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## Chapter 3

# Polyplacophora

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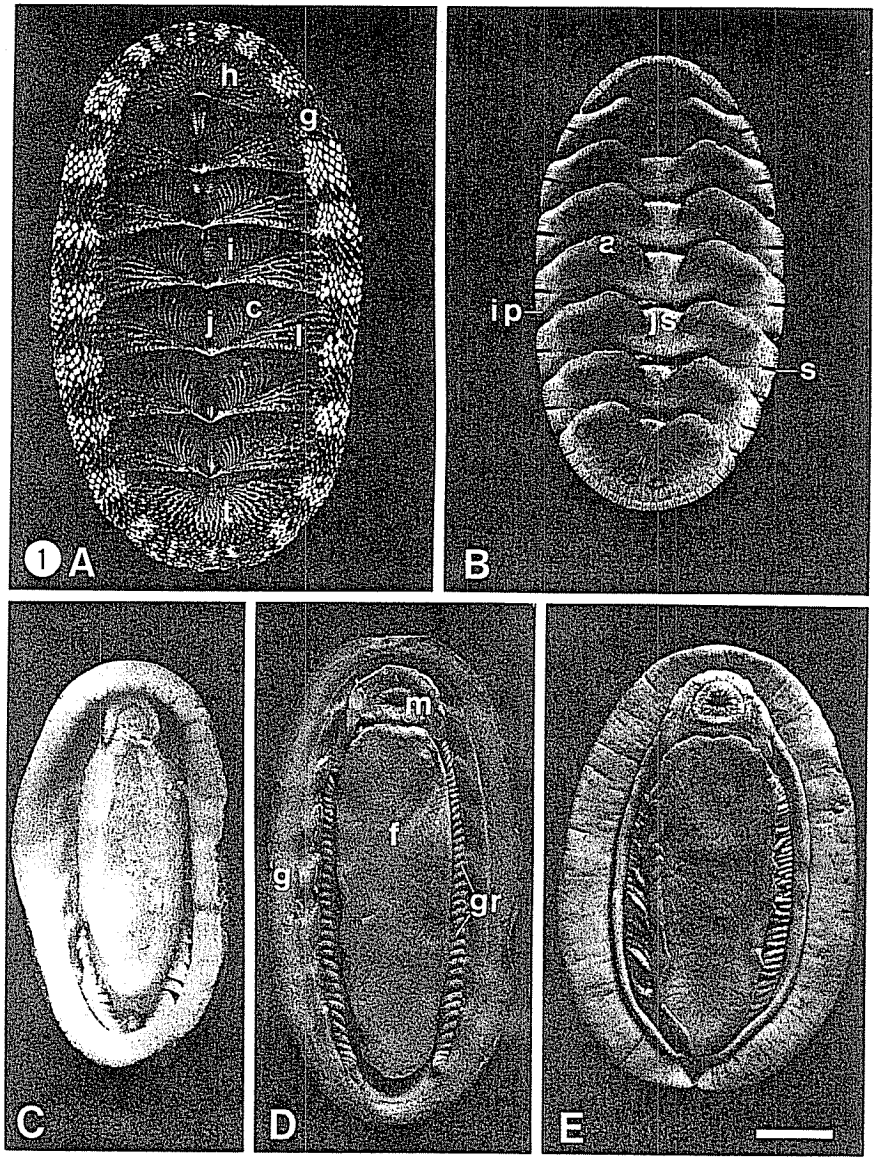
### INTRODUCTION

Polyplacophorans, also known as chitons, are bilaterally symmetrical molluscs distinguished by their eight overlapping shell plates (termed valves). The head and tail valves are hemispherical, separated by six butterfly-shaped medial valves, all articulating to form an oval to elongate dorsal or ventral profile (Fig. 1A,B). The valves' dorsal top layer, the tegmentum, is partly living tissue with sensory organs distributed in regular patterns on the valve surface supplied from below by a complicated system of nerve channels. Internally, the valves are attached firmly to eight double-cord pairs of muscle groups. On the distal margins the valves are embedded in tough, thick mantle tissue, the girdle (or perinotum), itself covered with numerous calcareous or corneous spines, scales, or bristles, as well as sensory organs (Fig. 1A).

Chitons attach firmly or creep over hard substrates with a broad sticky foot (Fig. 1C–E), feeding by scraping or biting with the mineral-reinforced teeth of the radula. The mantle cavity extends along either side of the foot anteriorly to the broad mouth platform and continues posteriorly behind the foot, surrounding the anus. Single-file gill rows are suspended like curtains from the mantle cavity roof on each side of the foot, forming either a continuous posterior arch surrounding the anus (Fig. 1C; suborder Lepidopleurina) or with considerable separation between the last gill in each row (Fig. 1D,E; other extant chitons). Most chiton species grow no longer than about 15 cm, many no longer than 1 cm, but the northern Pacific *Cryptochiton stelleri* reaches at least 33 cm (MacGinitie and MacGinitie, 1968). Approximately 830 living species are currently recognized in this exclusively marine group. Over half of all species live in intertidal to shallow subtidal habitats.

For a relatively small group, chitons were the subjects of a surprising number of conchological studies by prominent early malacologists, starting with Linnaeus (1758), who described the first four species and the genus *Chiton* to accommodate them, thus bringing chitons their first widespread attention. Much later, Pilsbry (1892–1894) and Thiele (1909–1910) provided monographs of worldwide chitons, and in recent years these classic stud-






ies have been extended by a systematic revision (Van Belle, 1983, 1985), and ongoing monograph series (Kaas and Van Belle, 1985a,b, 1987, 1990), species catalogues (Kaas and Van Belle, 1980; Van Belle, 1981), and a worldwide geographic distributional and nomenclatural database (Eernisse, in press).

Chitons are an ancient group, well-represented by the earliest Ordovician and of more disputed presence in the Cambrian periods. Runnegar et al. (1979) proposed a reconstruction of the North American and Australian Late Cambrian *Matthevia* Walcott, 1885 as a chiton, with detailed similarities to the somewhat later *Chelodes*. Yochelson (1966) had previously proposed *Matthevia* as the sole member of a new molluscan class. More recently there have been differing interpretations of small, nondescript, shelly remains from the Early Cambrian. Yu (1984a,b, 1987) added many nominal polyplacophoran taxa proposed from China, but these were rejected by Yi and Bengtson (1989; see also He and Xie, 1989). Evans and Rowell (1990) presented alternative reconstructions of Antarctic shelly fossils and, while they do not mention such a relationship, some of their reconstructions highly resemble chitons. Meanwhile, Yates et al. (1992) reported a preliminary reinterpretation of fossils from the South Australian Early Cambrian as chitons with three distinct valve types, the two presumed terminal valve types being rare. Among an estimated 300 recognized fossil chiton species (Van Belle, 1981; Eernisse, unpublished tally), only a few species are relatively well represented or known from fully articulated specimens, perhaps not surprising for a group mostly confined to hard-substrate erosional environments. *Pterochiton concinnus* is an exceptional species from the Carboniferous (ca. 300 MY), with most of over 200 articulated known specimens displaying well-preserved features of the radula (Yochelson and Richardson, 1979; Eernisse, personal observation).

The most current classification schemes (e.g., Van Belle, 1983, 1985) include all living chitons in a single order, Neoloricata. The most familiar and conspicuous chitons belong to the suborders Ischnochitonina (including Ischnochitonidae, Chitonidae, Mopaliidae, Lepidochitonidae, and several other common families) and Acanthochitonina (including Acanthochitonidae and Cryptoplacidae). Members of a third suborder, Lepidopleurina, mostly live in deep water and differ from the other chitons in their posterior (not lateral) gill arrangement (Fig. 1C), their nonprojecting or at least unslit shell eaves (insertion plates), which normally anchor the shells firmly in the mantle, their well-developed sperm acrosome (Hodgson et al., 1988; Buckland-Nicks et al., 1990), and their smooth egg hulls (Eernisse, 1988; Hodgson et al., 1988; Eernisse, unpublished manuscript). Members of the divergent monotypic genus, *Choriplax*, have been accorded full suborder status as Choriplacina by some recent authors (e.g., Gowlett-Holmes, 1987).

Fig. 1. External anatomy of representatives of Polyplacophora (chitons). All figures are oriented with anterior end at top. **A:** Dorsal view of *Chiton tuberculatus* (Ischnochitonina) (USNM 454640). **B:** Valves of *Chiton squamosus* in ventral view (USNM 845188.) **C:** Ventral view of *Ferreirella caribbeanensis* (Lepidopleurina) (18°23′ N, 75°03′ W on sunken wood at 530 m.; USNM acc. 383191). Note posterior gill placement with adanal gills posterior to the largest "post-renal" gill pair. **D:** Ventral view of *Chiton bowenii* (Ischnochitonina) (Chiloe Id., Chile, shallow subtidal, LACM 75-46). Note lateral gill placement with adanal gills posterior to the largest "post-renal" gill pair. **E:** Ventral view of *Mopalia hindsii* (Bordalais Id., B.C., Canada, intertidal, DJE coll.). Note lateral gill placement with abanal gills only; the most posterior gill pair is the largest "post-renal" gill pair. a, apophyses (sutural plates); c, central area (note that the anterior part of tail valve has a corresponding sculpture and esthete innervation pattern); f, foot; g, girdle elements (imbricating scales on dorsal surface); gr, gill row; h, head valve; i, intermediate valve; ip, insertion plates (here pectinate, a diagnostic feature of Chitonidae); j, jugal area; js, jugal sinus; l, lateral area (note that all the head valve and the posterior part of tail valve have a corresponding sculpture and esthete innervation pattern); m, mouth (anus is at opposite end of foot); s, slit rays (corresponding to esthete innervation); t, tail valve. Scale bars: A,B = 5.5 mm; C–E = 1 cm.

## EXTERNAL FEATURES

Recent overviews of anatomical diversity in chitons include those by Yonge (1960), Fischer-Piette and Franc (1960), Hyman (1967), Stasek (1972), Wingstrand (1985),

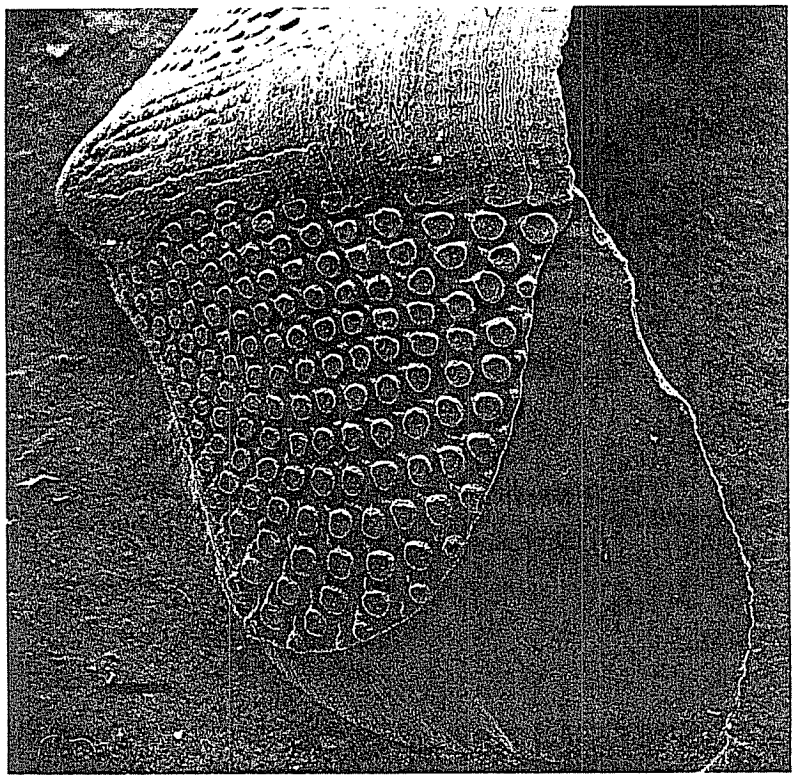


Fig. 2. Details of tegmental surface of valves. **A** (this page): *Acanthochiton fascicularis*. A middle shell plate, treated with KOH. SEM. A, articulamentum; L, lateral region; M, median area. Scale bar = 100 μm. **B** (facing page): *Callochiton achatinus*. The dorsal sculpturing of the lateral area of the intermediate valves, showing the distribution and alignment of the various surface features which are comparable with other areas covered by pigment spots. Ac, apical cap; Ps, pigment spot; Sc, subsidiary cap. Scale bar = 100 μm (A, from Fischer, 1979; B, from Baxter and Jones, 1984.)

and Salvini-Plawen (1985). Reviews of physiological, reproductive, and natural history aspects can be found in Boyle (1977), Pearse (1979), and Haderlie and Abbott (1980), respectively. The most important older anatomical descriptions are referenced in the above works. Figure 1 illustrates a chiton in dorsal and ventral aspects, with major anatomical features labeled.

### Shells

The best known diagnostic character of Polyplacophora is the serial repetition of eight, usually overlapping, dorsal valves in all living and fossil (Rolfe, 1981) chitons. Teratological specimens having five to seven or nine valves are known but are rare (Dell'-Angelo, 1982). The often colorful valves are highest along the longitudinal axis, with paired eaves forming a V-shaped to arching profile, distally embedded in the tough mantle tissue of the girdle or perinotum. Anterior insertion plates of valves 2 to 7 slide under the preceding valve in series, forming a flexible band to allow the animal to roll up or at least conform to irregular depressions or surfaces.

Having eight shells is widely regarded as diagnostic for chitons, but the attaching musculature is quite similar to that of monoplacophorans (i.e., members of Tryblidiida, including *Neopilina*). Wingstrand's comparison (1985) of the eightfold shell-attachment muscle groups in chitons and monoplacophorans led him to suggest that chitons and conchiferans are derived from an ancestor with eightfold serial repetition. Whether that ancestor had one, eight, or no shells is disputed. Considering only these three alternatives, it has been postulated that chiton valves are the result of a break-up of a single ancestral cap-shaped shell (Runnegar and Pojeta, 1974), that the ancestral multiple valves coalesced to a single shell in an ancestor to conchiferans (Haas, 1981), and that shells arose independently in chonchiferans and chitons (Scheltema, 1988). Consequently, the issue of whether the eight valves of chitons are even homologous with shells of conchiferan molluscs remains unsettled.



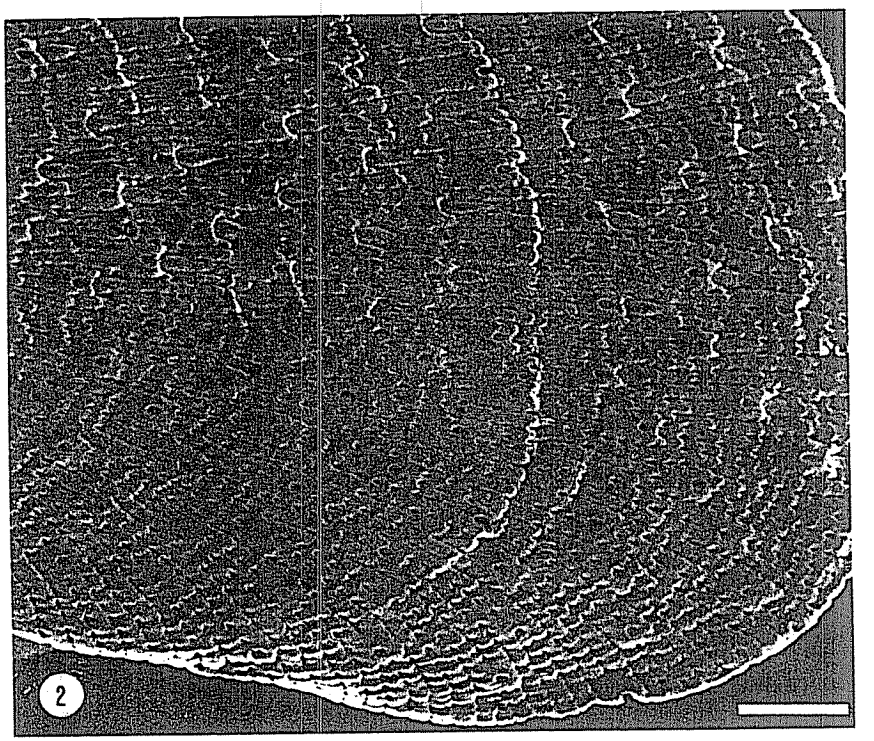



Figure 2B

A likely derived feature of chiton shells is the upper "tegmentum" shell layer, which is a partly living layer, not comparable to the purely inorganic shell layers of other molluscs (Fig. 2). The tegmentum is penetrated by branching canals (Fig. 3) that carry nerves to numerous sensory organs called esthetes and larger ocelli, when present. The ocelli are likely photoreceptors, as they have a well-developed lens structure. The function of the smaller esthetes remains in dispute, with photo-, mechano-, and chemosensory as well as secretory functions postulated; much of this may be due to real differences in esthete function in different taxa. Underlying the

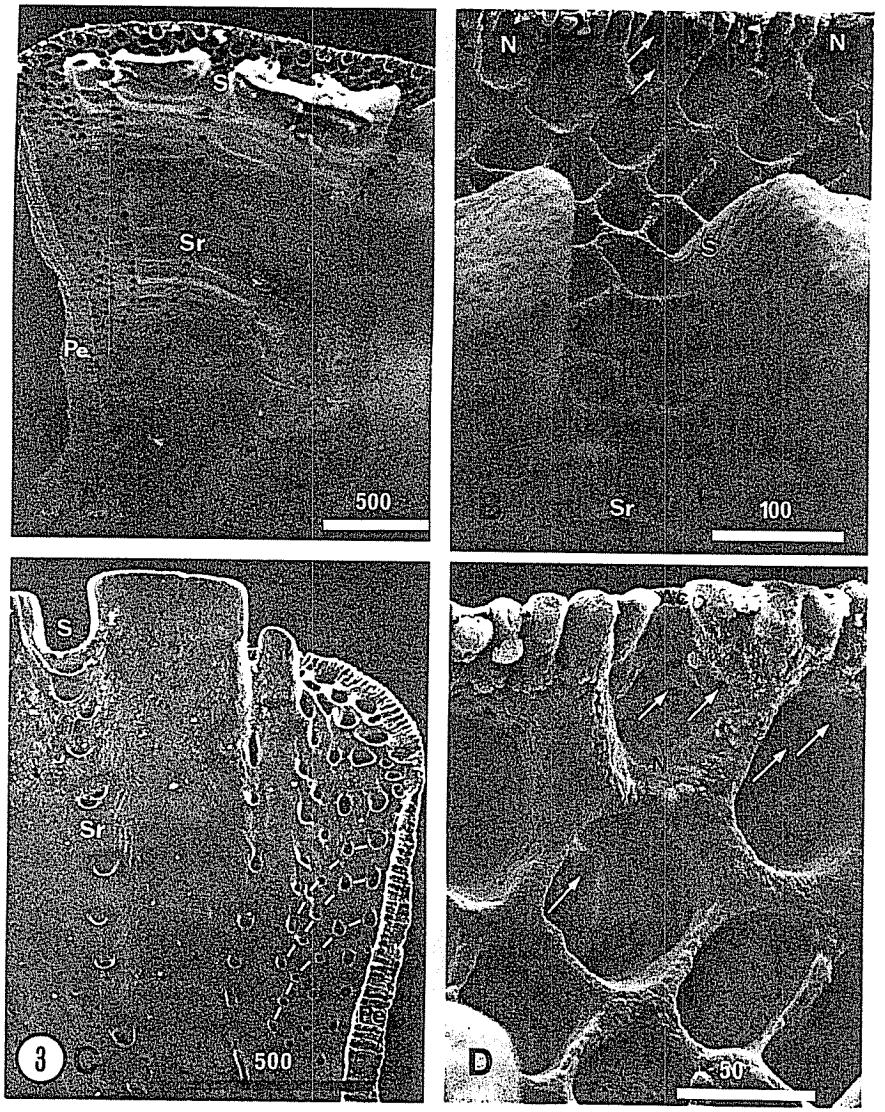



Fig. 3. *Callochiton achatinus* (SEM). **A:** The lateral eave tissue and posterior edge of an intermediate valve viewed from the ventral side showing the arrangements of holes penetrating these surfaces. **B:** The eave tissue in the region of a slit showing the diamond-grid pattern of holes and esthetes in early stages of formation near the dorsal surface. **C:** The posterior edge of the ventral surface of an intermediate valve with curving roles of holes mirroring the pattern on the dorsal surface in this region. **D:** A partially formed esthete complex exposed at the growing edge in the process of forming the apical cap; the number of micresthete channel openings visible in each hole declines as the latter pass ventrally. All scales in micrometers. Ac, apical cap; N, partially formed esthete complex; Pe, posterior edge; S, slit; Sr, slit ray; arrows, micresthete openings; dashed lines, alignment of holes on ventral surface at posterior edge of valve. (From Baxter and Jones, 1984.)



