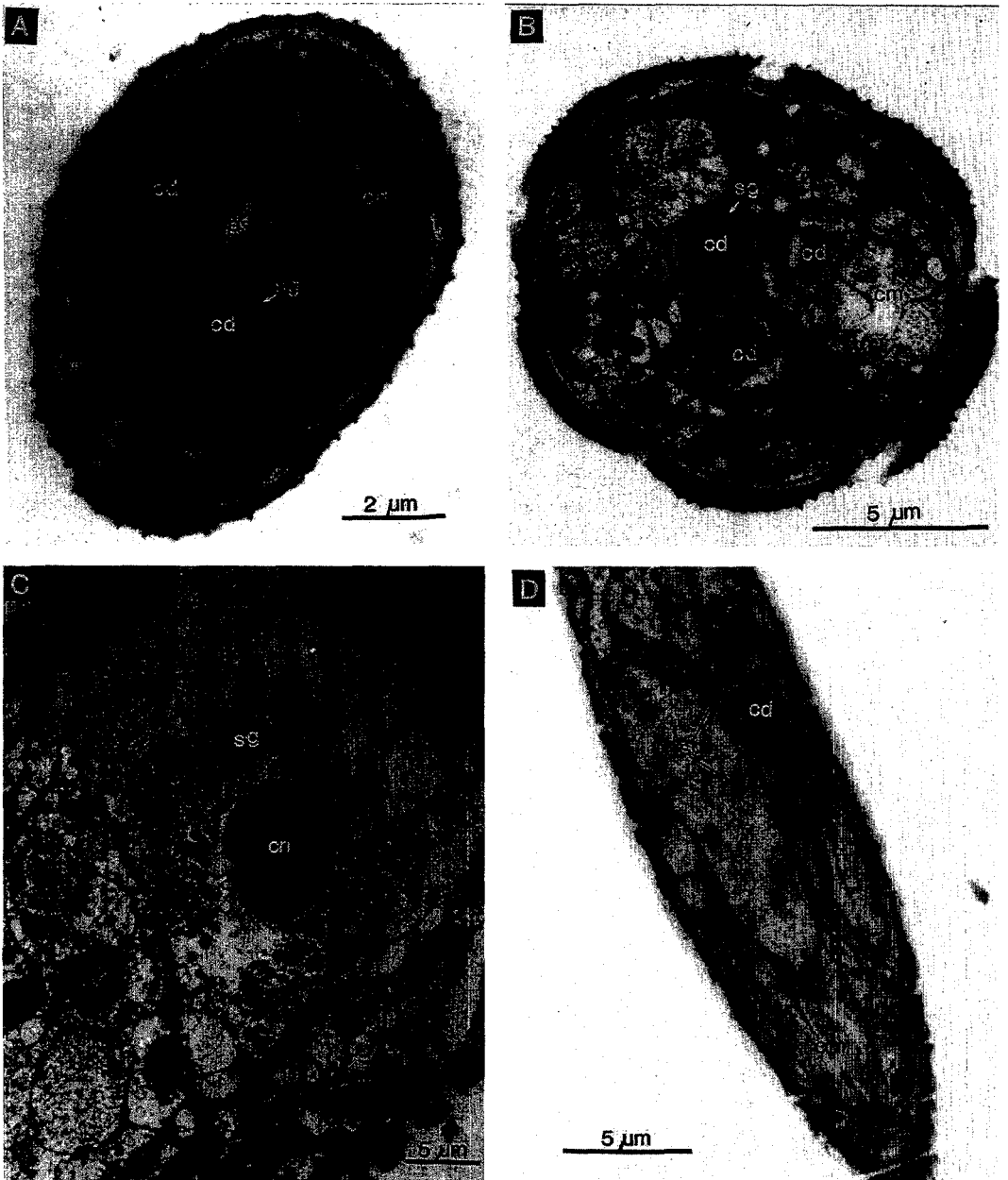


FIG. 6. Caudal glands. A,B) Transverse sections through tail. C) Longitudinal section through post-cloacal region. D) Longitudinal section through tail tip. cd = caudal gland duct, cm = caudal muscles, cn = caudal gland nucleus, ps = pseudocoel, sg = secretory vesicles.

therefore cheilostom. Only a short region in these species is secreted by arcade tissue.

We have described the anterior part of the buccal cavity in *G. australis* as enclosed by arcade tissue, following Van de Velde and Coomans (1991) and Van de Velde et al. (1994), and not cheilostom (Tchesunov,

1990a, 1990b), because its cuticle is not an extension of the external body cuticle. The buccal cavity of Linhomoeidae (Tchesunov, 1991) is rather different; the median zone expands in the lip region, but the cortical zone forms a strong ring within the mouth rim.

Yushin and Malakhov (1994b) surveyed the ultrastructure of the cuticle of many marine Chromadoria. The cuticles vary in form, often with more than four recognizable zones. The only species of Xyalidae described in the survey was *Steineria marsiana*. It possesses an epicuticle, and cortical and basal zones similar to that of *G. australis*, but has a much more elaborate median zone, with a fibrous base on which stand dense trabeculae crossing a lucent zone to support the cortical zone.