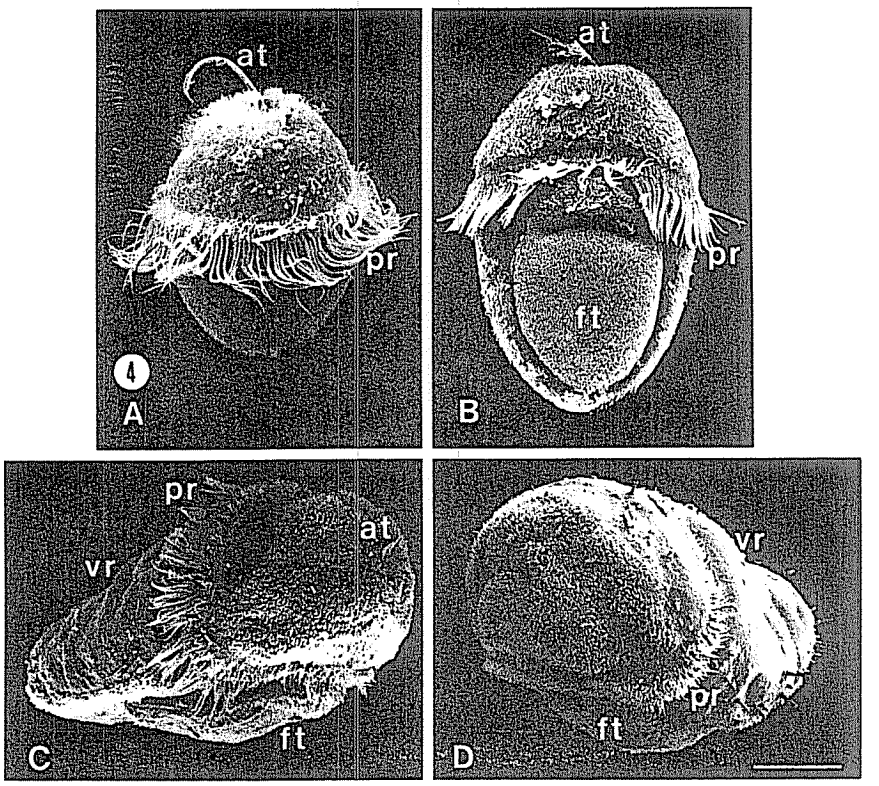



Fig. 4. Larvae near stage of hatching. SEM. **A:** *Lepidochitona hartwegii* (approximately 2 days old, fixed approximately 12 hours after synchronous hatching of a culture from one free-spawning female. **B:** *Lepidochitona caverna* (approximately 7 days old). **C:** *Lepidochitona thomasi* (approximately 9 days old). **D:** *Lepidochitona fernaldi* (approximately 9 days old). at, apical tuft; ft, foot; pr, prototroch; vr, valve rudiments. Scale bars: A–D = 50 μm. (From Eernisse, 1988.)

tegmental layer, other chiton shell layers are pure aragonite.

Shell formation begins dramatically at metamorphosis. Newly metamorphosed juveniles rapidly change their body profile, from flexible elongate creeping trochophore larvae (Fig. 4) to rigid oval discs (Fig. 5). By metamorphosis, they are already well fortified with girdle spines (Fig. 5G,H). As confirmed by polarized light microcope and SEM observations (Kniprath, 1980; Eernisse, unpublished and herein) (Fig. 5), valves develop only posterior to the recently discarded prototroch (cf. Naef, 1924–1926), in eight plate fields separated from each other by six, later seven, higher transverse epithelial ridges. The head valve will eventually grow to cover a porous, uncalcified precursor layer that extends into the pretrochal region (Fig. 5A,D). Shells will have grown to near full coverage of the dorsal surface by the time the eighth, most posterior, valve appears. Salvini-Plawen (1985) linked the late appearance of an eighth valve to a Paleozoic group of chitons,

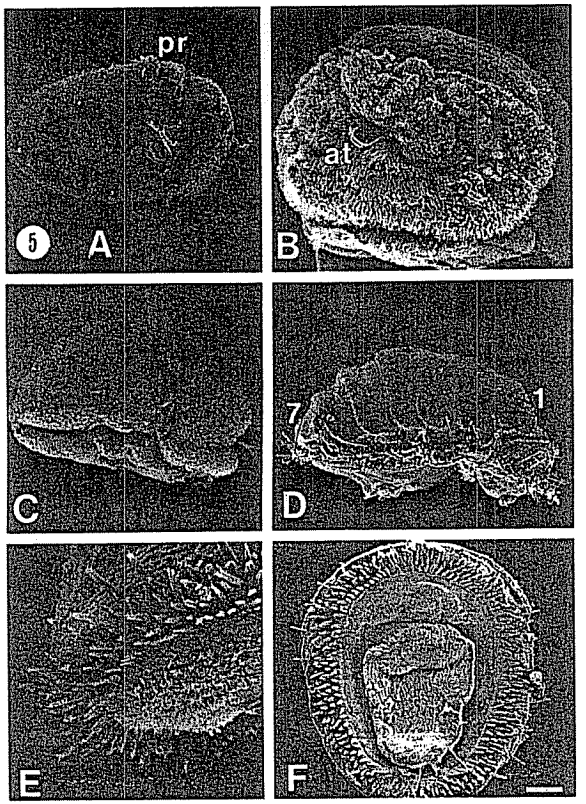



Fig. 5. Pre- and postmetamorphic larval/juvenile *Lepidochitona thomasi*. SEM. (See Eernisse, 1988, for details of laboratory rearing procedures). **A:** Late stage larva (exact age unknown) in dorsal view, fixed soon after collection from previously undisturbed brooder. Note porous valve precursors which are still uncalcified. **B:** Anterior view of larva at less than one day from hatching (approximately 10 days; see Fig. 4C). **C:** Lateral view of metamorphosing larva/juvenile of same age as in B. Note the partially discarded prototroch and uncalcified valve ridges. Foot is lowermost. **D:** Lateral view of juvenile less than 8 days from hatching. Note calcification of valves 1 to 7, not yet completely covering lateral and anterior porous shell precursor. Valve 1 is starting to extend over pretrochal region. Juveniles of this age are already active feeders. **E:** Ventrolateral view of juvenile collected as in A. Heavy spicule band believed to correspond to the marginal girdle spines of adults. **F:** Ventral view of juvenile of age as in D. at, apical tuft; pr, prototroch; 1, valve 1 precursor (head); 7, valve 7 precursor. Scale bars: D = 32 μm; F = 40 μm; A–C, E, unavailable. (Courtesy of D.J. Eernisse.)

Septemchitonidae, originally described with only seven valves. Rolfe (1981) had already noted that Septemchitonidae's "missing" valve was a previously unrecognized reduced head valve, thus having nothing to do with the eighth-appearing juvenile tail valve (Strathmann and Eernisse, 1987).

The early juvenile shells are produced by two cell types, "goblet" and "marginal" cells, acting typically to produce girdle cuticle. The shell material gradually extends anteriorly and posteriorly within each plate field (Kniprath, 1980). The stragulum, formed by long microvilli of bordering epithelial cells, completely covers and seals the plate field's crystallization chamber from the external seawater environment. At first only tegmentum is produced; then the central cells of the plate field flatten and the hypostracum is added. Under normal culture conditions, the valves are originally rod shaped, not composed of several granules (Kniprath, 1980). This observation contradicts a widely cited observation by Kowalevsky (1883) of "abnormal" early spiculose valves in some postmetamorphic chitons, which Kniprath (1980) instead attributed to the often high culture temperatures Kowalevsky was forced to use. Carter and Aller (1975) reviewed the long-popular hypothesis (Blumrich, 1891) that chiton shell valves have originated by fusion of ancestral cuticular spines, proposed more recently by Pojeta (1980) and especially Salvini-Plawen (1985). Salvini-Plawen compared the seven supposed spiculose ridges to seven spicule fields inferred from the famous, yet still unconfirmed, larval aplacophoran drawing and description of Pruvot (1891; redrawn in dorsal perspective by Salvini-Plawen based on Pruvot's narrative description). Carter and Hall (1990) proposed that chiton valves could also have originated through a modification of the mechanism of spicular calcification, rather than spicule fusion, to form subcuticular shell plates.

There are important differences in conchiferan and polyplacophoran shell formation and composition. Larval conchiferans typically have a discrete shell gland with a true pellicle, termed the periostracum, formed at the distal edge of the gland. This provides a crystallization chamber, with the outer shell layer precipitated against the periostracum, closed off to external influences. In constast, chiton shell formation occurs under a thin layer of cuticle across a broad plate field (Fig. 5), with a crystallization chamber provided by this and by the stragulum (Haas, 1981). Noting these differences, Haas (1981) and coworkers (Haas et al., 1979) have nevertheless concluded that chiton and conchiferan shell formation are fundamentally alike. By their interpretation, specialization of epithelial cellular surfaces proximal to the chiton shell plate indicates the presence of a "properiostracal groove," a more primitive, but homologous, type of shell-secreting system that produces an organic, weakly polymerized pellicle or "properiostracum" whose protein component might have some limited propensity for later action by tanning agents. They also hypothesized that the region between the proximal wall of the properiostracal groove and the shell edge was homologous with the outer mantle fold of the Conchifera, as earlier proposed by Haas (1972) and Stasek (1972) (see also Beedham and Trueman, 1967). Scheltema (1988) has presented an alternative view that emphasizes the different nature of shell formation in chitons and conchiferans, favoring an independent origin of shells in these groups.

Chiton valves and spicules are both completely aragonitic (reviewed by Haas, 1981; Scheltema, 1988; Carter and Hall, 1990). Variation in chiton valve crystalline structure has not been explored to nearly the extent that it has for bivalves and gastropods (Carter and Hall, 1990). Nevertheless, the few examined extant species all have cross-lamellar layers that are fundamentally different from the cross-lamellar shell layers of some advanced gastropods. Fully exposed, the chiton "properiostracum" outer layer is usually difficult even to document as present or distinct from the next layer, the tegmentum. The most complete characterizations are for *Tonicella marmorea* (Baxter and Jones, 1987), whose properiostracum is composed of a single layer of homogenous material only about 0.1 μm thick, and *Callochiton achatinus* (Baxter et al., 1990), whose properiostracum has a trilaminate composition up to about 20 μm thick. The latter has a thick central fibrous layer sandwiched between thin outer and inner layers of homogenous electron-dense material, the inner layer quite similar to the single layer of *Tonicella marmorea*. Baxter et al. (1990) relate the unusually thick properiostracum of *Callochiton achatinus* to their proposal of an active properiostracum secretion role for esthete organs. The inorganic portion

of the tegmentum varies from composite prismatic to irregular spherulitic prismatic (Haas, 1972, 1976), and this matrix supports an extensive system of sensory or secretory organs and innervations. Haas collectively referred to the remaining shell layers, which are much thicker than the tegmentum, as "p. [polyplacophoran] hypostracum." He found that this hypostracum is composed of irregular spherulitic prismatic, crossed lamellar, and irregular simple prismatic structures. Carter and Hall (1990) make more detailed characterizations of regional shell ultrastructure in the relatively large-shelled *Acanthopleura granulata*. According to their description, insertion plate extensions of the hypostracum known as the articulamentum have a predominantly rod-type crossed lamellar to crossed acicular structure. Most ventral is the myostracum layer of the hypostracum, to which muscles attach. The myostracum structure varies from irregular simple prismatic to homogeneous.

Crossed lamellar shells are known from certain, often derived, groups of gastropods, bivalves, and scaphopods. The crossed lamellar structure observed in chitons is unlike these shells in having third-order lamellae that are rod-like and not comprised of well-defined, laminar second-order lamellae. The articulamentum has been considered lacking in all ancient (i.e., pre-Devonian) chitons (Bergenhayn, 1930; Runnegar et al., 1979), with the only ultrastructural study of a Paleozoic "chiton" (Runnegar et al., 1979; Plate 1, Fig. 22) revealing a vastly different composition from that of living chitons. Nothing is known of shell ultrastructure of Devonian to early Cenozoic chiton fossils (Carter and Hall, 1990). A report of a calcium phosphatic shell structure in certain Paleozoic chiton fossils (Bischoff, 1981) could be alternatively interpreted as due to secondary replacement (Carter and Hall, 1990).

Chiton valves are divided into areas characterized by distinctive sculpturing (Bergenhayn, 1930), which also coincides with patterns of esthete innervation (e.g., Knorre, 1925; Baxter and Jones, 1984). The members of Acanthochitonina differ from other chitons in generally lacking the division of the tegmentum into central and lateral areas (e.g., Fig. 1A vs. Fig. 2A), in having a more pronounced median (jugal) region, and in having large pustular tegmental sculpturing. Members of Lepidopleurina differ in their generally poorly developed or at least unslit lateral and terminal insertion plates (Laghi and Russo, 1981; Fischer, 1988). In other living chitons, these insertion plates extend laterally from the articulamentum layer, anchoring each valve firmly in the girdle, and have distinct slits corresponding to major paths of esthete innervation.

### Girdle

The girdle typically has numerous calcareous or corneous elements scattered across the dorsal surface (Fig. 6). These may include imbricating scales, simple or well-developed spines (sometimes in tufts), strap-like or branching bristles, and hairs with clapper-like structures at their tip (Fischer et al., 1988) (Fig. 7). There is usually (perhaps always) a fringe of marginal spines, which are especially conspicuous in postmetamorphic juveniles (Fig. 5G,H). The flat ventral girdle surface usually has rectangular, close-packed, calcareous scales, except in members of *Ferreirella* (Fig. 1C), which lack ventral girdle calcareous elements (Ferreira, 1980; 1986; Sirenko, 1988; Jones and Gowlett-Holmes, 1992). Among or associated with especially the dorsal inorganic elements are sensory structures, in many ways similar to the tegmental esthetes, discussed in detail later as conspicuous elements of the nervous system.

### Epidermis

Early examinations of chiton integument include those by Blumrich (1891), Thiele (1891, 1892a,b), Prenant (1923), Frieboes (1923), and especially Knorre (1925), partially reviewed by Hyman (1967). More recently, Beedham and Trueman (1967, 1968, 1969), Stasek and McWilliams (1973), Haas and Kriesten (1974, 1975, 1977), and Haas (1981) have characterized the integument and its secretory products in comparison to other molluscs.



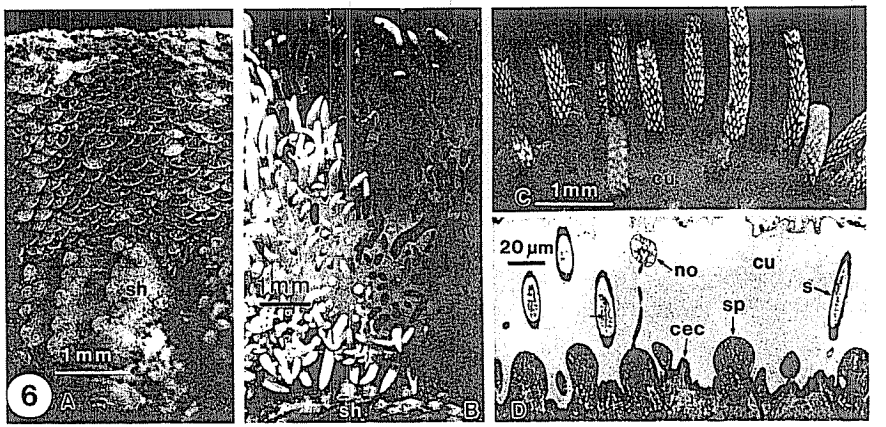



Fig. 6. Variation in girdle elements **A:** Dorsal integument of *Lepidozona cooperi* demonstrating overlapping scales. **B:** Dorsal integument of *Acanthopleura granulata* displaying calcareous spines. Cuticle is visible between spines. **C:** Dorsal hairs of *Placiphorella velata*. Numerous spicules (arrows) are embedded near the surface of each hair. A–C, SEM. cu, cuticle; sh, shell. **D:** Transverse 1 µm section through the decalcified integument of *Mopalia muscosa*. Spicules (s) produced by spiniferous papillae (sp) contain brown pigment granules (arrow). One spiniferous papilla has produced a sensory nodule (no). Part of its stalk is not in the plane of this section. Common epidermal cells (cec) occur between papillae (A–C, from Leise, 1988; D, from Leise and Cloney, 1982.)

The integumentary structures of chitons vary by region. Encircling and anchoring the eight shell valves is the girdle or perinotum, consisting of mantle epidermis covered by a thick glycoproteinaceous cuticle in which are embedded calcareous (aragonitic) spines, scales, or corneous bristles. There are epidermal pockets at the mantle edge that secrete a fine properiostracum (Haas, 1981) over the dorsal valve surface. The mantle epidermis is continuous with a thin epithelium in close contact with the valve undersurface and extending through pores to the dorsal esthete organs. The girdle epidermis is covered by unusual packets of secretory cells, similar to those in aplacophorans (Hoffman, 1949). The clusters of tall, spaced cells surround one or two low epidermal cells and together secrete the various types of girdle elements already described, the form and location of which vary with species.

As the epidermis extends beyond the mantle to the pallial groove, the cuticle is absent and the cells become cylindrical and heavily ciliated, increasingly interspersed with mucous goblet cells toward the foot. In places these form tracts of tall gland cells, intermixed with peculiar, distally expanding, ciliated cells, which Knorre (1925) has pointed out have great similarity to the wall of the shell gland in aplacophorans. The foot epidermis consists of slender ciliated epithelial cells interspersed with both secretory and adhesive gland cells (Knorre, 1925; Högland and Rahemtulla, 1977).

## MUSCLE AND CONNECTIVE TISSUE

A chiton attaches to the underlying substrate using its single large ventral foot, aided by mucous secretion, muscular action, and the surrounding ventral surface of the muscular girdle (Fig. 1C–E). Movement occurs by coordinated waves of opposing muscular foot

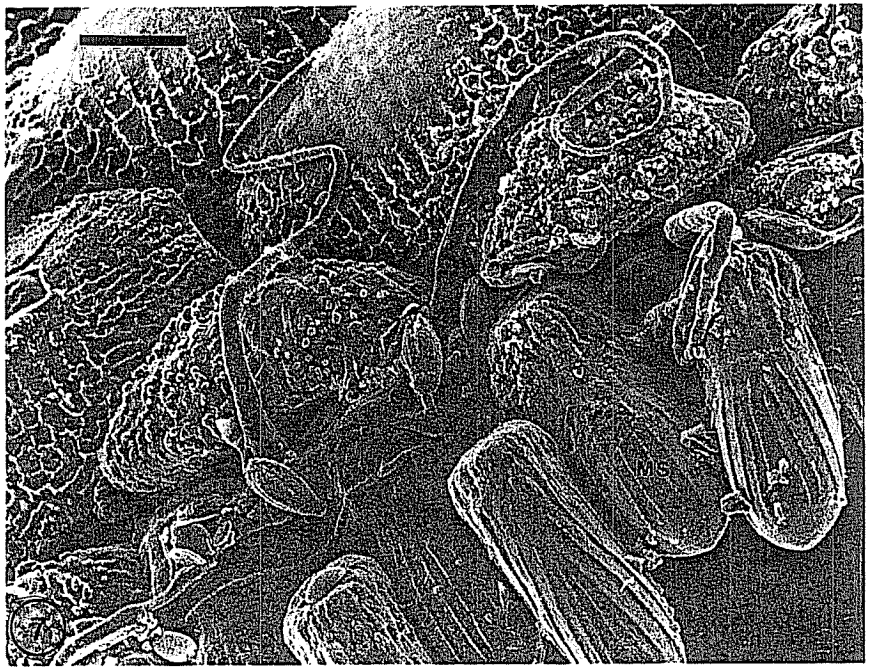



Fig. 7. *Rhyssoplax olivacea*. The girdle margin. SEM. CL, clapper; DS, dorsal scale; H, hair; MS, marginal spine. Scale bar = 20 μm. (From Fischer et al., 1988.)

contractions (Sampson, 1894, 1895; Voltzow, 1988) to enable a forward or backward sliding motion. The foot musculature is exceedingly complex, and has not been well-studied at an ultrastructural level. The musculature involved with shell articulation and movement was described by Sampson (1894, 1895) and Henrici (1912), while girdle muscle was examined by Castello (1973) and that of the radular apparatus by Plate (1896). The latter "buccal" musculature is notable for its high concentrations of myoglobin, which colors the muscles a deep red. Extending earlier studies, Wingstrand (1985) has made careful comparisons of the major chiton muscle systems to the corresponding ones of monoplacophorans.

The anatomical organization of chiton muscle groups has been summarized by Hyman (1967). There are four main groups of shell muscles: the rectus muscles (which are median narrow muscle bands that originate from the body wall beneath one valve and insert on the jugal sinus of the following valve), the broader oblique muscles (which have a similar insertion but originate in the lateral body wall), the dorsoventral transverse muscles (which are found between the imbricated valves), and the lateral longitudinal muscle (which runs along the outer edge of all valves). In addition, six bundles of muscle fibers pass from each valve into the foot, and many fibers pass from the valves into the mantle, which can be distinguished as inner and outer mantle muscles (Hyman, 1967).

Fischer (personal communication) has examined the ultrastructure of muscle in *Lepidochitona monterosatoi* and distinguished two types: bright red musculature of the buccal region (Fig. 8A) and white muscle (Fig. 8B) in the rest of the body. Ultrastructurally, both cell types possess elongate cells with central or peripherally located nuclei, and no systematic arrangement of thick and thin myofilaments. Furthermore, collagen was found between muscle cells of both types, with no specialized cell contacts between individual muscle cells observed. The thick filaments of buccal muscle have a patchy distribution, separated by clear cytoplasm or clusters of mitochondria, and have a diameter of 20–40 nm with no regular periodicity. Smooth endoplasmic reticulum (SER) penetrates both contractile and noncontractile myoplasm and forms subsarcolemmal cisternae. Buccal muscle cells are also characterized by the presence of many electron-dense bodies, associated with the cell membrane or free in the cytoplasm (Fig. 8A). White body musculature, in contrast, has much thicker filaments (45–70 nm), with a more rigidly parallel orientation and little cytoplasm or mitochondria between filaments (Fig. 8B).



## DIGESTIVE SYSTEM

During feeding, a chiton extends the anterior portion of a long rasping feeding organ, the radula, and a sensory tongue-like subradular organ (Fig. 9). Chitons eat a varied diet consisting mostly of diatoms, detritus, fleshy algae, or encrusting colonial animals. Specialists on sponges, crustose corallines, kelp or sea grasses are not uncommon. Members of *Ferreirella* (Fig. 1C) are specialist feeders on sunken wood, usually at great depths (Sirenko, 1988), while other chitons live associated with hydrothermal vents (Saito and Okutani, 1990). *Placiphorella velata* is unusual in its carnivorous habit of entrapping free-swimming crustacean prey with a rapid clamping movement of its anterior mantle edge (McLean, 1962). Members of genera in two other families, *Loricella* and *Spongiochiton* (= *Craspedochiton*), have a similarly expanded anterior mantle flap and may feed in a similar manner as ambush predators (Saito and Okutani, 1992).

The digestive system, which accounts for a large portion of the visceral mass, lies ventral to the gonad in the hemocoel. Chitons have a straight anterior to posterior digestive tract, except for the substantial looping of the intestine. The most complete treatment of the polyplacophoran digestive system is Fretter's comparative study (1937) of four species; the digestive tract physiology has been studied (Greenfield, 1972), but ultrastructural studies are lacking. The mouth is located ventrally at the center of the "head," which is separated from the foot and surrounded by a preoral unpaired veil of mantle tissue that Wingstrand (1985) compared with the "velum" of *Neopilina*. The mouth opens into a short buccal tube or cavity, from which the blind subradular and radular sacs extend posteriorly (Fig. 9). The subradular organ lies within the subradular sac; as the muscular organ is protruded like a tongue prior to feeding, it has been presumed to "taste" the substrate (Heath, 1903). This contact chemosensory function has been deduced from direct observations of feeding and is consistent with ultrastructural observations. Boyle (1975) described microvillous epithelial cells with electron-opaque secretory granules, interspersed with ciliated cells. Nerves extend from the epithelium to the paired subradular ganglia, among the most obvious concentrations of nervous tissue in chitons. Above the subradular sac and projecting into the buccal cavity, the radula is enclosed within a sheath of connective tissue, the radular sac, and supported by the odontophore (see below).

The pharynx is posterior to the radula (Fig. 9) and receives the secretions of the buccal glands (termed the salivary glands by Fretter, 1937), which are compound in larger species. A mucous groove and ciliated infoldings of unknown function are also found in this region. More posteriorly, the wall of the digestive tract is usually expanded to form lateral diverticula. These pharyngeal glands,

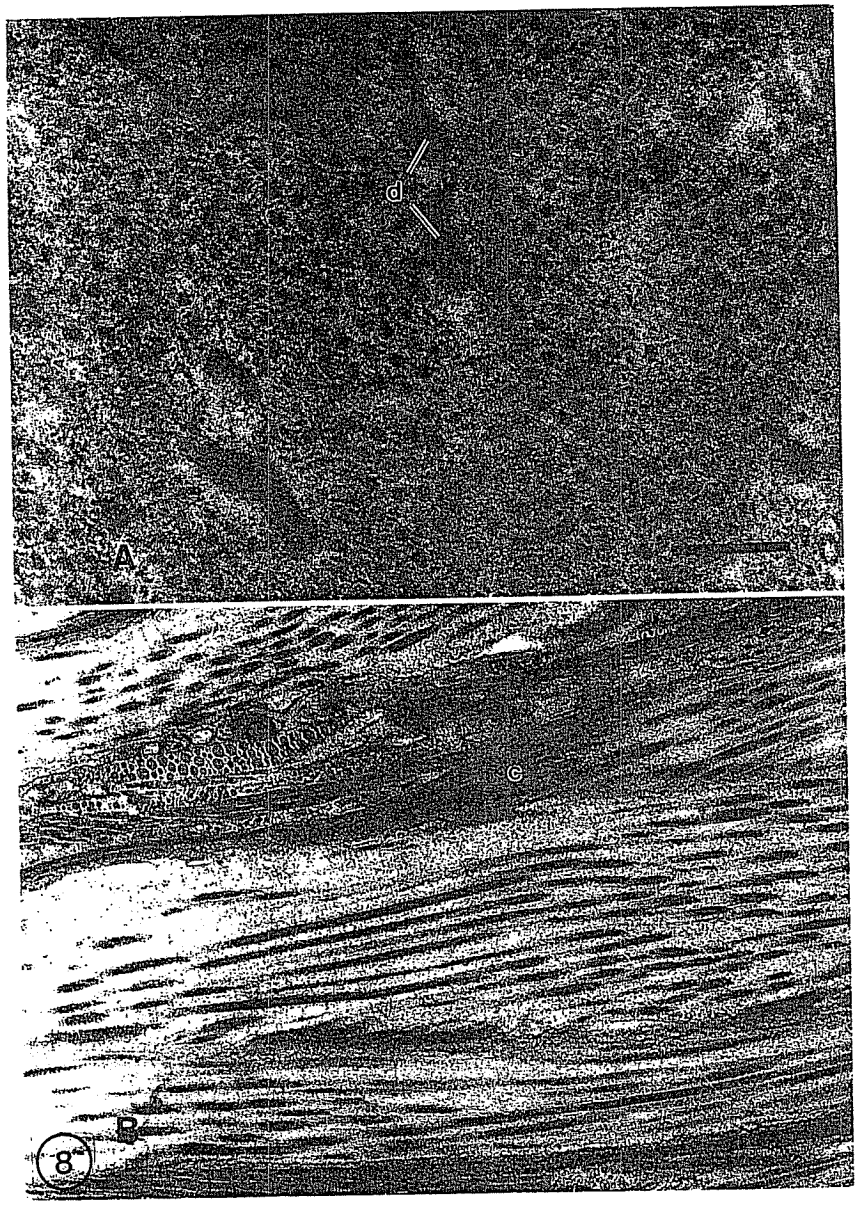



Fig. 8. Muscle from *Lepidochitona monterosatoi*. **A:** Cross section through a "red" muscle fiber of the buccal region. Note arrangement of thick and thin filaments and dense bodies (d). TEM. Scale bar = 0.2 μm. **B:** Longitudinal section through a "white" muscle fiber, with surrounding collagen (c). TEM. Scale bar = 1 μm. (Both courtesy of F.P. Fischer.)



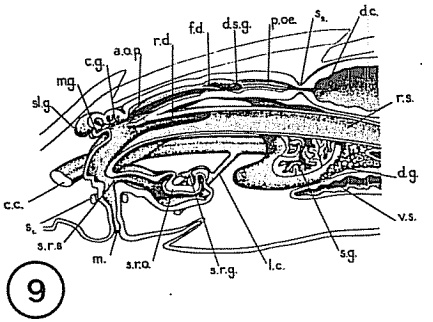



Fig. 9. Sagittal section of the anterior part of the alimentary canal of *Lepidochitona cinerea*. All cut surfaces are unshaded. a.o.p., anterior esophageal pouch; c.c., cerebral commissure (= cerebrobuccal ring); c.g., ciliated groove; d.c., dorsal channel; d.g., digestive gland; d.s.g., duct of sugar gland; f.d., fold dividing posterior esophageal pouch; l.c., labial commissure (= subcerebral commissure); m., mouth; m.g., mucous gutter; p.oe., posterior esophagus; r.d., diverticulum of radular sac; r.s., radula sac; $s_1$., sphincter muscle, which closes passage from mouth to buccal cavity; $s_2$., sphincter muscle, which closes passage between posterior esophagus and dorsal channel; s.g., sugar gland; sl.g., salivary gland; s.r.g., subradular ganglion; s.r.o., subradular organ; s.r.s., opening of subradular organ into buccal cavity; v.s., ventral sac of stomach. ×9.7 (approximately). (From Fretter, 1937.)

also referred to as "sugar" or "oesophageal" glands by Fretter (1937) and "pharyngeal" or "salivary" glands by Hyman (1967), open into the esophagus but are constricted from the esophagus and not an integral part of it, as in the pharyngeal glands of prosobranchs (Fretter, personal communication). The lumen of the pharyngeal glands is thrown into numerous villi, each lined by glandular epithelium that produces carbohydrate-digesting enzymes (Meeuse and Fluegel, 1958, 1959). Wingstrand (1985) hypothesized the monoplacophoran pharyngeal diverticula as homologous with the pharyngeal glands of chitons. Based on inferior material, Lemche and Wingstrand (1959) had originally reported these structures as separate smaller pharyngeal diverticula and larger "coelomic sacs."

The esophagus runs a short distance before being separated from the stomach by a sphincter (Fig. 9). The stomach is a large and muscular though flexible sac and is shaped largely by the surrounding lobes of the midgut or digestive gland. Two ciliated bands run longitudinally along the dorsal wall of the stomach, extending into the ciliated intestine. The digestive gland is bilobed, the right lobe extends anteriorly over and around the anterior stomach, while the larger left lobe is located over the posterior stomach and among the coils of the intestine. Histological findings suggest that the cells of the digestive glands are composed of digestive and basophilic cells with calcareous granules (Fretter, 1937), similar to the digestive glands examined by electron microscopy in other molluscan groups (e.g., gastropods: Mason and Simkiss, 1982; Mason et al., 1984; bivalves: Owen, 1970; scaphopods: Reynolds, 1991).

The intestine is thrown into several loops, and an anterior and posterior portion are separated by a valve. The two longitudinal ciliated bands of the stomach continue into the anterior intestine, which histologically resembles the stomach (Fretter, 1937). The intestinal valve was described by Fretter (1937) as consisting of distinct anterior and posterior regions. The anterior region is a section of intestine that is ciliated and longitudinally ridged, with a layer of circular muscle and some longitudinal fibers embedded in collagen beneath the epithelium. Dilation of this region can at least equal that of the anterior intestine, while contraction of valve musculature obliterates the lumen. The posterior region of the valve is a bulbous expansion, which is also muscular, ciliated and longitudinally ridged, and separated from the posterior intestine by a constriction. Contraction of the anterior valve musculature isolates a portion of the food string in the posterior valve, which then rotates the material into a firm fecal pellet (Fretter, 1937). The posterior intestine is extensively coiled and lined with ciliated and mucus-producing epithelium. It leads to the rectum, a short ciliated tube that passes through body musculature and opens to the exterior through the anus, located at the posterior end of the ventral surface of the body.

## RADULA

By molluscan standards, radular variation in living chitons is slight. The radula nearly always has 17 (rarely 15 or 13) teeth or plates per row, with approximately 25 to 150 rows.

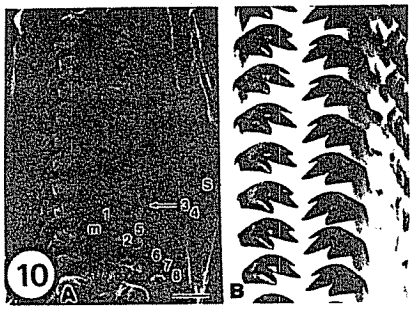



Fig. 10. Chiton radulae. **A:** Anterior one-fifth of radula of *Nuttallina californica* (Gaviota, California; DJE coll.). m. medial tooth, 1–8, lateral teeth or plates 1–8; s, membranous radular sheath. Note wear of prominent second laterals at the anterior end. SEM. Scale bar: = 300 μm. **B:** A part of the radula of *Cryptochiton stelleri:* eight rows of paired mature major lateral tooth caps. The marginal teeth show increasing pigmentation toward the anterior of the radula (top). Scale unknown. SEM. (A, courtesy of D.J. Eernisse; B, from Nesson and Lowenstam, 1985.)

Each row has conspicuous paired black denticle-capped major lateral (second) teeth, which are among the first three (besides fifth and eighth lateral) pairs of teeth to appear in early postmetamorphic juveniles (Eernisse and Kerth, 1988). The radula (Fig. 10A) is supported by a complex muscular buccal mass (Fig. 9), with antagonistic attachments enabling the radula to be rocked over a cartilaginous odontophore at its most anterior exposed end, very similar to the apparatus found in monoplacophorans and patellogastropod limpets (Wingstrand, 1985). Teeth are alternately splayed open or rolled back into a tube that articulates like a zipper. The biting action varies, depending on diet and the orientation of the major lateral teeth (Bullock, 1988), but the effective feeding action usually is a combination of posterior to anterior scraping, combined with rolling the opposed teeth together to scrape or tear off chunks of food, which then are moved up internally into the overlying esophagus. The teeth are already functional within a day or two of metamorphosis, with early tooth rows lacking the full 17 teeth per row (Eernise and Kerth, 1988).

The mineralization of each row's black-capped second or major lateral teeth is noteworthy, because these teeth are covered by a thick denticle coating of magnetite ($FeO \cdot Fe_2O_3$), a magnetic ferrous mineral, which provides tooth hardening. Biogenic magnetite was first identified in chitons (Lowenstam, 1962) and is otherwise only known to be produced in trace amounts in relatively few other biotic sources (e.g., birds and insects that are known to exhibit a magnetite-based magnetoreception). In radular teeth, the magnetite occurs as an ordered matrix of organic fibrils (Runham, 1963; Carefoot, 1965; Towe and Lowenstam, 1967; Kirschvink and Lowenstam, 1979; Lowenstam and Weiner, 1985), deposited first as ferrihydrite distributed within the organic tooth matrix and then transformed into magnetite.

On the chiton radula, magnetite is restricted to the caps of the disproportionately large major lateral teeth (Nesson and Lowenstam, 1985). The magnetite-covered cusps are typically one- to four-cusped or shovel shaped, quite commonly with prominent tricuspid shiny black caps as in the major lateral cusps of *Nuttallina* and *Cryptochiton* (Fig. 10A,B, respectively). The magnetite caps another ferric mineral, reddish lepidocrocite (γ-FeO[OH]), which itself overlies the greyish basal tooth layers, composed of polysaccaride, chitin, and approximately 10% by weight protein in close association with a calcium biomineral, hydroxyapatite ($Ca_5OH[PO_4]_3$), which may be fluoride or carbonate substituted (Lowenstam and Weiner, 1985, 1989; Evans et al., 1990, 1991, 1992). Some patellogastropod limpet radular teeth, which have a similar role in benthic feeding, are capped by another ferric mineral tooth covering, goethite, which is nonmagnetic (Lowenstam, 1962; Runham et al., 1969). Goethite is also present in at least some chiton teeth (Kim et al., 1989).

The elongate fifth lateral teeth appear to be closely associated with the major lateral cusps (Fig. 10A), perhaps protecting soft tissues from contact with sharp teeth (Eernisse and Kerth, 1988). New rows of teeth are continuously produced from the posterior to replace the worn rows gradually sloughed off at the exposed anterior end (Fig. 10A). Teeth are calcified after most iron deposition has occurred, approximately halfway along the radula (Kim et al., 1986). The zone of mineralization is more posterior within the radular sac and extends over at least 10 rows of teeth in *Lepidochitona hartwegii* (Nesson and Lowenstam, 1985) (Fig. 11A). Superior epithelial cells, with intracellular membrane-bound vesicles containing ferric granules obtained by endocytosis, are brought into contact with the cusps of the major laterals (Carefoot, 1965). This cellular iron was identified as partly ferritin, an iron transport protein present in the circulatory system, and partly as a less-ordered electron-dense particulate ferrihydrite material similar to vertebrate hemosiderin (Towe and Lowenstam, 1967; Webb and Macey, 1983; Kim et al., 1986; Evans et al., 1990, 1991). Polyplacophoran use of ferritin for large-scale iron transport is shared by at least the patellogastropod limpets mentioned above and by certain vertebrates such as rats and amphibians. The posterior radular sac has a dorsal sinus with cartilaginous support and, in most families (Plate, 1898–1901), lies entirely within a large blood vessel, the visceral artery, which receives oxygenated blood from the head sinus after it comes directly from the dorsal aorta. It is therefore likely that blood supply is ample within the zone of mineralization. Mineralization of the earliest formed tooth rows in chiton juveniles is unusual because zinc is incorporated, along with iron, into the mineral coatings of the second lateral cusps (Eernisse, unpublished data).

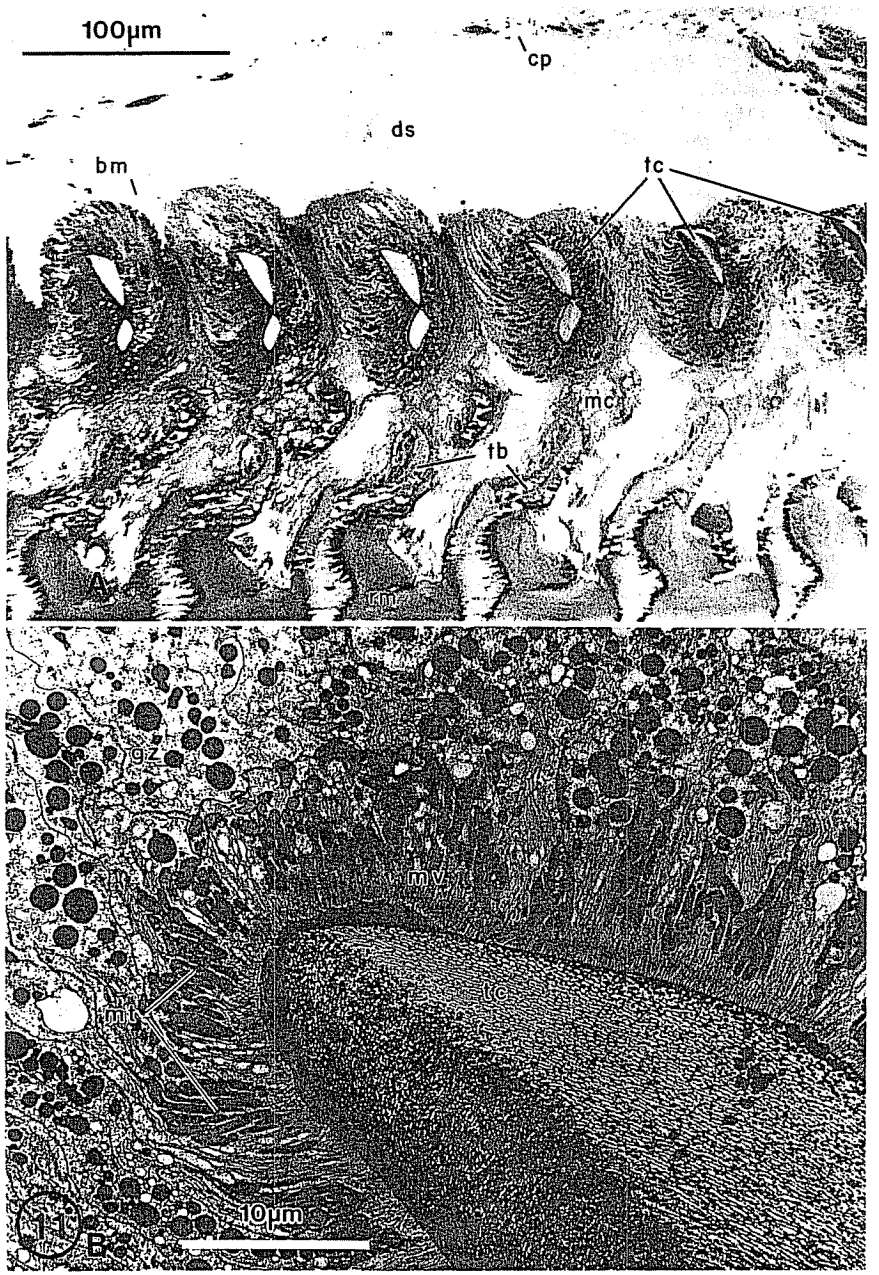
The cellular mechanism of mineralization is still partly unknown but involves the apical end of "cap" cells, each with bundles of 500–1,000 microvilli that arise in the apical mitochondrion-rich cytoplasm and, in an unusual manner, terminate at the surface of the tooth cap (Nesson and Lowenstam, 1985) (Fig. 11B). Specialized endoplasmic reticulum cisternae located in a specific region of the mineralization zone may prevent exocytosis of the iron-containing granules to the extracellular spaces, which instead must undergo terminal processing for secretion by the microvilli.



## RESPIRATION

The chiton mantle cavity is the continuous space surrounding the foot behind the oral region. Multiple adjacent gills are suspended in the mantle cavity and are connected by interlocking cilia to form paired continuous curtains. These gill rows may meet in the region posterior to the foot, as they do in the 100 or so members of Lepidopleurina (e.g., Fig. 1C), or remain well separated on either side of the foot, as they do in the remaining over 700 species of chitons (e.g., Fig. 1D,E). In juveniles, the first gill pair is added immediately posterior to the left and right renal openings; then, gills are added either to both the posterior and anterior (adanal condition; Fig. 1C,D) or the anterior only (abanal; Fig. 1E). Yonge (1939) considered the functional arrangement of gills in three chiton species, but most subsequent authors have cited only his first abanal example (*Lepidochitona cinerea*) as being typical for chitons (Fig. 1E). About one-third of all chitons have an abanal condition (Eernisse, 1984), and of the remaining adanal chitons about four-fifths have an arrangement of gills that is functionally similar to the abanal chitons in their lateral placement of gills alongside the foot with an interspace between the most posterior left and right gill series (Fig. 1D). Single representatives of disparate genera were shown to exhibit correlations between adult size and gill numbers and size, with larger chitons having longer gill rows composed of more gills, but each gill smaller relative to body size (Johnson, 1969). The same relationship was observed for closely related members of *Lepidochitona* that varied in adult size (Eernisse, 1984).

The following description of water flow is based on the data of Yonge (1939) and on direct observations (Eernisse, unpublished).






Both abanal and adanal chitons with lateral placement of gills circulate water into the pallial groove from raised areas of the mantle margin. Ctenidial cilia pump water through the gill filaments (Russell-Hunter, 1988), and water is actively expelled past the posterior anus. Certain chitons (e.g., *Mopalia ciliata*) have a terminal cleft in the girdle that can form a dorsally directed chimney, and this could conceivably direct exhalent flow beyond the thin boundary layer blanketing the generally flat animal. In species with lateral gills, the lateral regions of the mantle cavity are separated by the gill curtain into inhalent (distal) and exhalent (proximal) regions. Water can enter through one or more everchanging narrow channels formed by deformation of the mantle either toward the anterior, as observed by Yonge (1939), or anywhere to the extreme posterior of the inhalent chamber (Arey and Crozier, 1919; Eernisse, personal observation). Water flow appears to be mainly due to ciliary pumping, as it stops almost instantaneously if the animal is disturbed. At the posterior end, the inhalent and exhalent regions are kept separate either by the last and largest gill resting against a flap of the mantle (abanal species) or by an outward-turning series of ever-smaller gills (adanal species). In either case, the posterior end of the row seals the gill curtain against the outer mantle wall, defining the posterior extent of the inhalent region. The members of Lepidopleurina also add adanal gills, but these gills extend to form a continuous arch in the posterior mantle cavity Fig. 1C), as described by Yonge (1939) for *Leptochiton asellus*. The distinction between lepidopleurid and other chiton gill arrangements was confirmed for about one-fourth of all recognized chiton species surveyed for gill condition (Eernisse, 1984, 1985), including representatives of most genera (see also Pilsbry, 1892–1894; Pelseneer, 1897; Plate, 1898–1901). The lepidopleurid's continuous semicircular curtain of gills behind the anus necessarily involves a different division of the pallial cavity into inhalent and exhalent chambers. In lepidopleurids, the exhalent current still exits to the extreme posterior, but the dorsal roof of the cavity is part of the inhalent region. Moreover, the anterior part of the gill row (Fig. 1C) extends to only the same region that is most posterior in the majority of nonlepidopleurids (e.g., Fig. 1D,E). As observed in flow chambers with unobtrusive dyes, the circulation current velocities generated by *Leptochiton rugatus* were consistently lower than in various nonlepidopleurids similarly examined (Eernisse, unpublished data). Water flow patterns in lepidopleurids remain to be extensively investigated, as Yonge (1939) recognized in his pioneering study.