Nematode spermatozoa vary in form, but, unlike other animal spermatozoa, they lack an acrosome and a flagellum (Baccetti et al., 1983; Bird and Bird, 1991). Locomotion is amoeboid with a prominent anterior pseudopodium. The chromatin becomes highly condensed and electron-dense, but with two known exceptions, the nuclear envelope is lost when the sperm completes development. A nuclear envelope is present in *Mesacanthion* spp. (Baccetti et al., 1983) and *Enoplus* spp. (Yushin and Malakhov, 1994a), both marine Enoploidea, but not in marine *Sphaerolaimus* (Noury-Srairi et al., 1993) or *G. australis*, both Monhysteroidea. Further research may show whether the retention of a nuclear envelope in the mature spermatozoon distinguishes Enoploidea from other nematodes. *G. australis* spermatozoa possess multimembrane bodies, characteristic of many nematode spermatozoa while still within the male gonoduct, and dense bodies that from their location and appearance are probably homologous with the dense bodies described in *Sphaerolaimus* (Noury-Srairi et al., 1993). However, we were unable to resolve their fibrous nature.

Most Adenophorea possess three caudal glands, usually but not always located post-annually, that produce an adhesive secretion through a spinneret at the tip of the tail (Bird and Bird, 1991). *Theristus caudasaliens* and *Perepelsonema conifera* possess two additional caudal glands producing a releasing secretion, associated in *T. caudasaliens* with an unusual hopping locomotion (Adams and Tyler, 1980). In the caudal glands of *G. australis*, the nucleus, secretory vesicles, and ducts resemble the adhesive glands in *T.*

caudasaliens, but we have not observed an extensive rough endoplasmic reticulum or Golgi complex as was described in the latter species. Secretory activity possibly is cyclical, as was observed in *Chromadorina germanica* (Lippens, 1974). This nematode possesses three adhesive glands, but they differ in the way their secretions are formed, whereby secretory vesicles condense on microvilli in ramifications of the gland ducts (Lippens, 1974).

LITERATURE CITED

- Adams, P. J. M., and S. Tyler. 1980. Hopping locomotion in a nematode: Functional anatomy of the caudal gland apparatus of *Theristus caudasaliens* sp.n. *Journal of Morphology* 164:265-285.
- Baccetti, B., R. Dallai, S. Grimaldi De Zio, and A. Marinari. 1983. The evolution of the nematode spermatozoon. *Gamete Research* 8:309-323.
- Baldwin, J. G., and C. D. Eddleman. 1995. Buccal capsule of *Zeldia punctata* (Nemata: Cephalobidae): An ultrastructural study. *Canadian Journal of Zoology* 73:648-656.
- Bird, A. F., and J. Bird. 1991. The structure of nematodes, 2nd ed. San Diego: Academic Press.
- De Ley, P., M. C. Van de Velde, D. Mounport, P. Baujard, and A. Coomans. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae, and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). *Nematologica* 41:153-182.
- Lippens, P. A. 1974. Ultrastructure of a marine nematode, *Chromadorina germanica* (Buetschli, 1874) I. Anatomy and cytology of the caudal gland apparatus. *Zeitschrift für Morphologie der Tiere* 78:181-192.
- Noury-Srairi, N., N. Gourbault, and J. Justine. 1993. The development and evolution of actin-containing organelles during spermiogenesis of a primitive nematode. *Biology of the Cell* 79:231-241.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17:208-212.
- Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 20:31-43.
- Stewart, A. C., and W. L. Nicholas. 1994. Fine structure of the head and cervical region of *Ceramonema carinatum* (Chromadorida: Ceramonematidae). *Journal of Nematology* 26:188-211.
- Tchesunov, A. V. 1990a. Cephalic cuticular structures in some free-living nematodes from the superfamily Monhysteroidea (Chromadoria, Monhysterida) Communication 1. *Zoologicheskyy Zhurnal* 69(11):18-24. [In Russian].
- Tchesunov, A. V. 1990b. Cephalic cuticular structures in some free-living nematodes from the superfamily Monhysteroidea (Chromadoria, Monhysterida) Communication 2. *Zoologicheskyy Zhurnal* 69(12):13-23. [In Russian].
- Tchesunov, A. V. 1991. On the structure of the ce-

phalic cuticle of free-living marine nematodes of the family Linhomoeidae, (Nematoda, Chromadoria, Siphonolaimoidea). Zoologicheskyy Zhurnal 70:21-27. [In Russian].

Van de Velde, M. C., and A. Coomans. 1991. The ultrastructure of the buccal cavity of the monhysterid nematodes *Geomonhystera disjuncta* and *Diplolaimella divengatensis*. Revue Nematologie 14:133-146.

Van de Velde, M. C., P. De Ley, D. Mounport, P. Baujard, and A. Coomans. 1994. Ultrastructure of the

buccal cavity and cuticle of three Cephalobidae (Nematoda: Rhabditida). Nematologica 40:541-563.

Yushin, V. V., and V. V. Malakhov. 1994a. Ultrastructure of sperm cells in the female gonoduct of free-living marine nematodes from genus *Enoplus* (Nematoda: Enoplida). Fundamental and Applied Nematology 17:513-519.

Yushin, V. V., and V. V. Malakhov. 1994b. Ultrastructure of the body cuticle of free-living marine nematodes. Russian Journal of Nematology 2:83-98.