The fine structure of the chiton gill has been examined in only one species, *Rhyssoplax olivacea* (Fischer et al., 1990). The approximately 50 lamellae (26 μm mean length) alternately branch from a central axis (Figs. 12, 13A,B). The size of lamellae is similar regardless of age or size of gill; smaller gills of juveniles simply possess fewer lamellae. The columnar epithelial cells have a dense covering of microvilli, ciliated tufts are found scattered over the lamellar surface, and a band of relatively long cilia runs continuously along the center of each lamella

Fig. 11. Radula of *Lepidochitona hartwegii*. **A:** Longitudinal section through the early mineralization zone of the radula sac. From posterior to anterior (left to right), there are two colorless tooth caps (tc), two brown ferrihydrite-impregnated ones, and the first two magnetite-containing caps. Magnetite is visible as the dark material on the posterior surface of the anteriormost tooth cap. The plane of sectioning passes through two prongs of the tricuspid tooth caps. The basement material (bm) is discernible mainly where it has lifted off the superior epithelial cells during fixation. The cap cells (cc) form discrete tissue masses surrounding each cap, separated from each other by strands of minor cells (mc). Within the tissue mass, cap cells extend from the dorsal sinus (ds) to the tooth cap surface. cp, cartilaginous plate; rm, radula membrane; tb, tooth bases. One-micrometer Epon section; toluidine blue, azure II. **B:** A ferrihydrite-impregnated tooth cap (tc) and the apical pole of the cap cells. The cells terminate nearly perpedicularly to the tooth surface. The iron-containing electron-dense granules adjoin a mitochondrion-rich apical cytoplasm (mt), which forms into bundles of microvilli (mv) that extend directly to the tooth surface. The pattern of ferrihydrite deposits follows the underlying matrix fibers (which are visible toward the tooth tip). Note the much greater ferrihydrite density in the posterior half of the tooth. Observe the amorphous layer over the tooth tip and continuing along the posterior surface of the cap. gz, granule zone. TEM. (From Nesson and Lowenstam, 1985.)

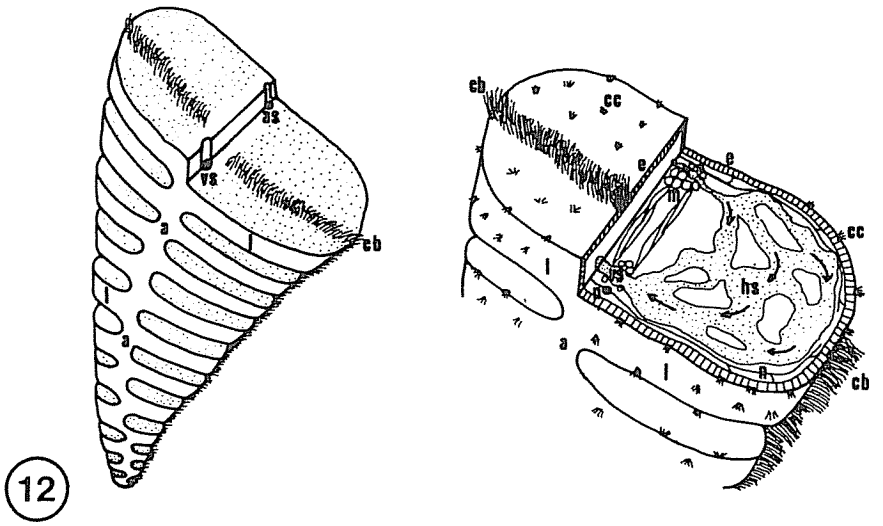



Fig. 12. *Rhyssoplax olivacea* gill. Left, detached gill; right, one gill lamella opened to show hemolymph sinuses and direction of hemolymph flow. a, main axis; as, arterial sinus; cb, ciliated band; cc, ciliated cell; e, epithelium; hs, hemolymph sinus; l, lamella; m, muscle cells; n, nerve; vs, venous sinus. (From Fischer et al., 1990.)

(Figs. 12, 13A,B). Ultrastructural features distinguish the ciliated cells, particularly those of the ciliary band, from unciliated epithelium. These include greater numbers of mitochondria, filamentous content of microvilli, glycogen stores, and consistent orientation of the ciliary central microtubule elements and of the long, anchored basal roots, all of which indicate active, aligned beating of the band cilia. Sparsely distributed interstitial cells, which possess long microtubule-filled processes toward the basal lamina, were found within the epithelium. Epithelial goblet cells, with membrane-delimited granules, are rare but present near the gill base.

Beneath the epithelium, a variety of connective tissue cells are associated with collagen fibers. Parallel bundles of thick collagen fibers with a concentration of typical smooth molluscan muscle cells (thick myofilament periodicity of 13 nm consistent with a paramyosin composition) are found in the main axis (Fischer et al., 1990). Scattered, thin collagen fibers and muscle cells are present in the lamellae, producing irregular hemolymph sinuses. Longitudinal arterial (inner) and venous (outer) sinuses run within the main axis (Fig. 12A) and supply and drain capillary sinuses within each lamella (Fig. 12B). The medial to lateral blood flow is a countercurrent to respiratory water movement within the chiton pallial cavity. Two main nerves extend through the gill axis adjacent to the main venous and arterial sinuses, from which branches extend into each lamella. Nerve processes that extend through the basal lamina are regularly found in close association with the interstitial cells and are suggested by Fischer et al. (1990) to mediate sensory reception in the gill epithelium.

## CIRCULATORY SYSTEM

Aspects of the circulatory system of chitons have been described at the histological level



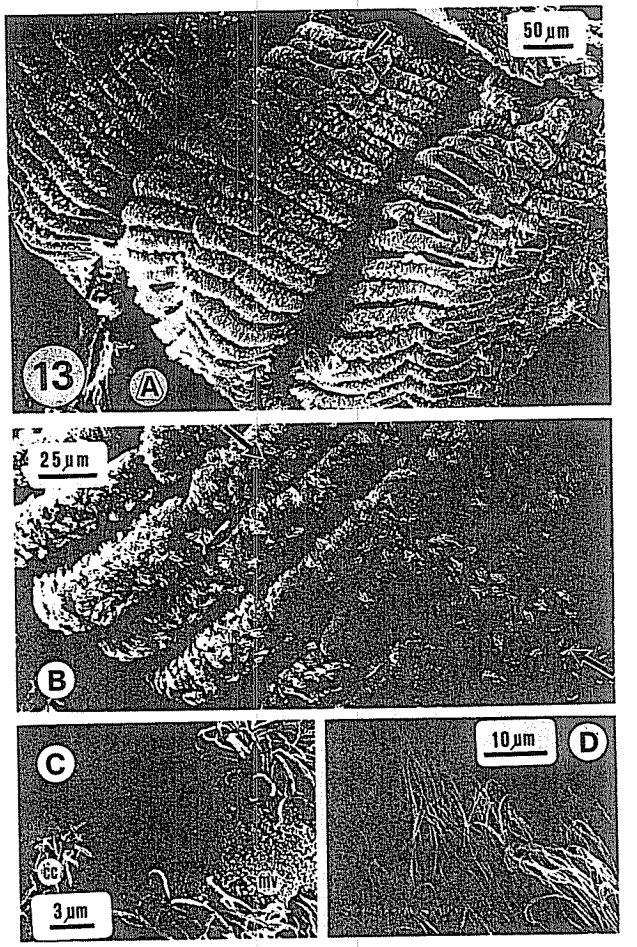



Fig. 13. *Rhyssoplax olivacea* gill. SEM. **A:** Lateral view of three gills. The girdle has been moved to permit access to the gills. Arrow, main axis; double headed arrows, ciliated band. **B:** Detail showing the alternating position of the lamellae in relation to the main axis. Near the main axis, ciliated cells are found less frequently than elsewhere. Arrows, main axis. **C:** Higher magnification of B. **D:** Detail of the ciliated band. cc, ciliated cell; c, cilia; gl, girdle (seen at top right); l, lamella; mv, microvilli. (From Fischer et al., 1990.)

by Haller (1882), Plate (1898–1901), Heath (1905), Seth et al. (1975), and Hyman (1967), who also reviewed earlier literature. Generally, the system consists of extensive hemocoelic sinuses, a few main channels, and a posterior, dorsal heart within a spacious pericardium that is attached dorsally to the body wall below the two posterior shell valves. As observed in other molluscan classes with the exception of the Cephalopoda, blood vessels are not lined by endothelium; nevertheless, channels are often dis-

tinguished by a surrounding layer of connective tissue. After passing though capillary sinuses within the gill lamellae, blood is passed via the venous sinus within each gill to the efferent branchial channels (see above) (Fischer et al., 1990) and then to the lateral auricles through the auricular pores. These number from two to several pairs, the first pair sometimes referred to as the arterial sinuses, and an additional median pore may be located posteriorly where the two auricles are confluent behind the blind posterior end of the median ventricle (Hyman, 1967; Seth et al., 1975). One to four pairs of muscular valves or auriculoventricular ostia lead from each auricle to the ventricle, but may differ in number between the two sides (Hyman, 1967).

The single dorsal aorta, which in *Chiton tuberculatus* divides into two (Seth et al., 1975), extends anteriorly from the ventricle to a position between the first two shell valves. Along its length, the aorta gives off channels ventrally to gonadal tissue, unpaired dorsal intersegmental, and paired lateral channels to valve muscles. The aorta opens anteriorly into the head sinus, surrounding the buccal apparatus, which is separated in most chitons from the visceral sinus by a connective tissue partition. From the head sinus, a number of main longitudinal channels arise. These are a median pedal sinus, which originates from the buccal ring sinus, and paired pedal and neuropedal sinuses; the latter surrounds the pedal nerve cords and may include a visceral artery that opens into the visceral sinus. Associated with the pallial groove are the neurolateral sinuses, surrounding the lateral nerve cords, and the afferent and efferent branchial vessels that serve the gills. Venous blood moves from the visceral sinus to the median pedal sinus and other channels within the connective tissue of the foot; posteriorly, these channels drain into the afferent branchial sinuses, with a pair of transverse sinuses usually joining the median sinus with the afferent branchial sinuses (Hyman, 1967). Hyman (1967) also discusses exceptions to this general arrangement, e.g., *Choneplax*, *Cryptoplax*, and *Cryptochiton*, all members of Acanthochitonina. The afferent branchial sinuses collect blood from other channels within the foot and give off a branch into each gill, the branchial arteries, which then supply the capillary sinuses within each gill lamella (Fischer et al., 1990).

Ultrastructural studies on the polyplacophoran circulatory system are limited to Økland's studies (1980, 1981) of the heart and pericardium. The cellular lining of the pericardial cavity is continuous, comprised of the pericardial endothelium and the ventricular and auricular epicardia (Økland, 1980). The fine structure of the heart wall in *Leptochiton asellus* and *Tonicella marmorea* follows the general pattern of most molluscan classes, with an epicardium successively underlain by basal lamina and a myocardium. An endocardium is absent. While the ventricular epicardium is composed of simple epithelium, podocytes are found within the auricular epicardium of both species (Fig. 14B). This suggests the auricles as the site of blood ultrafiltration in chitons and is discussed further in the next section.

The myocardium of the chiton heart is poorly developed in comparison with the larger molluscan classes. Only a few muscle bundles are present in auricular and ventricular myocardia and are predominantly found in longitudinal orientation in both species examined by Økland (1980), *Leptochiton asellus* and *Tonicella marmorea;* a similar arrangement is found in the neomenioid aplacophoran myocardium (Reynolds et al., in press). Bundles, each consisting of two to six fibers, are surrounded by basal lamina and are embedded in a collagen matrix. There is no evidence of coupling between muscle cells or of sarcomeral organization within fibers, although a loose alignment of dense bodies and attachment plaques occurs in a supercontracted state (Økland, 1980). Sarcoplasmic reticulum is well developed, extending between myofilaments. The ventricular myocardium is more developed than that of the auricles, being thicker and with dorsoventral



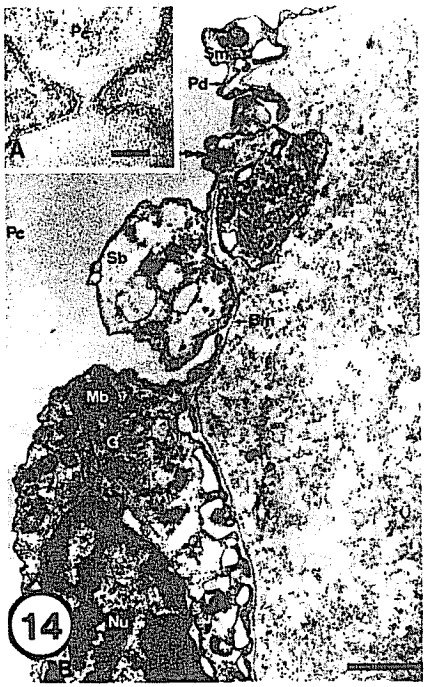



Fig. 14. *Leptochiton asellus* pericardium. TEM. **A:** An ultrafiltration slit, showing the lack of a diaphragm between two pedicels. Pc, pericardial cavity. Scale = 0.1 μm. **B:** Transverse section through the auricular wall showing an epicardial cell body. G, Golgi complex; Mb, membrane body; Nu, nucleus of epicardial cell. Stout branches (Sb) and small branches (Sm) with pedicels (Pd) radiate from the cell body. Inside the basal lamina (Bm) is seen a muscle fiber (Mf) and a nerve process (N). Note the desmosome (double-headed arrow) between the two cell processes and the lack of cover of the basal lamina (arrowhead). Pc, pericardial cavity. Scale bar = 0.5 μm. (From Økland, 1980.)

trabeculae crossing the ventricle lumen. Slight differences were noted between *Leptochiton asellus* and *Tonicella marmorea* in size of dense bodies, attachment plaques, thick filament diameter, and the presence of desmosomes and Golgi bodies (Økland, 1980). Innervation of the heart musculature in *Leptochiton asellus* and *Tonicella marmorea* is effected by neuromuscular junctions with several nerve processes that run within the collagen matrix surrounding muscle bundles or within the bundle itself.

The auriculoventricular ostia of *Leptochiton asellus* and *Tonicella marmorea* consist of tubes composed of several layers of muscle cells, tapering to a single cell layer as the tube extends into the ventricular lumen. In ultrastructural characteristics, the musculature resembles that of the ventricle (Økland, 1980). A similar study, at the light microscope level, is reported for *Chiton tuberculatus* by Seth et al. (1975).

The fine structure of the pericardium has been examined in five chiton species by Økland (1981). It is composed of simple, though irregularly branching, epithelial cells that are underlain by a basal lamina and a muscle layer embedded in a collagen matrix. There are two types of muscle cells: obliquely oriented smooth molluscan muscle and longitudinal muscle similar to auricular myocardial cells. Nerve processes with associated glio-interstitial cells are also found within the collagen matrix, and synaptic junctions with both types of muscle cells have been described but may be independently innervated (Økland, 1981). A contractile pericardium is also found in *Cryptochiton stelleri* (Greenberg, 1962), scaphopods (Reynolds, 1990), and neomenioid aplacophorans (Reynolds, et al., in press).

Heath (1905) characterized the general pattern of circulation of *Cryptochiton stelleri*, and Martin et al. (1958) estimated the blood volume to comprise about 44% ± 9% of the wet weight of *Cryptochiton stelleri* not including the valves. The respiratory pigment found in the hemolymph of chitons is hemocyanin (Manwell, 1958; Ryan et al., 1985; Hamilton et al., 1989). Myoglobins or hemoglobins also occur, especially in the buccal apparatus (Terwilliger and Read, 1969, 1970), but rarely also in other soft tissues (Eernisse et al., 1988). Two classes of cells have been distinguished in the hemolymph of chitons, leukocytes and leukoblasts, primarily on the basis of cytoplasm volume (Arvy and Gabe, 1949). Killby et al. (1973) studied

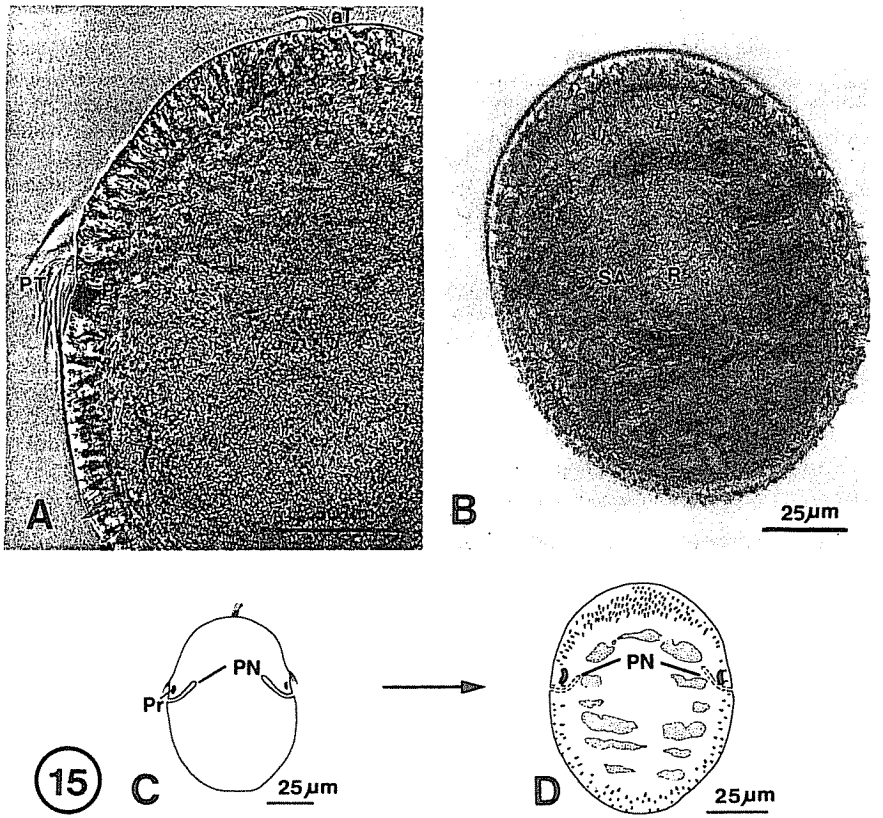



Fig. 15. *Lepidochitona cinerea* larval protonephridia. **A:** Trochophore larva. aT, apical tuft; Pr, larval ocelli; PT, prototroch; calcareous spicules are also visible about 1 hour after hatching. **B:** Older larva with the anlage of six shell plates (SA) and the radula-anlage (R). **C:** The protonephridia (PN) are situated almost at the height of the ocelli (Pr) of a newly hatched larva. **D:** In an older larva, the protonephridia (PN) are located underneath the anlage of the third shell plate. (From Bartolomaeus, 1989.)

the ultrastructure of phagocytic cells circulating in the bloodstream, but found also in foot and gill tissue, and suggested that these cells form part of an immune response system in chitons.

## EXCRETORY SYSTEM

The protonephridia of larval *Lepidochitona cinerea* (Figs. 15, 16) were located and investigated by Bartolomaeus (1989), who, based on ultrastructural and developmental similarities, concluded that the protonephridia are likely homologous with those in other groups of molluscs that have been investigated (i.e., gastropods and bivalves). The protonephridia are surrounded by muscle cells, situated dorsolaterally to the ventral nerve cords. They open to the outside caudal to the larval eyes (Fig. 15C,D). Each organ consists of one terminal cell, about 15 duct cells, and one neph-

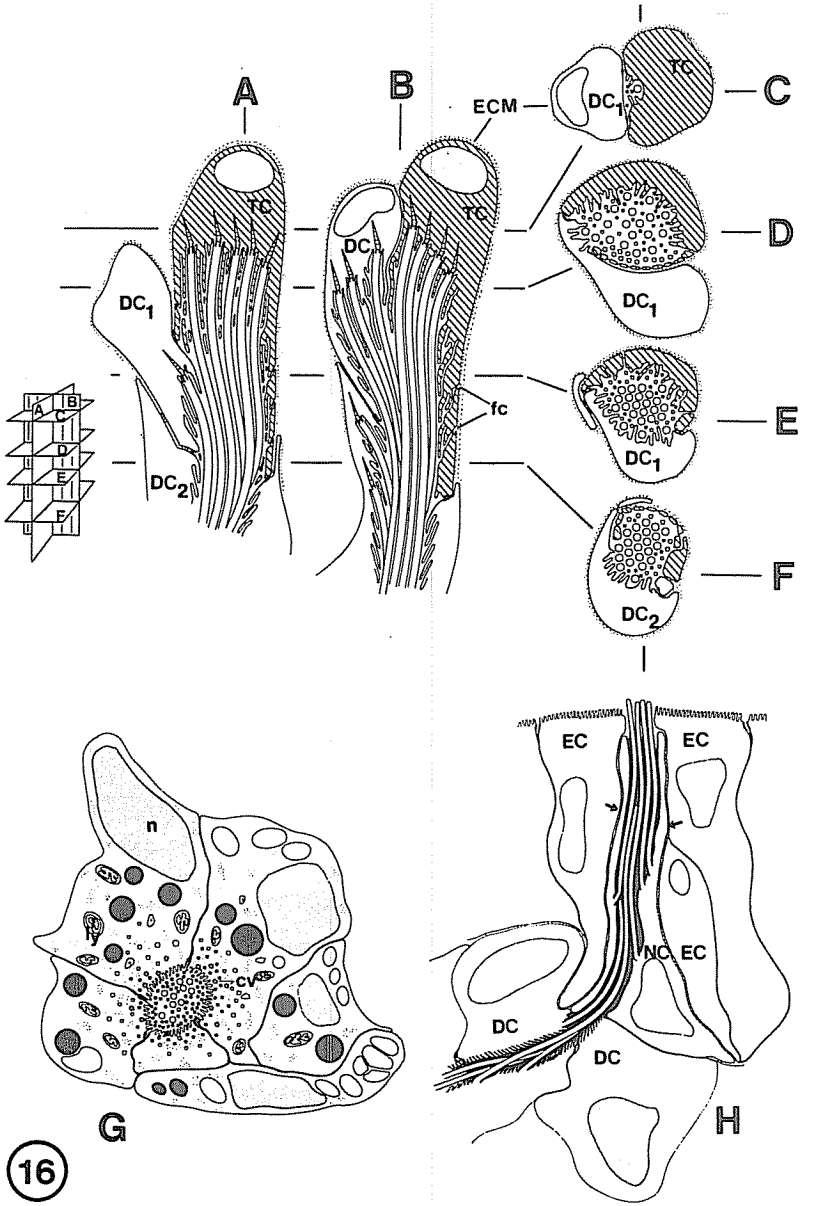



Fig. 16. *Lepidochitona cinerea* newly hatched larva, protonephridia. **A–F:** Scheme of the terminal cell (orientation of the sections as indicated). A,B: Longitudinal sections. C,F: Cross sections. **G:** Drawing of a cross section of the duct. **H:** Reconstruction of a longitudinal section of the nephropore cell. The cell has no microvilli and is surrounded by extracellular matrix. cv, coated vesicles; DC, duct cell; EC, epidermal cell; ECM, extracellular matrix; fc, clefts in the cytoplasic lobe of the terminal cell; ly, lysosome; n, nucleus; NC, nephropore cell; TC, terminal cell. (From Bartolomaeus, 1989.)

ropore cell. These cells are multiciliated, one striated rootlet fixing each cilium to the cytoplasm. Between the cilia, microvilli are found at the apical cell surface but are absent in the nephropore cell. Duct cells contain coated vesicles revealing receptor-mediated endocytosis of material from the lumen (Fig. 16G).

Podocytes, interposed between hemocoel and pericardial coelomic spaces, are generally considered to represent the site of blood ultrafiltration in postmetamorphic molluscs. In addition to physiological and morphological evidence from the vertebrate nephron (for review, see Farquhar, 1982), ultrafiltration of blood into the pericardial lumen has been demonstrated physiologically in prosobranch gastropods (Harrison, 1962; Little, 1965), coleoid cephalopods (Harrison and Martin, 1965; Martin and Aldrich, 1970), and bivalves (Jones and Peggs, 1983; Hevert, 1984). The limits of the bivalve podocyte ultrafilter have been examined using transmission electron microscopy and electron-opaque tracers (Morse, 1987). In light of this evidence, the presence of podocytes in the auricular wall of chitons (Økland, 1980) strongly suggests the heart as the site of blood ultrafiltration and is consistent with other molluscan classes examined to date (Aplacophora: Reynolds et al., in press; Cephalopoda: Schipp and Hevert, 1981; Gastropoda: Andrews, 1981; Bivalvia: Pirie and George, 1979; Scaphopoda: Reynolds, 1990). Unlike some representatives of these classes, however, the podocyte pedicels of chitons do not possess slit diaphragms (Fig. 14A). The contractile pericardium of chitons (see above) is thought to function in excretion, specifically in circulation of the fluid within the pericardial lumen (Økland, 1981) and to the kidney lumina, via a pair of ciliated renopericardial ducts.

The kidneys of chitons are located ventrolaterally along the length of the visceral cavity floor. They exhibit great variation in form, surveyed by Plate (1898–1901) and summarized by Hyman (1967), ranging from tubes with simple diverticula that possess short renopericardial ducts (e.g., *Callistochiton*) to those with an extended renopericardial duct that connects to the inner canal of a U-shaped kidney (e.g., *Chiton*). In *Acanthopleura echinata*, extensive diverticula from the outer canal extend anteriorly and posteriorly into the foot, where they are associated with the medial and transverse pedal blood sinuses (Hyman, 1967). The nephrocytes consist of cuboidal epithelium, with yellow intracellular granules (Hyman, 1967); Andrews (1988) reported that preliminary ultrastructural observations on *Lepidochitona cinerea* revealed the nephrocytes of both canals to be similar, possessing microvilli and secretory vacuoles that contain granular material. Generally, a saclike region leads to each of the nephridiopores, which open into the pallial groove before (in abanal chitons) or among (in adanal species) the last gills (Hyman, 1967)—specifically, anterior to the "post-renal" gill, which is the first gill to appear in juveniles.

The nephrocytes of *Nuttallina californica* (Fig. 17A–C) (Reynolds, unpublished data) possess a highly infolded basal cell membrane, abundant mitochondria, and extensive secretory vacuoles, all features associated with active transport. Microvilli of the apical cell membrane are usually well developed, but are sparse in cells actively undergoing merocrine secretion. In addition to the secretory vacuoles, which have electron-transparent contents, intracellular excretory granules with a more electron-opaque core occur throughout the cytoplasm (Fig. 17B) and also in the kidney lumen after release. These granules are occasionally found with parallel arrays of dense granular material (Fig. 17C), which resemble intracellular crystalline ferritin. Although they did not examine isolated kidneys, Kim et al. (1988) found iron in all the major tissues of *Acanthopleura hirtosa* and identified the major iron-binding protein in each organ to be ferritin. The highest levels of iron were found in the radula and associated tissues; the epithelial cells involved in radular mineralization were found by Kim et al. (1989) to contain ferritin aggregates similar to those seen in the *Nuttallina californica* kidney granules. Similar ferritin arrays have been described from gastropods, e.g., the intracellular granules of the digestive gland and gonad of *Biomphalaria glabrata* (Heneine et al., 1969) and mantle pore cells and oocytes of *Helisoma duryi* (Miksys and Saleuddin, 1986, 1987).



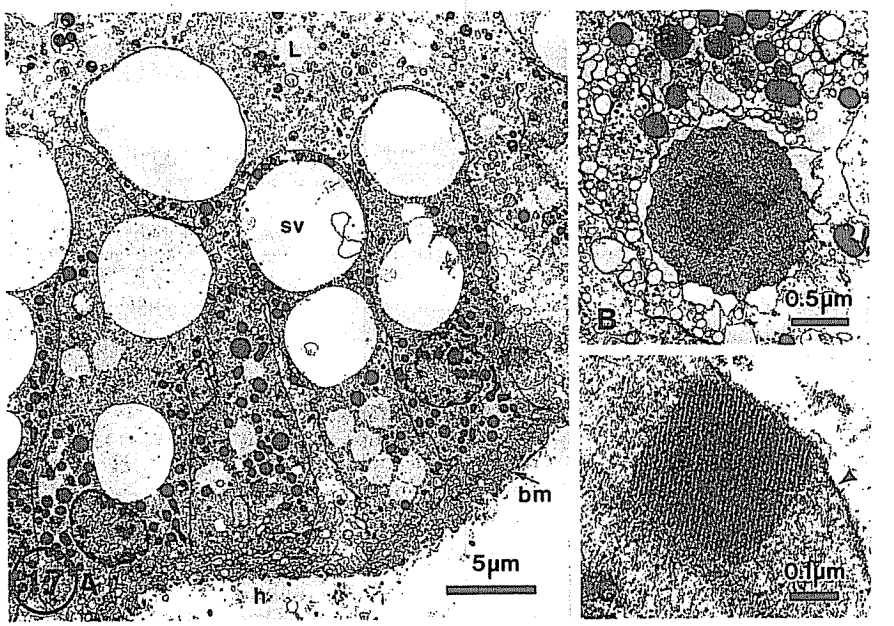



Fig. 17. Kidney of *Nuttallina californica*. **A:** Nephrocytes of the kidney. bm, infolded basal cell membrane underlain by basal lamina; h, hemocoel; L, kidney lumen; sv, secretory vacuole. **B:** Intracellular granule of nephrocyte. Note the more opaque core. **C:** Ferritin-like array of material within an intracellular excretory granule. Arrowhead, granule surface. (Courtesy of P.D. Reynolds.)

## NERVOUS SYSTEM AND SENSORY ORGANS

The main elements of the nervous system of *Lepidochitona monterosatoi* are shown in Figure 18, and an expanded lateral view of the anterior or cerebral nerves is presented in Figure 19 (Gantner, 1987). Although some differences exist between species, the general pattern of a circumesophageal nerve ring giving off paired, nonganglionated lateral and pedal nerve cords is consistent throughout the class (see Ihering, 1877; Burne, 1896; Plate, 1898–1901; Vincente and Gasquet, 1970; also Fischer, personal communication). Connectives between lateral and pedal nerve cords and between pedal nerve cords form the ladder-like amphineury typical of chitons, monoplacophorans, and many aplacophorans. The lateral nerve cords, located above the pallial groove, give off two nerves to each gill, associated with venous and arterial blood sinuses (Fischer et al., 1990). In addition to these elements, two sets of nerves each give rise to a pair of ganglia in the anterior region. The esophageal connectives extend dorsally and anteriorly from the lateral region of the cerebrobuccal ring, along the sides of the buccal cavity. They terminate in the esophageal ganglia, which are joined by the dorsal esophageal commissure. Arising from each esoph-

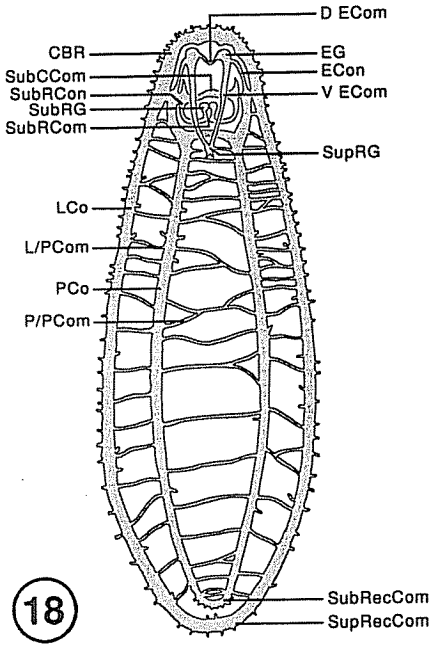



Fig. 18. Nervous system in *Lepidochitona monterosatoi*. CBR, cerebrobuccal ring; D ECom, dorsal esophageal commissure; ECon, buccal connective; EG, esophageal ganglion; LCo, lateral nerve cord; L/PCom, lateropedal commissure; PCo, pedal nerve cord; P/PCom, pedal commissures; SubCCom, subcerebral commissure; SubRCom, subradular commissure; SubRCon, subradular connective; SubRG, subradular ganglion; SubRecCom, subrectal commissure; SupRG, supraradular ganglion; SupRecCom, suprarectal commissure; V ECom, ventral esophageal commissure. (Modified from Ganther, 1987.)

ageal ganglion, a ventral esophageal commisure travels posteriorly around the esophagus, joining and forming a single supraradular ganglion between the esophagus and radular sac. From the posterior cerebrobuccal ring, subradular connectives extend dorsally and posteriorly to form subradular ganglia between the radular and subradular sacs. Hyman (1967) summarizes Plate's description (1898–1901) of a similar system in *Acanthopleura echinata;* further details of nerve processes in the buccal region can be found in Plate (1898–1901) and Vincente and Gasquet (1970).

Ultrastructural aspects of the polyplacophoran nervous system have been examined by Vincente and Gasquet (1970) in *Acanthochitona discrepans* and *Rhyssoplax olivacea* and by Fischer (personal communication) in *Rhyssoplax olivacea* and *Lepidochitona monterosatoi*. Vincente and Gasquet (1970) described synaptic junctions and two types of neurons based on size and neurosecretory characteristics. Fischer (personal communication) found nerves and ganglionic swellings to have a variety of small and large profiles (0.07–2.0 μm), with cell bodies normally located at the periphery (Fig. 20). Larger diameters were found in esthete nerves. There is no continuous cellular sheath around nerves or ganglia, although flat enveloping cells are occasionally found (Fig. 20). Normally, nerve system elements are embedded in collagen (Vincente and Gasquet, 1970; Fischer, personal communication). A juxtacommissural system in *Acanthochitona discrepans* and *Rhyssoplax olivacea* consists of two to three cells interposed between nerve cords and adjacent blood lacunae, muscle, or other tissues, although separated by a collagen layer; these cells possess ultrastructural characteristics indicative of high metabolic activity, with well-developed Golgi, rough endoplasmic reticulum, and mitochondria (Vincente and Gasquet, 1970; Fischer, personal communication). Vincente and Gasquet (1970) propose a neuroendocrine regulation of gametogenesis for the neurosecretions of neurons and juxtacommissural cells.

A glio-interstitial system has been described in the *Rhyssoplax olivacea* gill epithelium (Fischer et al., 1990) and is found in various parts of the *Acanthochitona discrepans, Rhyssoplax olivacea*, and *Lepidochitona monterosatoi* body (Vincente and Gasquet, 1970; Fischer, personal communication). The cells of this system possess long processes that form a complex network, adjoining a variety of tissues and all parts of the nervous system, although often separated by a layer of connective tissue (Fischer et al., 1990, personal communication). The glio-interstitial



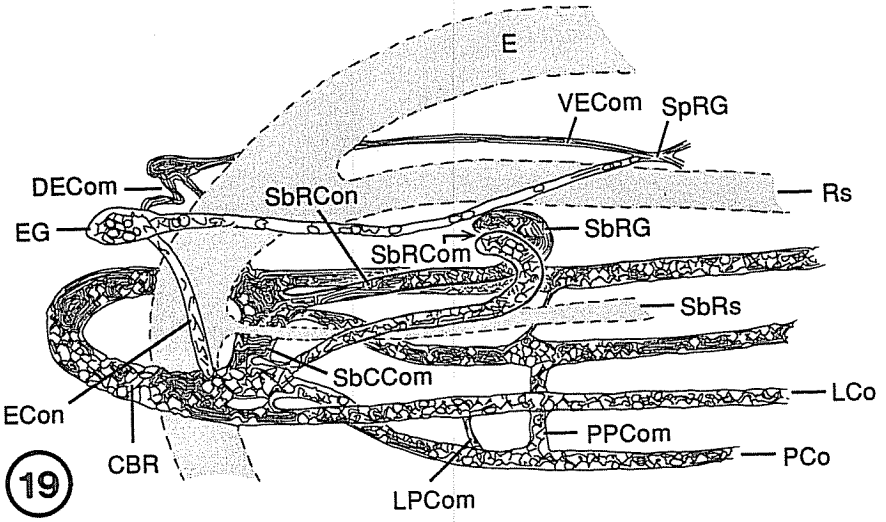



Fig. 19. Nervous system of the head region in *Lepidochitona monterosatoi*. CBR, cerebrobuccal ring; DECom, dorsal esophageal commissure; E, esophagus; ECon, buccal connective; EG, esophageal ganglion; LCo, lateral nerve cord; LPCom, lateropedal commissure; PCo, pedal nerve cord; PPCom, pedal commissures; Rs, radular sac; SbCCom, subcerebral commissure; SbRCom, subradular commissure; SbRCon, subradular connective; SbRG, subradular ganglion; SbRs, subradular sac; SpRG, supraradular ganglion; VECom, ventral esophageal commissure. (Modified from Gantner, 1987.)

cells are characterized by electron-opaque membrane-bound vesicles and rough endoplasmic reticulum. No specialized synaptic junctions occur between the glio-interstitial and other cells.

### Larval Ocelli

Posterior to the prototroch of the trochophore larva in chitons occur a pair of red pigmented ocelli. Their histology was described by Kowalevski (1883) and Heath (1904), but more accurate descriptions at the ultrastructural level have been given for *Katharina tunicata* by Rosen et al. (1979) and for *Lepidochitona cinerea* by Fischer (1980). In both species, the ocellus is ovoid, approximately 15–18 μm in diameter, and found adjacent to the lateral nerve cord. It is composed of a cup-shaped arrangement of eight or nine columnar pigment cells that are characterized by intracellular electron-opaque granules (<1 μm) and an apex with short microvilli (Fig. 21). At the base of this hemispherical arrangement, one (*Katharina tunicata*) to three (*Lepidochitona cinerea*) sensory cells are found that give rise to an array of long microvilli that fill the central space, forming a rhabdomeric photoreceptor. Other ultrastructural features of the sensory cell include abundant mitochondria, microtubules, cytoplasmic vesicles, and one (*Katharina tunicata*) or perhaps two (*Lepidochitona cinerea*) 9 + 2 cilia. The ocellus is embedded in the unspecialized epithelium, although the central cavity may open to the exterior through an ocellar aperture (Rosen et al., 1979).

Despite their name, larval ocelli persist through metamorphosis and are observable with a light microscope until the valves gradually become too opaque (Eernisse, 1988). In juveniles, the overlying epithelium may function as a lens to focus light on the ocelli (Kowalevsky, 1883; Heath, 1904; Rosen et al., 1979). These lenses are likely the two bright

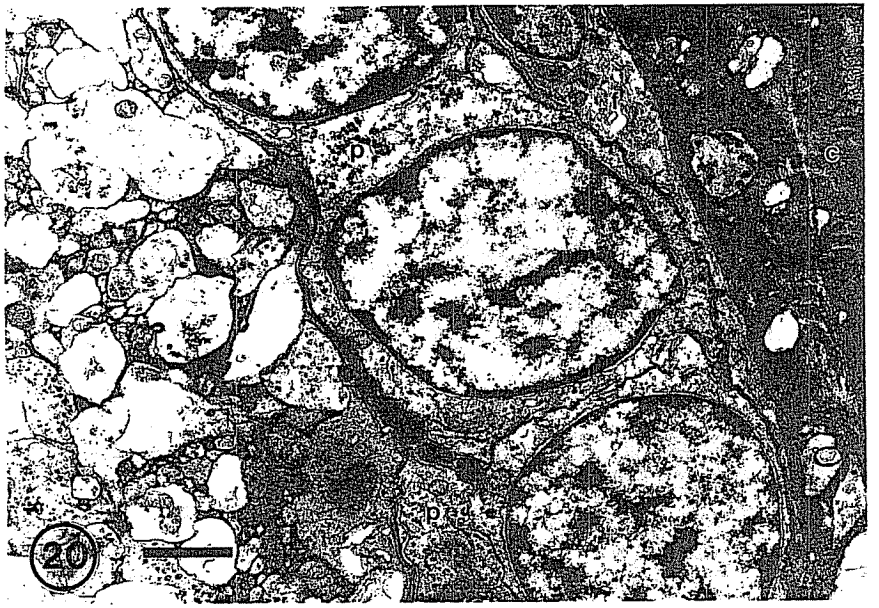



Fig. 20. Section through the lateral nerve cord of *Lepidochitona monterosatoi*, showing variation in nerve fiber diameter (to left). TEM. c, surrounding collagen; f, partially enveloping flat cells; p, peripherally located cell bodies. Scale bar = 1 μm. (Courtesy of F.P. Fischer.)

areas that are typically observed with polarized light microscope observation of metamorphosed juveniles, above the eyespots but well below the newly calcified shell plates (Eernisse, unpublished observation).

### Esthetes

Moseley (1885) first identified sensory receptors embedded in the shells of chitons. He provided histological descriptions of canals within the tegmentum and the organs found within them. Moseley coined the term aesthete (=esthete) and further distinguished microaesthetes (=micresthetes) from megalaesthetes (=macresthetes) on the basis of size viewed where the organs open to the shell surface. Moseley also described much larger "eyes" or ocelli in members of Acanthopleurinae (e.g., *Acanthopleura, Onithochiton, Tonicia*) and especially in *Schizochiton incisus*, interpreting them as modifications of macresthetes. Although Moseley proposed a mechanoreceptive function for the esthetes, Blumrich (1891), Boyle (1972), Fischer (1978, 1988), and others have provided ultrastructural or behavioral evidence for a photoreceptive modality, and Fischer (personal communication) has successfully correlated electrical recordings with photoreceptive stimuli. Baxter and Jones (1987) and Baxter et al. (1990) questioned the generality of a photoreceptive function and proposed instead a primarily chemosensory and periostracal secretory role for macresthetes and a solely secretory role for micresthetes. While Fischer (1988) found no evidence for secretion by the micresthetes, he has suggested that the central and secretory cells of macresthetes may function in prevention of desiccation, predation, and fouling by epibionts. Moseley's classic study was followed by several studies of esthete histology (e.g., Plate, 1898–1901; Nowikoff, 1907, 1909). The fine structure of esthetes has received considerable attention (Omelich, 1967; Boyle, 1969a,b, 1974, 1976; Fischer, 1978, 1979, 1988; Haas and Kriesten, 1978; Baxter and Jones, 1987; Baxter et al., 1990; Sturrock and Baxter, 1993). Only a few systematists have figured esthetes from light microscope observations (e.g., Leloup, 1956) or SEM images (e.g., Bullock, 1988; Watters, 1990), so comparative data are still lacking.



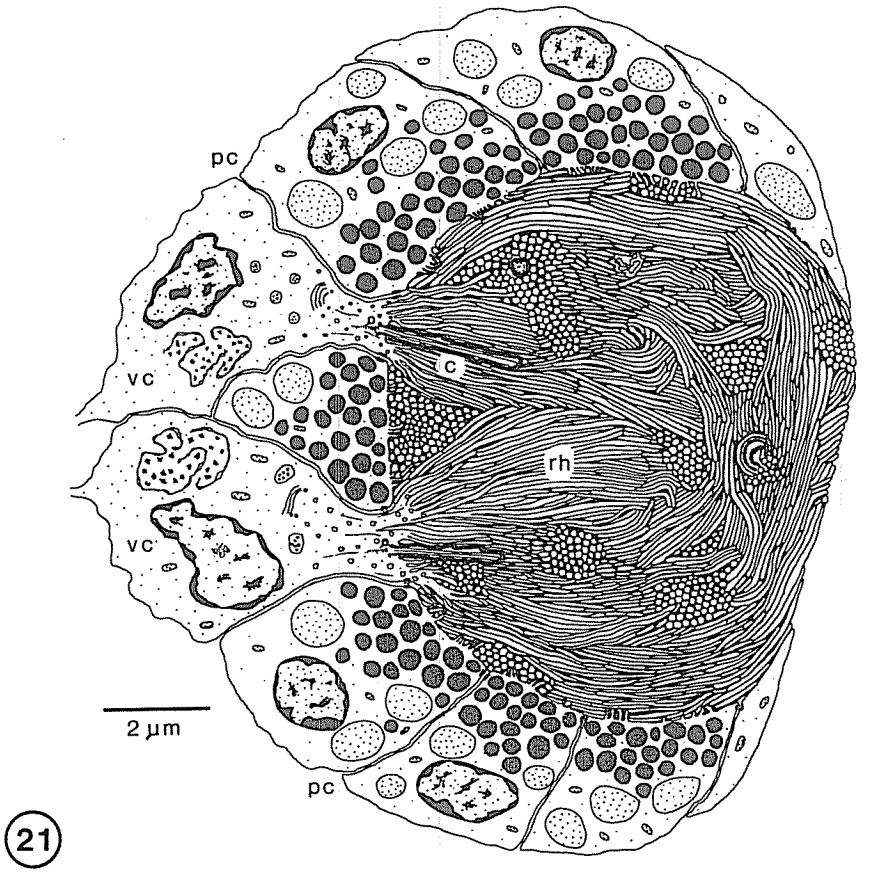



Fig. 21. Schematic diagram of the larval ocellus in *Lepidochitona cinerea*. c, cilium; pc, pigment cell; rh, rhabdome; vc, visual cell. (From Fischer, 1980.)

Esthetes consist of elongated clusters of microvillous, nerve, secretory, photoreceptive, and peripheral cells that are found in the tegmental shell layer of chitons, terminating at the shell surface (Figs. 2, 22–29). The main body of the esthete, the macresthete, gives off several branches, the micresthetes, which form a secondary termination at the shell sur-

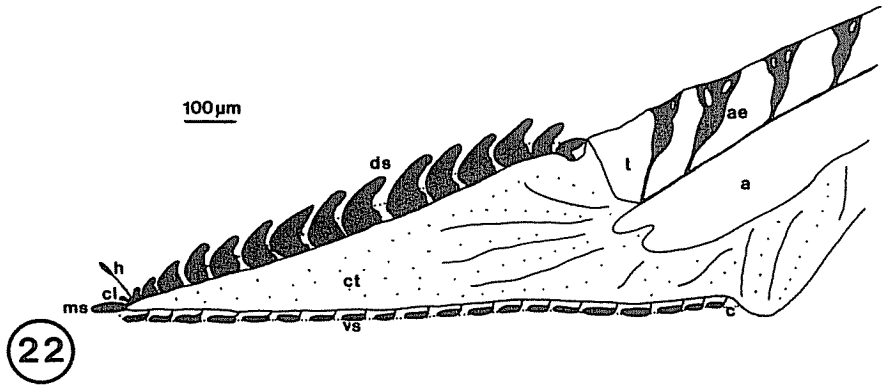



Fig. 22. Schematic cross section through the girdle of *Rhyssoplax olivacea*. a, articulamentum; ae, esthetes in the tegmental shell layer; c, cuticle; cl, clapper; ct, connective tissue; ds, dorsal scales; h, hair; ms, marginal spines; t, tegmentum; vs, ventral scales. (After Maile, 1981, from Fischer, 1988.)

face (Figs. 2B, 22, 23, 28A–C). Esthetes are usually present in high numbers; abundances of up to 250 macresthetes and 4,200 micresthetes per mm$^2$ have been reported (Baxter and Jones, 1987; Fischer, 1988; Sturrock and Baxter, 1993).

Despite variation among species, some generalizations in esthete ultrastructural characteristics can be made from available data. The apex of both esthete types is capped by a fibrous, organic extracellular material (Omelich, 1967; Boyle, 1974), similar to the periostracum that usually overlies them (Baxter and Jones, 1987; Baxter et al., 1990) (Figs. 24A,B, 25D). When this periostracum is eroded or removed, the macresthete (or apical) and sometimes the micresthete (or subsidiary) caps appear to be perforated (Figs. 25C, 28D) (Boyle, 1976; Fischer, 1988). Fischer (1978, 1988), Baxter and Jones (1987), and Baxter et al. (1990) describe the release of secretory products through the macresthete cap pores.

Although the fine structure of esthete caps is fairly consistent, distribution patterns in shell valves and the situation of the apical and subsidiary caps vary substantially from one species to another (e.g., Moseley, 1885; Boyle, 1976; Haas and Kriesten, 1978; Fischer, 1979, 1988; Baxter and Jones, 1984, 1987). For example, apical and subsidiary caps in *Callochiton achatinus* produce a regular six-row recurring linear pattern; up to 24 micresthetes branch from the macresthete and are found in aligned grooves (Baxter and Jones, 1984) (Fig. 2B). By contrast, the esthetes of *Lepidochitona cinerea* and *Lepidopleurus cajetanus* are found on ribs or protuberances, with subsidiary caps surrounding the apical cap (Knorre, 1925, and Fischer, 1988, respectively; Fig. 25A,B). In *Acanthochitona fascicularis*, esthete caps are found on the concave apices of shell tubercles (Fig. 26), which form a regular array on the lateral valves (Fig. 2A) (Fischer, 1979; see also Watters, 1990; Currie, 1992a).

Of the cell types that make up the macresthete, the central core is composed of several microvillous cells, each with a single (9 + 2) cilium (Fig. 23). These cells contain longitudinally oriented microtubules, numerous mitochondria, and clear vesicles, with a granular cytoplasm. This core is surrounded by several secretory cells; in addition to the presence of Golgi, rough endoplasmic reticulum, and mitochondria, there are large secretory vesicles, often with contents of high electron opacity (Fig. 27A). In some species, a second secretory cell type may be present (Baxter and Jones, 1987; Baxter et al., 1990). Nerve elements have been noted among the secretory and central cells. One to a few photoreceptor cells are usually peripherally associated with the macresthetes, but notably absent in *Tonicella marmorea* (Baxter and Jones, 1987). These cells are characterized by the presence of a rhabdome (Fig. 27B), which consists of a mass of microvilli and a few cilia (9 + 2) (Fischer, 1978, 1988; see also Boyle, 1974). Rough and smooth endoplasmic reticulum, which appear as membranous cisternae or lamellate bodies, are present and produce small vesicles; microtubules, mitochondria, and glycogen are also present. Peripheral



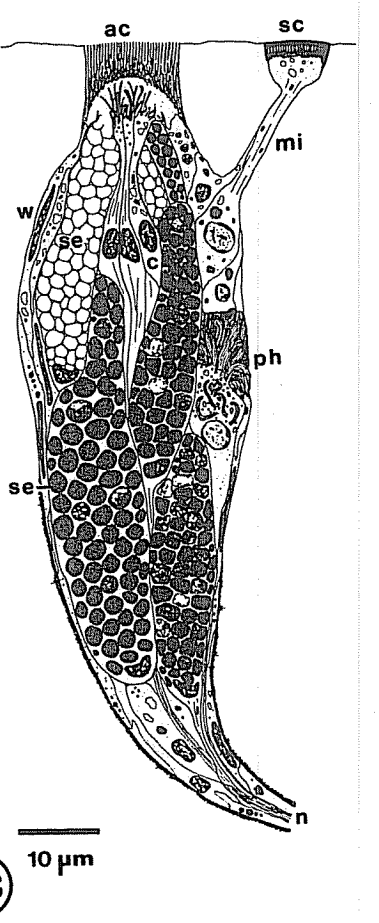



Fig. 23. Schematic longitudinal section through an esthete of *Lepidopleurus cajetanus*. ac, apical cap; c, central cell; mi, micresthete; n, neurites; ph, photoreceptor cell; sc, subsidiary cap; se, secretory cell; w, peripheral cell. (From Fischer, 1988.)

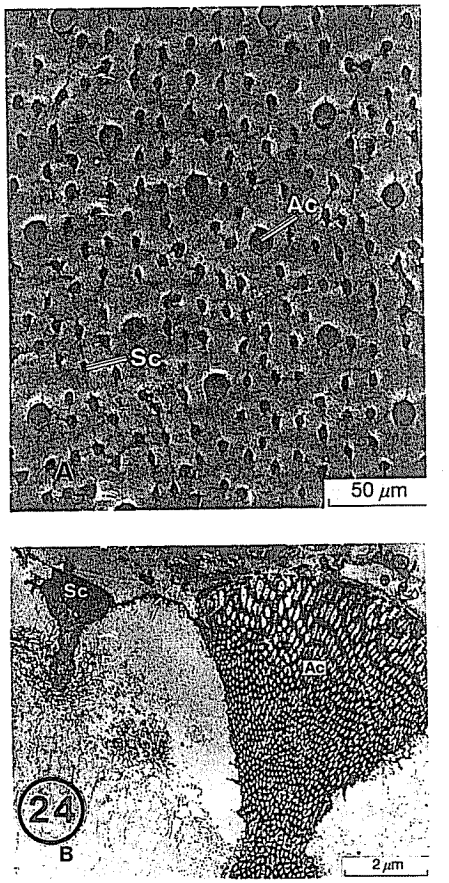



Fig. 24. Apical caps of tegmentum of *Tonicella marmorea*. **A:** Typical sculpturing of dorsal surface of valve showing apical caps located on only very slightly raised dorsal papillae and subsidiary caps roughly arranged in lines. SEM. **B:** A vertical section through both an apical cap and a subsidiary cap showing the thin layer of periostracum at the valve surface. TEM. Ac, apical cap; Sc, subsidiary cap; arrows indicate periostracum. (From Baxter and Jones, 1987.)

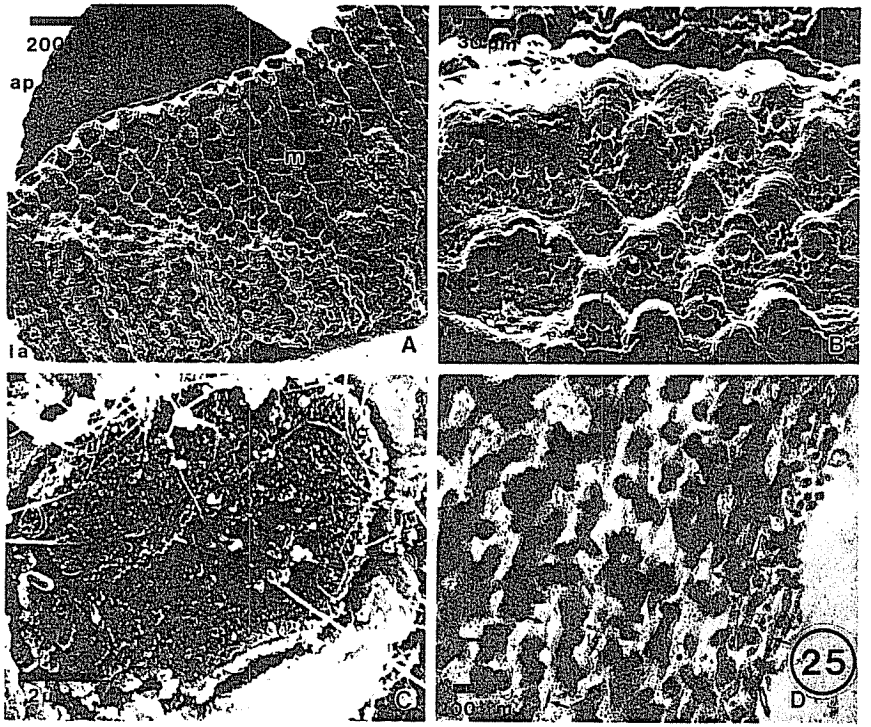



Fig. 25. Apical caps of tegmentum of *Lepidopleurus cajetanus*. **A:** Left half of an intermediate shell valve. KOH treated. **B:** Higher magnification of the lateral triangle. KOH treated. Arrows indicate several smaller micresthete openings surrounding a macresthete opening. **C:** Surface of an apical cap. The cap is perforated by numerous small pores. **D:** Longitudinal section of the basal area of an apical cap consisting of a network of larger and smaller (arrows) organic filaments. (From Fischer, 1988.)

cells are found in the outer cell layer of the macresthete and probably function in support of the organ. These cells often possess electron-opaque secretory granules and granular cytoplasm, with deeply infolded membranes and microvilli adjacent to the macresthete canal (Boyle, 1974) or extracellular filaments that extend into the surrounding shell material (Fischer, 1988).

A few to several micresthetes branch from the macresthete cluster of cells (Figs. 22, 23, 28A–C). Each micresthete consists of a single cell extending to the shell surface and associated cap (Fig. 24); the main cell body, including the nucleus, remains within the macresthete cluster of cells. Peripheral cells may surround the micresthete cell proximally. Micresthete cytoplasmic characteristics include the presence of many microtubules, mitochondria, and secretory granules, with the distal tip extending microvilli into the cap material (Boyle, 1974; Fischer, 1978, 1979, 1988; Baxter and Jones, 1987).

The esthetes are ultimately joined to the lateral and dorsal epithelium via the esthete canals (Fig. 28A,B), which run close to the



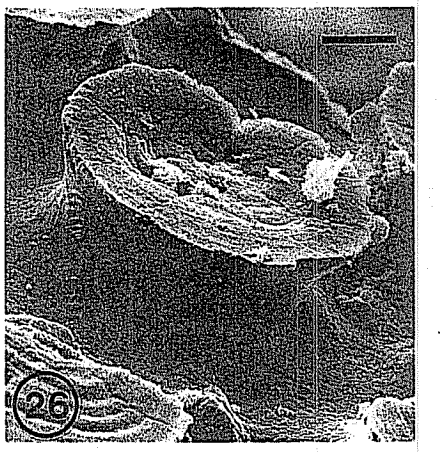
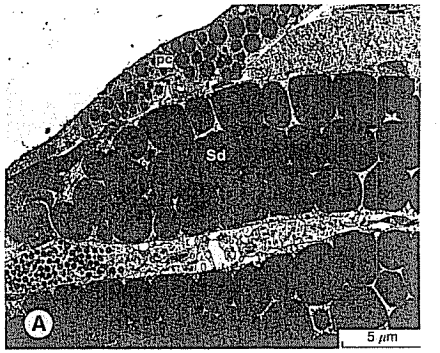



Fig. 26. Tegmental tubercles of the lateral shell region in *Acanthochitona fascicularis* (see also Fig. 2A). SEM. Scale bar = 20 μm (From Fischer, 1979.)

base of the tegmental layer to the lateral epithelium (Moseley, 1885). These canals sometimes pass through the articulamentum to the dorsal epithelium (Boyle, 1974). The esthete canals are lined with peripheral and secretory cells and nerve elements (Boyle, 1974; Fischer, 1988).

The esthetes of some chitons show intracellular pigmentation of cells forming a pigment cup that partially surrounds the photoreceptor cell(s), distinguishing them from those described above. These "intrapigmental aesthetes" have been described by Haas and Kriesten (1978) from *Chiton marmoratus* and by Baxter et al. (1990) from *Callochiton achatinus*. In members of *Callochiton*, these correspond to the black pigment spots that are visible on the tegmental surface under low magnification (see Baxter and Jones, 1984). In both species, the intrapigmental body is



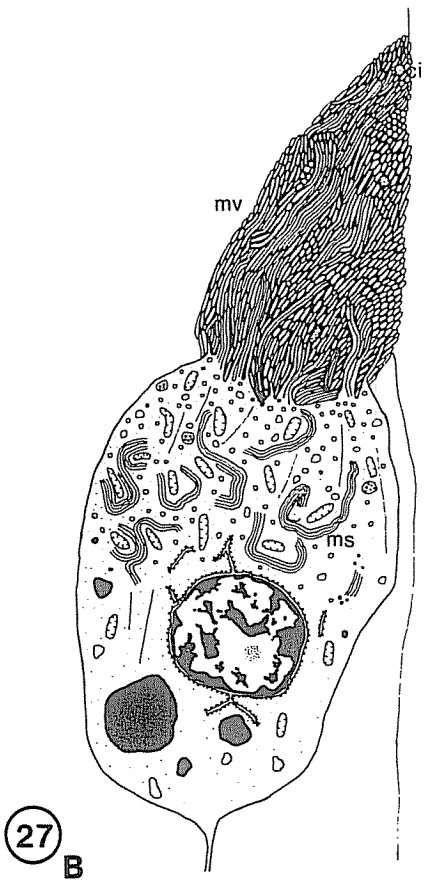




Fig. 27. **A:** *Tonicella marmorea*. Large central cells in the macresthete body packed with large, electron-dense secretory droplets (Sd). pc, peripheral cell. (From Baxter and Jones, 1987.) **B:** Photoreceptor cell of *Acanthochitona fascicularis*, schematic drawing. ci, cilium; ms, membranous structures; mv, microvilli. (From Fischer, 1978.)

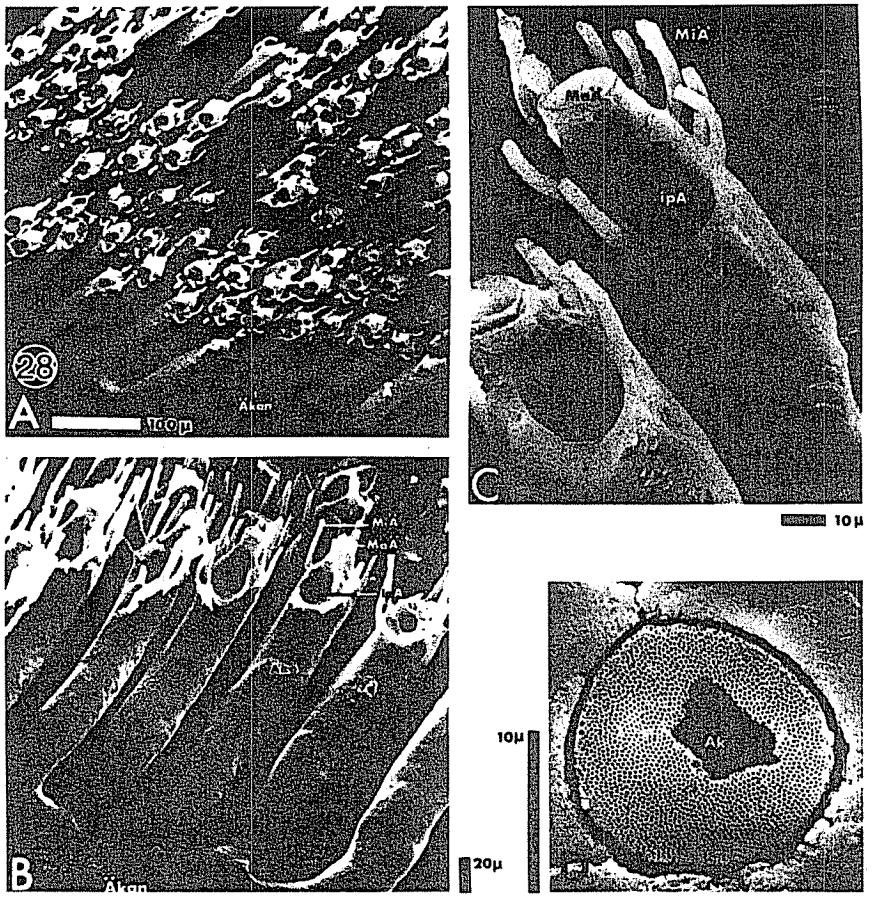



Fig. 28. *Chiton albolineatus* esthete organs. **A–C:** Cast of tegmentum canals showing esthete shape and branching pattern of micresthetes. **D:** Shell surface showing fractured macresthete cap. Ā, esthete, Āk, macresthete cap, 1 Ākan, esthete canal; Ākō, esthete body; ipA, intrapigmented esthete organ (impressions left by the lens-like structure of the tegmentum); MaĀ, macresthete; MiĀ, micresthete; Sch, shell. (From Haas and Kriesten, 1978.)

found on the dorsal side of the macresthete cluster of cells, the region of the rhabdome not enclosed by pigmented cells directed toward the shell surface (Fig. 29A,B). Rhabdome cilia exhibit 9 + 2 and 9 + 0 microtubule arrangements (Baxter et al., 1990). Membranous whorls form lamellate bodies in the photoreceptor cytoplasm adjacent to the rhabdome microvilli, within the pigment cup, and in the cell bodies of the photoreceptor cells beneath the pigment cells (Haas and Kriesten, 1978; Baxter et al., 1990). The structure of the tegmental layer above the intrapigmented body has been interpreted as a lens-



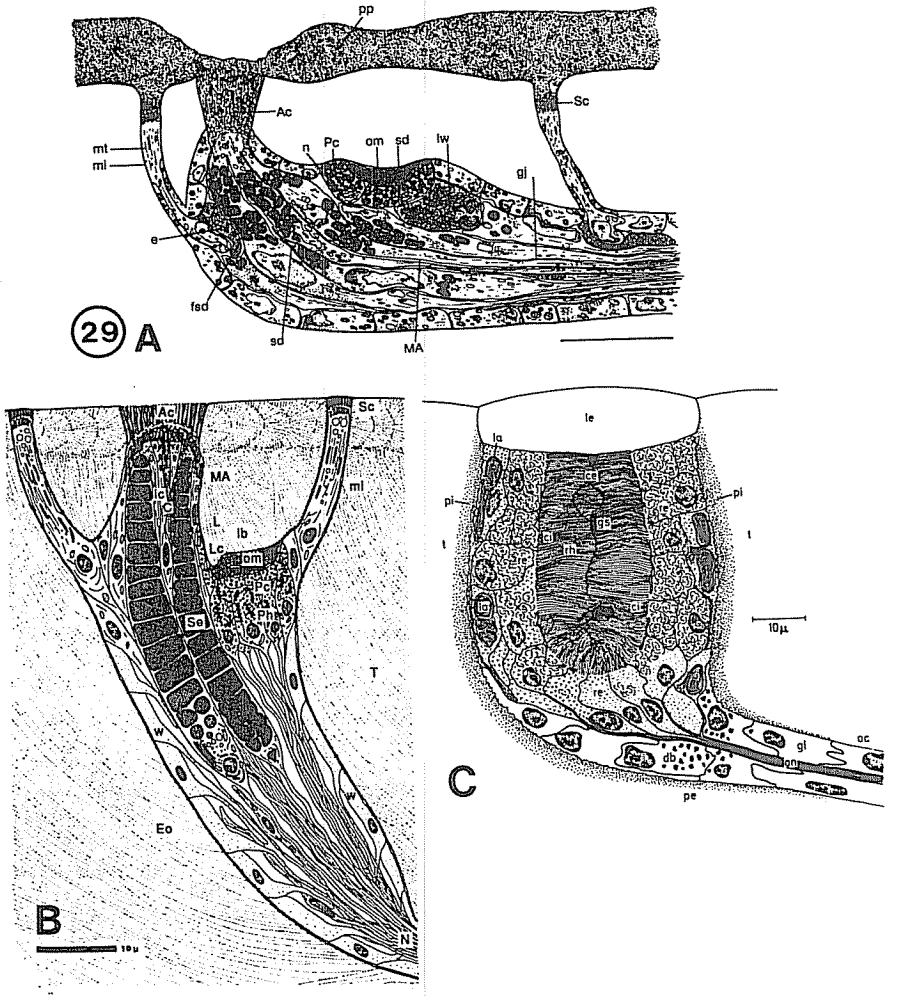



Fig. 29. Esthete and ocellus structure. **A:** Diagrammatic representation of an intrapigmented esthete of *Callochiton achatinus*, showing the location of the intrapigmental body relative to the macresthete body and the dorsal surface of the valve. Ac, apical cap; e, electron-dense core secretory droplet; fsd, folded secretory droplet; gj, gap junction; lw, lamellate whorls; MA, macresthete; mi, micresthete; mt, microtubule; n, nucleus; om, rhabdomeric microvilli; Pc, pigment cell; pp, properiostracum; Sc, subsidiary cap; sd, septate desmosome. Scale bar = 20 µm. (From Baxter et al., 1990.) **B:** Esthetes with intrapigmental bodies from *Chiton marmoratus*. Schematic drawing. Ac, apical cap; C, central cell; Eo, esthete organ; ib, intrapigmented body; ic, interstitial cell; L, lens; Lc, lens cone; MA, macresthete; mi, micresthete; N, nerve fibers; om, rhabdomeric microvilli; Pc, pigment cells; Ph, photoreceptor cells; Sc, subsidiary cap; Se, secretory cell; T, tegmentum; w, peripheral cell. (From Haas and Kriesten, 1978.) **C:** Diagram of a generalized vertical section of an ocellus, through the optic canal, based on electron and light microscopy. ce, central axis; ci, cilia; db, dense body; gl, glial cells; gs, ground substance; la, lamellar body; le, lens; oc, optic canal; on, optic nerve; pe, peduncle; pi, pigment; re, retina cell; rh, rhabdome; t, tegmentum. (From Boyle, 1969a.)

like structure, which may gather light to the pigment cup and rhabdome (Haas and Kriesten, 1978) (Fig. 28B,C, 29B). Baxter et al. (1990) suggest that a swelling in the tegmental layer above the intrapigmental body of *Callochiton achatinus* (Baxter and Jones, 1984) may represent a primitive lens structure. Intrapigmented esthetes usually comprise a subset of the entire esthete complement; in *Callochiton achatinus*, intrapigmented esthetes are restricted to the head valve, anterior lateral areas of the intermediate valves, and the posterior region of the tail valve. Normal esthetes are found elsewhere (Baxter and Jones, 1984).

More complex ocelli, or "extrapigmented aesthetes," have been described from certain chitons by Moseley (1885) and especially by Boyle (1969a,b, 1976), who examined the fine structure of ocelli in *Onithochiton neglectus*. The two known species of *Schizochiton*, including *Schizochiton incisus* studied by Moseley, possess the most spectacular ocelli, but these have not been studied on an ultrastructural level. In the species examined by Moseley and Boyle, the ocelli comprise a small percentage of the total macresthete complement, from which these "eyes" are thought to be derived (Moseley, 1885; Fischer, 1978, 1988; Baxter and Jones, 1987). Ocelli are typically arranged in regularly spaced, radiating rows, either at the junction or internal to the lateral areas of the intermediate valves, or all around the terminal valves. In *Onithochiton neglectus*, many photoreceptor or "retinal" cells line a cup-shaped depression in the tegmentum, with the surrounding shell material pigmented (Fig. 29C). Microvilli from the retinal cells form a central rhabdome within which some 9 + 2 cilia are found, and the whole is overlain by a lens formed from the tegmentum. Extracellular lamellate bodies are derived from ciliary membranes. Nerve processes extend from the retinal cells into an optic canal, which passes through the tegmentum and to the esthete canal complex. The optic canals are lined with glial cells (Boyle, 1969a) and appear equivalent to the usual nerve and peripheral cell arrangement of the esthete canals.

### Girdle Sensory Structures

The morphology of girdle sense organs (Fig. 7) has been studied at the ultrastructural level by several authors (Haas and Kriesten, 1975; Fischer et al., 1980, 1988; Leise and Cloney, 1982; Leise, 1986, 1988). The anatomical basis for girdle sensory reception is epidermal projections containing neuronal dendrites, often called stalked nodules, which have been described from chiton integument by early anatomists (see Leise, 1988, for review). These projections arise from epidermal papillae; nerve elements emerge basally from these papillae and presumably represent nodule cell axons (Figs. 6D, 30–32). These structures have long been considered mechanoreceptors (Leise, 1988), supported by the demonstration by Fischer et al. (1988) of avoidance behavior in *Rhyssoplax olivacea*. In *Acanthochitona fascicularis*, however, photoreceptor cells are also found within the epidermal papillae (Fischer et al., 1980).

Stalked nodules are usually found at the base of calcareous spicules, which range from simple shafts to compound, articulated hairs as in some Mopaliidae (Figs. 6, 7, 30–32). In certain members of Lepidochitonidae, Mopaliidae, and Acanthochitonidae, however, stalked nodules without associated spicules are embedded in the cuticle (Fischer et al., 1988; Leise, 1988) (Fig. 32). These dendritic processes also vary in form; claviform nodules in *Katharina tunicata* lack a stalk, while the girdle hairs in *Placiphorella velata* are innervated but lack nodules (Leise, 1988). Furthermore, not all spicules or spines of the chiton girdle are innervated (Fischer, 1979; Leise, 1988). However, dendritic processes have been found in association with dorsal, marginal, and ventral spicules among all species examined to date and appear to be a general feature of the class.

Fischer et al. (1980, 1988) described the ultrastructure of the epidermal papillae and nerve elements of sensory girdle elements in



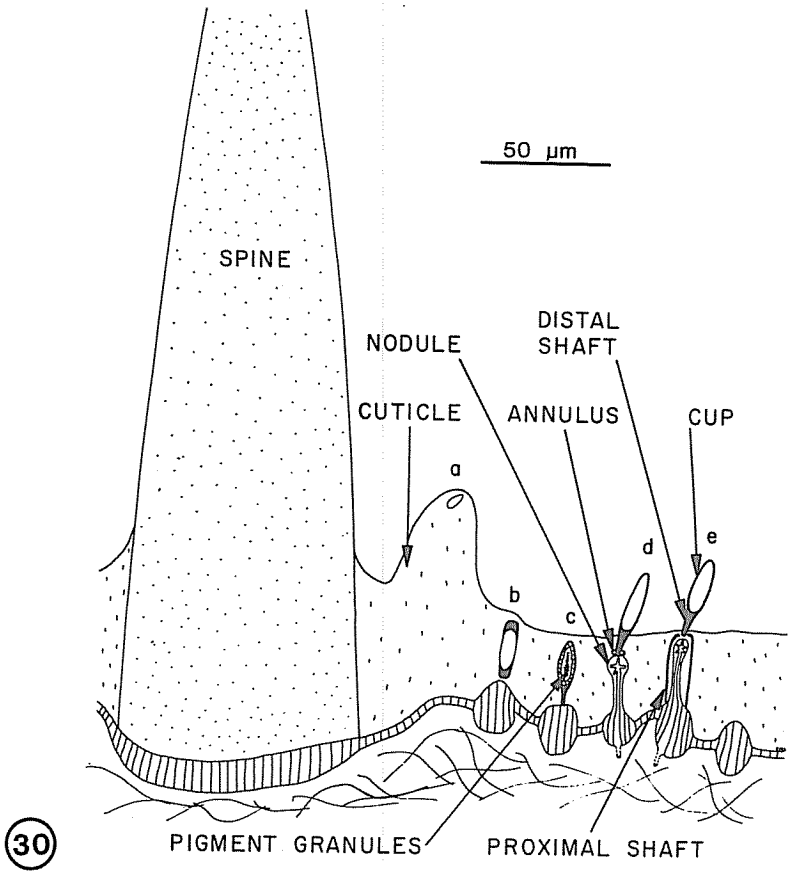



Fig. 30. Diagram of five types of spicules and one spine of the polyplacophoran girdle. a, primary spicule from a newly metamorphosed juvenile; note thin chitinous cup. b, spicule with apically and basally thick cup. c, spicule with pigment granules and shaft. d, spicule with an annulate shaft surmounting a sensory nodule. e, spicule as in d but with an articulated shaft. (From Leise, 1988.)

*Rhyssoplax olivacea* and *Acanthochitona fascicularis*. Regardless of spicule form, a spicule cell is attached to the base of the calcareous element by its microvillous apical membrane (Fig. 32). An adjacent ciliary cell extends into the cuticle, with its single cilium at the base of the spicule and consistently oriented towards the girdle surface. Processes from the ciliary cell project into the spicule cell; a dense network of microfilaments is associated with the cell membrane of the corresponding spicule cell invagination. Several secretory cells surround both cell types, and nerve processes have been noted within the papilla (Fig. 32). Epidermal papillae in *Acanthochitona fascicularis* also possess a photoreceptor cell and a stalked nodule, which extends into the cuticle (Fischer et al., 1980)

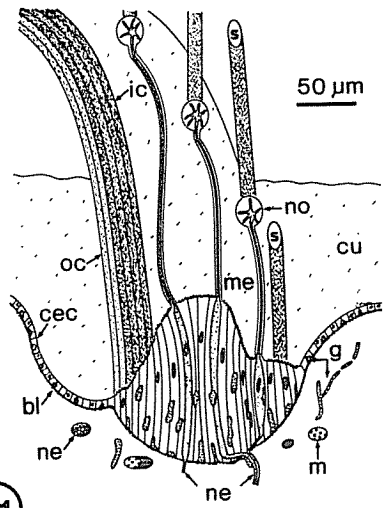



Fig. 31. Diagrammatic longitudinal section through the base of a hair of *Mopalia muscosa*, drawn passing through the mesial groove and two spicules (s). Dendrites from three sensory neurons terminate in nodules (no). In mature hairs, the sensory neurons occur in clusters, not as single cells, as they are drawn here, for clarity. Two nerves (ne) cross the basal lamina (bl) as they emerge from the base of the papilla. cec, common epidermal cells; cu, cuticle; g, pigmented glial cells; ic, inner cortex; m, muscle fiber; me, medulla; oc, outer cortex. (From Leise, 1988.)

(Fig. 32). Fischer et al. (1988) state that the basic structure of epidermal papillae found in *Rhyssoplax olivacea* is similar to that found in *Lepidopleurus cajetanus* and *Lepidochitona cinerea* (Haas and Kriesten, 1975) and that the distal swelling of papillae, as in the girdle hairs of *Rhyssoplax olivacea*, is similar to the stalked nodules found in *Acanthochitona fascicularis* (Fischer et al., 1980) and *Mopalia muscosa* (Leise, 1986). Fischer et al. (1988) propose the general ultrastructural features of *Acanthochitona fascicularis* as a model of girdle sense organs in Polyplacophora.

### Osphradia

The comparative morphology of the chiton osphradia, also known as adanal sensory stripes, has been examined by Haszprunar (1987); histological accounts of the sensory organs in the chiton mantle cavity are given in several early studies, including Burne (1896), Plate (1898–1901), Pelseneer (1899), and Yonge (1939).

Two types of pallial sensory epithelia are found in members of Lepidopleurina, as originally described by Burne (1896) and generally referred to as "branchial" and "lateral" sense organs. Haszprunar (1987) considered both to be secondary structures, not homologous to the osphradia of other molluscs, and hypothesized that osphradia were suppressed in lepidopleurids due to their posterior placement of gills, which he presumed was also a derived condition (but see Conclusions). Haszprunar did not regard the branchial and lateral organs to be true sensory organs because of their poorly developed state. In the species Haszprunar examined, *Lepidopleurus cajetanus*, the so-called branchial sense organs are found in the inhalent chamber of the mantle cavity. Within the epithelium of these organs, supporting cells surround free nerve processes that bear 9 + 0 and 8 + 0 cilia. They arise from the efferent ctenidial nerve. Lateral sense organs are found in the anterior region of the inhalent chamber in a series (approximately 35) of small patches on the outer wall of the mantle cavity, innervated by the lateral nerve cord. In addition to supporting and secretory cells, ciliated sensory cells that are innervated by subepithelial nerve processes are present.

Members of Ischnochitonina and Acanthochitonina examined by Haszprunar (1987) show some osphradial variation. Three species, *Ischnochiton rissoi*, *Lepidochitona cinerea*, and *Lepidochitona corrugata*, have the osphradia located on the roof of the mantle cavity posterior to the ctenidia, innervated from the suprarectal commissure. The osphradia are adjacent to the pallial mucous tract. Microvillous supporting cells with intracellular pigment granules impart a yellowish-brown coloration to the tissue, and ciliated sensory cells adjoin nerve processes that extend subepithelially (Fig. 33). The osphradia



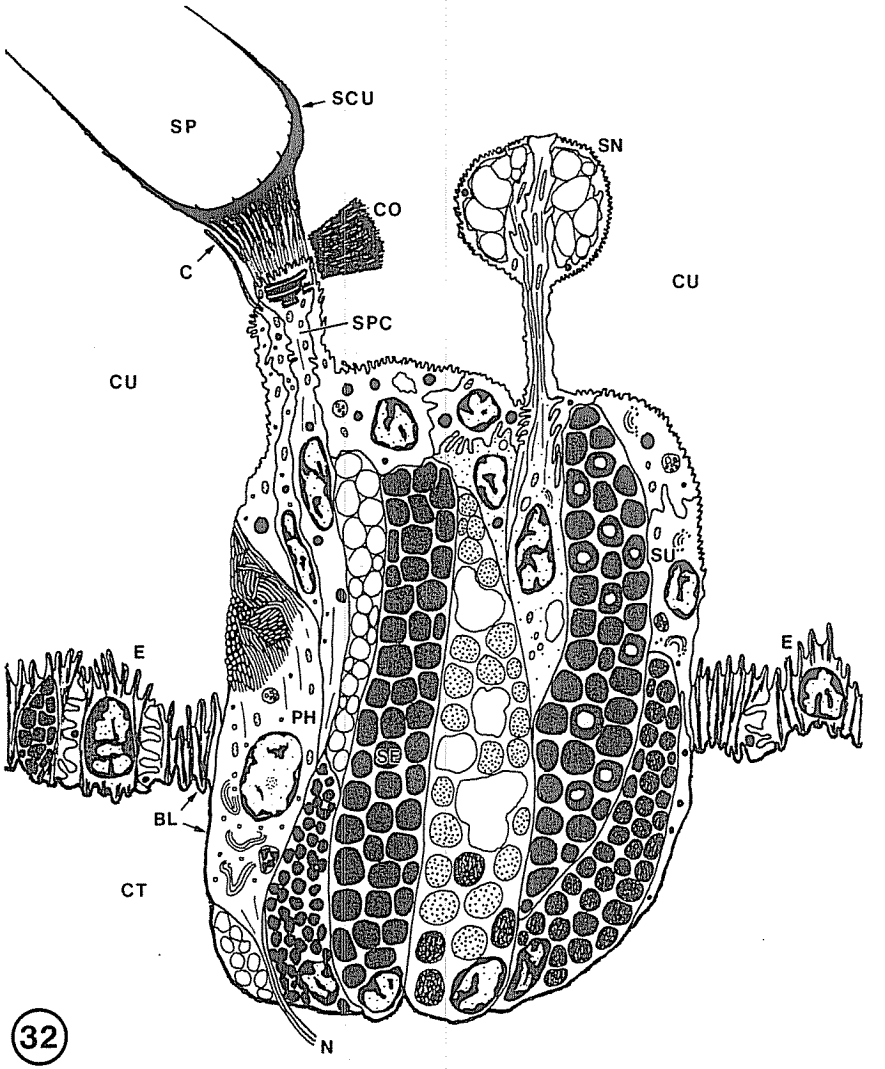



Fig. 32. Schematic longitudinal section through an epidermal papilla of the girdle in *Acanthochitona fascicularis*. BL, basal lamina; C, cilium; CO, collar; CT, connective tissue; CU, cuticle; E, mantle epithelium; N, neuronal process; PH, photoreceptor cell; SCU, spicule cup; SE, secretory cell; SN, stalked nodule; SP, spicule; SPC, spicule cell; SU, supporting cell. (From Fischer et al., 1980.)

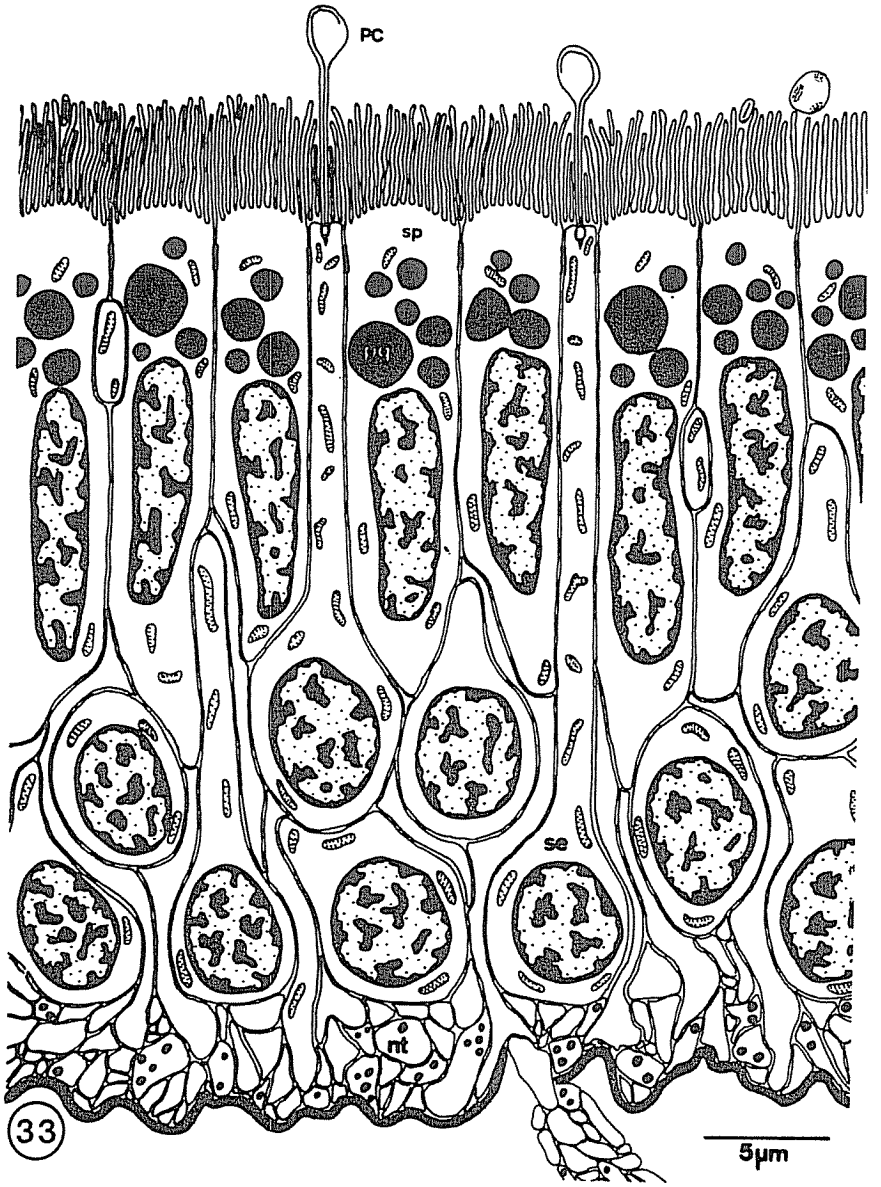



Fig. 33. Section of the osphradium of a member of Ischnochitonina (semischematic). nt, nervous tissue; PC, paddle cilia; pg, pigment granules; se, sensory cells; sp, supporting cell. (From Haszprunar, 1987.)



of *Rhyssoplax olivacea* and *Rhyssoplax coralina* show a similar ultrastructure, although they differ in their position relative to the posterior ctenidia and mucous tract (Haszprunar, 1987). The same can be said of *Acanthochitona fascicularis*, in which the osphradium is located posterior to the anus and consists of a lateral secretory epithelium and a medial sensory groove. Apart from the short, irregular cilia of the branchial pallial organ in *Lepidopleurus cajetanus*, all sensory cells examined using ultrastructural methods bear "paddle" or discocilia. In these cilia the axoneme curves apically, and the ciliary membrane forms a broad flange; discocilia have been found in putative chemoreceptor epithelia of most molluscan classes (Haszprunar, 1987), but were also considered to be generally artifactual by Nielsen (1987) and Short and Tamm (1989).

Debates over the function of the molluscan osphradium as a photoreceptor (Kraemer, 1981), mechanoreceptor (Yonge, 1939, 1947, 1977), or chemoreceptor (Sokolov and Zaitseva, 1982; Haszprunar, 1987) do not focus directly on Polyplacophora. Haszprunar (1987), however, suggested that the location of osphradia in the exhalent current of some Polyplacophora argues against a "protection receptor" function, involved in testing respiratory currents for sediment loading or noxious chemicals. Instead, Haszprunar (1987) proposed a reproductive role for the osphradium of most molluscs, specifically in the coordination of spawning and search for a mate.

## REPRODUCTION

Chitons are nearly all gonochoric. The only known exceptions are two small hermaphroditic species (Heath, 1907; Eernisse, 1988), which are also exceptional because they apparently self-fertilize predominantly (Eernisse, 1988). Most chitons free spawn eggs or sperm and usually develop into free-swimming trochophore larvae. Over 30 chiton species are known to brood embryos inside their mantle cavities, with emerging larvae sufficiently developed to remain attached throughout their lives (Pearse, 1979; Strack, 1987; Eernisse, 1988). A few chitons such as the well-studied *Stenoplax heathiana* are known to attach eggs to rocks in a sticky gelatinous matrix, and these larvae likewise emerge at a late developmental stage (Heath, 1899). One report of ovovivipary awaits confirmation; Plate (1899) noted several juveniles within the gonad of a *Calloplax vivipara* specimen. Compared with the dramatic differences noted for other marine metazoan groups between brooding and broadcasting members, egg diameter and larval development remain similar despite the difference of free spawning and brooding/egg mass laying and the corresponding difference in stage of larval emergence (Eernisse, 1988). Development is rather direct, but several changes typify metamorphosis (Figs. 4, 5), including change of body shape, calcification of the girdle, followed by shell (Kniprath, 1980; Leise, 1984) and radular formation (Eernisse and Kerth, 1988).

### Female Reproductive System

Recent light microscopic and histochemical studies of chiton oogenesis include those by Selwood (1968, 1970) and Richter and Götting (1974), as reviewed by Pearse (1979) and Richter (1986). An elaborate series of experiments performed by Yoshioka (1987, 1988, 1989) has shown that oogenesis of *Acanthopleura japonica* depends on complex environmental (lunar/tidal/daylength) control. The generality of his findings is suggested by repeated instances of observed reproductive synchrony in chitons, for example, within populations of the brooders *Lepidochitona thomasi, Lepidochitona caverna*, and *Lepidochitona fernaldi* (Eernisse, 1988). When mature, the ovary (or testes) occupies much of the dorsal internal space (Fig. 34). Mature egg diameter (without hull) is similar in nearly all chiton species, usually about 150–300 μm when spawned (Pearse, 1979; Strathmann and Eernisse, 1987). One exception is the brooder *Hemiarthrum setulosum*, whose eggs approach 1 mm in diameter (Simpson, 1977).

Chiton eggs are remarkable in having an

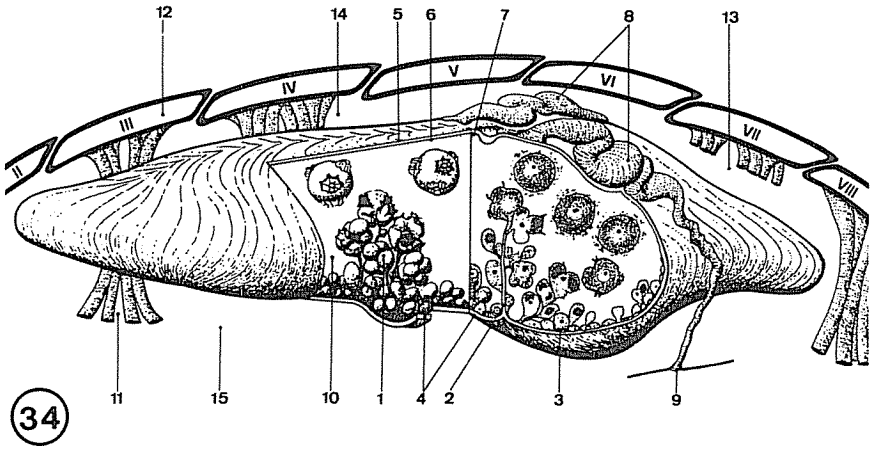



Fig. 34. The ovary of *Lepidochitona cinerea* inside the body cavity. Note the oocyte-free dorsal wall and the germinal folds arising from the ventral wall. On the left, oocytes are drawn three dimensionally; on the right, oocytes as they are represented on thin sections. 1, oocyte of stage IV; 2, germinal folds of the nutritive tissue; 3, germinal epithelium; 4, ventral gonadal wall; 5, dorsal gonadal wall; 6, ciliated epithelium; 7, ciliated groove; 8, oviduct; 9, gonopore; 10, gonocoel; 11, dorso-ventral muscle; 12, shell plate; 13, location of the pericardium; 14, location of the aorta; 15, gastrointestinal region. Oocyte stages not in correct scale. Scale bar = 1 mm. (From Richter, 1986.)

elaborate extracellular egg "hull." The term hull is used instead of chorion because it appears that the oocyte contributes much or most of the hull materials, instead of the follicle cells as in true chorions. The follicle cells are nevertheless active participants of hull formation, likely helping to shape the complex layers of acidic or neutral mucopolysaccharides and proteins into cups, cones, or spines, the number of which corresponds to the number of follicle cells surrounding each egg during its formation (Garnault, 1888; Lyngnes, 1924; Anderson, 1969; Selwood, 1970; Richter, 1976). Hull variation, especially as imaged by scanning electron microscopy (SEM) was found to be of taxonomic value at both species and higher levels within chitons (Eernisse, 1982, 1984, 1988). Figure 35 illustrates variations found in six *Lepidochitona* spp. Several authors have also published SEM images of egg hulls for particular species (Pearse, 1979; Durfort et al., 1982; Buckland-Nicks et al., 1988a,b). In an SEM survey of the hulls of about 40 species, Eernisse (1984) distinguished five basic shape categories: smooth, cup, cone, flap, and spine. The shape category "smooth" was restricted to the only lepidopleurid (*Leptochiton asellus*) eggs reported in mature state, based on spawned eggs examined with light microscopy (Christiansen, 1954) and more recently confirmed by TEM observations (Hodgson et al., 1988). The latter TEM studies also revealed that the smooth hull of *Leptochiton asellus* is more than one order of magnitude thicker (14–30 μm) than that of other chiton hulls examined (e.g., <2 μm), an observation that is likely related to the more ordinary molluscan acrosome found in the sperm of *Leptochiton asellus* (see below). Cone-like (Fig. 35) and flap-like (see Fig. 38A, below) hulls were concluded to be variations on a cup-like hull, with spines more divergent. Members of Chitonidae and certain members of Ischnochitonidae have hull spines with petaloid tips, perhaps indicating a phylogenetic affinity. The spiny hulls of *Chaetopleura apiculata* are unusual in their complicated spiraling patterns (Grave, 1932; Buckland-Nicks, personal communication).



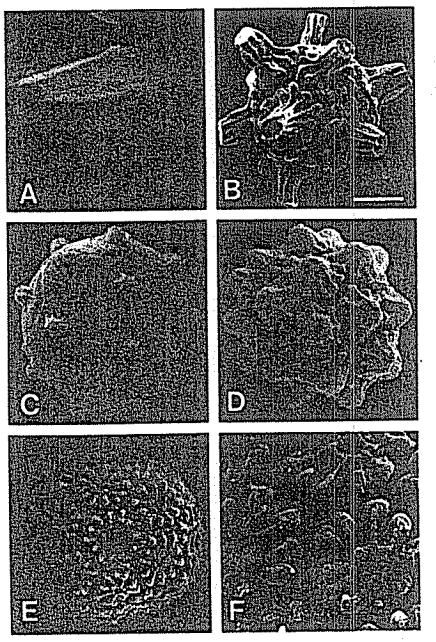



Fig. 35. Eggs of six species of *Lepidochitona*. All eggs were dissected from animals with mature gonads but, except for a slightly smaller egg diameter, appear very similar to eggs that are spawned. The eggs correspond to **A:** close-up of *Lepidochitona hartwegii* egg hull; **B:** *Lepidochitona berryana;* **C:** *Lepidochitona caverna;* **D:** *Lepidochitona dentiens;* **E:** *Lepidochitona thomasi;* and **F:** close-up of *Lepidochitona fernaldi* egg hull surface. Scale bars: A,F = 20 μm; B = 63 μm; C = 48 μm; D = 54 μm; E = 51 μm. (From Eernisse, 1988.)

The function of the striking egg hulls is not clear, and competing hypotheses are not necessarily mutually exclusive. Hulls could function to slow sinking, deter predators, prevent crushing or otherwise maintain spacing, or hold the eggs together in chains or to jelly secretions attached to the substrate (Eernisse, 1988; Buckland-Nicks, 1993). The possibility that at least some hull structures may need to open for fertilization to occur (Buckland-Nicks et al., 1988b, 1990) suggests that their shape may, in part, be attributable to fertilization requirements (see below). Eernisse (1988) noted a relationship between the likely multiple origins of brooding in chitons and a regular tendency for brooders to have less projecting egg hulls, although taxon-characteristic shape patterns remain apparent even with the hull reduction. Eernisse proposed that the reduction could be either due to escape from planktonic requirements or imposed by close-packing constraints of a limited brood chamber. Since egg mass layers with benthic but nonbrooded embryos may not be subjected to close-packing constraints, it would be interesting to determine whether egg hulls tend to be reduced in the egg mass layers. Eernisse (1984) observed hulls of two species of only three known benthic egg mass layers, but these were inconsistent, one with projecting hulls and one without.

### Male Reproductive System

The typical chiton sperm is remarkable for its highly divergent structure in comparison with the sperm of other metazoans. Recent comparisons of chiton sperm (Buckland-Nicks et al., 1988a; Hodgson et al., 1988), and investigations of fertilization (Buckland-Nicks et al., 1988b, 1990; Buckland-Nicks and Eernisse, 1993) reveal a highly unusual interaction between sperm and egg. Spermiogenesis has been described most completely by Buckland-Nicks et al. (1990), who also summarized earlier studies by Hodgson et al. (1988) and Sakker (1984). Spermiogenesis was concluded to be highly similar in taxonomically disparate species, with the sole exception being the only member of Lepidopleurina thus far examined, *Leptochiton asellus* (Fig. 36), whose "smooth" egg hull was mentioned in the last section.

Hodgson et al. (1988) observed that the sperm headpiece of *Leptochiton asellus* has a large acrosome (Fig. 36) in place of the thread-like anterior nuclear extension otherwise invariably found in mature chiton sperm (see below). The *Leptochiton asellus* acrosome is rather typical of mollusc and marine metazoan free-spawners in general (Franzén, 1956) but is considerably more elongate than

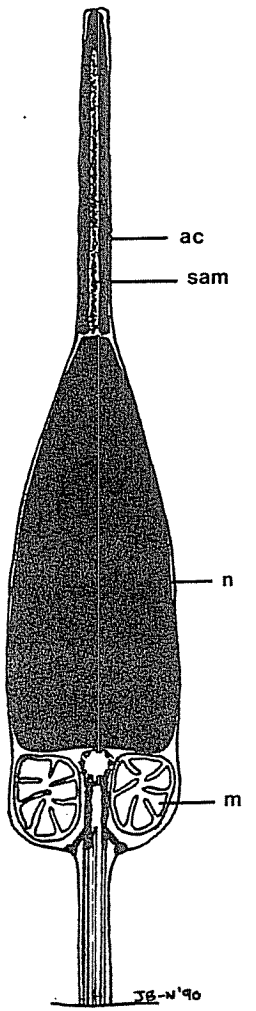



Fig. 36. *Leptochiton asellus* sperm. ac, acrosomal cap; n, nucleus; m, mitochondrion; sam, subacrosomal material. (From Buckland-Nicks et al., 1990.)

figured by Hodgson et al. (1988), presumably because those authors did not observe a perfectly bisecting section (Buckand-Nicks, personal communication).

The other two suborders, Ischnochitonina and Acanthochitonina, are each well represented in the previous sperm comparisons and are quite similar. In early spermiogenesis, small vesicles with lysosome activity are released from the Golgi body, which is situated next to the centrioles (Fig. 37A). The nucleus begins to change from spherical shape with granular chromatin to elongate with increasingly aggregated chromatin (Fig. 37B). The chromatin then becomes twisted into fibers as condensation continues (Fig. 37B,C). One fiber group separates into a bundle and begins to form the anterior filament (Figs. 37C,D, 38B). The small vesicles originating in the Golgi body then migrate through the spermatid cytoplasm into the tip of the anterior filament. Meanwhile, the anterior filament lengthens, and some or all of the mitochondria migrate posteriorly; lateral mitochondria are retained in certain genera. The centriole satellite complex, annulus, and flagellum become well integrated and, in most species, a fibrous body (or in some cases a thickening of the plasma membrane) begins to form on the outside of the axoneme. The flagellum increases in length to over 50 μm with a normal 9 + 2 arrangement of doublet microtubules, ending with a tapering endpiece containing first 9 + 2 singlet microtubules, then only the two central singlet microtubules, which extend to the end of the roughly 5 μm long end-piece. Buckland-Nicks et al. (1990) speculated that the endpiece shape and length matched the anterior filament at the other end of the sperm, perhaps hydrodynamically balancing it.

As the sperm becomes mature, the anterior filament fully extends. Five independent groups of investigators of sperm ultrastructure (Pearse and Woollacott, 1979; Russell-Pinto et al., 1983, 1984; Sakker, 1984; Al-Hajj, 1987; Hodgson et al., 1988) confirmed that the anterior filament is filled with an extension of the nucleus, not an acrosome as was supposed by Franzén (1956). All of these authors failed to find an acrosomal vesicle either in the mature anterior filament or present during spermiogenesis, so a consensus was



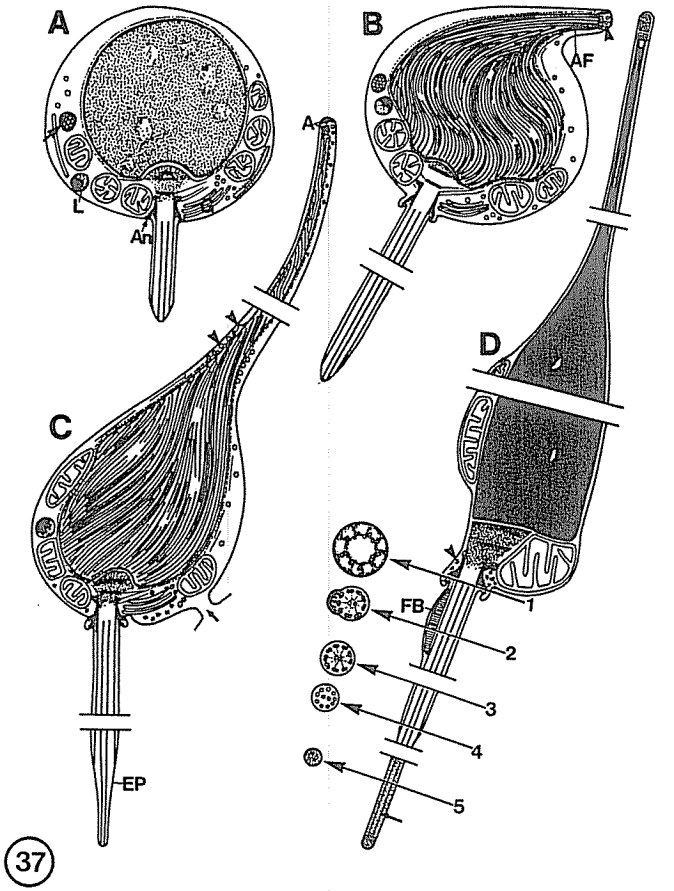



Fig. 37. Diagrammatic representation of spermiogenesis in *Cryptochiton stelleri*. **Stage A:** Golgi body (G) releases small proacrosomal vesicles that migrate anteriorly. Distal centriole is attached to plasma membrane by centriolar satellite complex and annulus (An). Microtubules emanate from both centrioles. Lysosome (L) and multivesicular body (arrow) are sometimes visible. **Stage B:** Nuclear chromatin is condensing into fibers, a bundle of which projects forward into the anterior filament (AF). Proacrosome is developing from fusion of small vesicles at the tip of anterior filament (arrowhead). Golgi body is posterior. **Stage C:** Anterior filament containing nuclear extension is elongating. Proacrosome (A) is visible at tip of nucleus. Nucleopores (arrowheads) are visible in anterior filament. Mitochondria are aggregating posteriorly. Proximal and distal centrioles are bound together by dense material and are housed in an indentation of nucleus. Endpiece (EP) is elongating. Position of cytoplasmic bridge to adjacent spermatid is shown (arrow). **Stage D:** Completed acrosome is at tip of anterior filament. Nucleus is uniformly dense with two small lacunae. Glycogen granules (arrowhead) surround centrioles. Fibrous body (FB) is visible just posterior to midpiece on one side of spermatid. Endpiece (arrow) tapers to a fine filament that contains only two central microtubules. Cross sections through 1, distal centriole; 2, fibrous body; 3, flagellum; 4, first portion of endpiece, showing singlet microtubules; 5, filamentous end-piece. (From Buckland-Nicks et al., 1990.)

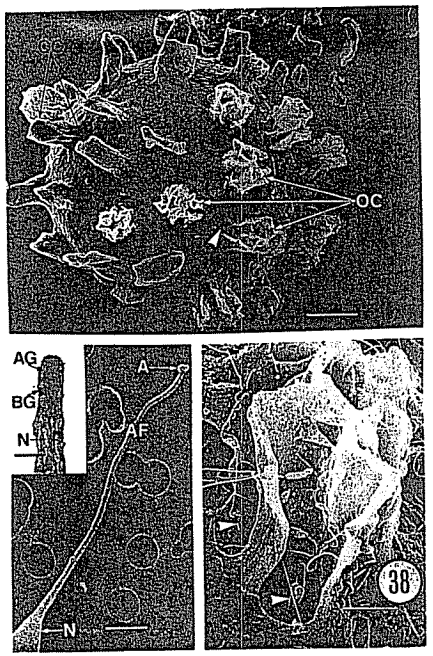



Fig. 38. Fertilization in *Tonicella lineata*. SEM. **A:** A zygote with both open (OC) and closed (CC) hull cupules. Sperm are attracted to open cupules and penetrate the hull at their bases (arrowhead). Scale bar = 40 μm. **B:** Sperm resting on a 0.45 μm nucleopore filter. Acrosome (A) bent upward, anterior filament containing extension of nucleus (AF) and a portion of main body of nucleus (N). Scale bar = 0.45 μm. **Inset:** Tip of sperm anterior filament showing apical (AG) and basal (BG) granules in acrosome separated from tip of nucleus (N) by a basal plate. Scale bar = 0.08 μm. **C:** Part of figure at higher magnification showing sperm penetrating hull (arrowheads) within an open cupule. Scale bar = 10 μm. (From Buckland-Nicks et al., 1988b.)

emerging that the acrosome must be absent. In the absence of evidence for an acrosome, some argued that a micropyle might be involved to facilitate sperm penetration (Sakker, 1984; Al-Hajj, 1987). Buckland-Nicks et al. (1988a,b, 1990) first demonstrated the presence of a small acrosome, visible at the tip of the anterior filament (Fig. 38B), but found no evidence that a micropyle was involved in fertilization. They further demonstrated that the acrosome was divided into discrete apical and basal acrosomal granules (Fig. 38B, inset) and confirmed the lysosomal nature of these vesicles with acid phosphatase staining. The formation of the acrosome at the tip of the sperm completely independent of the Golgi body is the only known case in which small proacrosomal vesicles migrate independently to a predetermined site at the anterior of the spermatid (Buckland-Nicks et al., 1990).

The confirmation of an acrosome-mediated sperm–egg fusion (Buckland-Nicks et al., 1988a) has led to new interpretations of the fertilization mechanism in all observed chitons (Fig. 39) (Buckland-Nicks et al., 1988b) except *Leptochiton asellus*. Depending on the sort of egg hulls present, mature sperm appear to fertilize the egg at particular sites, e.g., either at the center of opened cupules (Fig. 38A,C) or at the adjoining bases of closed, cone-like hulls (Fig. 35) (Buckland-Nicks, 1993; Buckland-Nicks and Eernisse, 1993). Buckland-Nicks et al. (1988b) have postulated that the presumed enzymatic activity of the apical acrosomal granule is expended while the sperm's anterior filament is penetrating the egg hull, and this exposes the basal acrosomal granule, which is expanded during passage through the vitelline membrane. The removal of the basal granule exposes the inner acrosomal membrane, which initiates fusion of the anterior filament tip with a single egg microvillus. This fusion is accompanied by elevation of the plasma membrane around the microvillar base, forming a membranous tube surrounded by a small fertilization cone through which sperm nuclear chromatin, devoid of the nuclear membrane and unaccompanied by sperm organelles, achieves penetration of the vitelline and oocyte membranes. In this way, chiton fertilization is similar to the acrosomal fertilization process observed in other molluscs, various marine invertebrates, and some vertebrates such as lamprey, salamander, and chicken.

If one assumes that the sperm of other members of Lepidopleurina, besides *Leptochiton asellus*, likewise have a large acrosome and that *Chaetoderma argenteum* is an appropriate outgroup for establishing sperm character polarity (Buckland-Nicks and Chia, 1989), then members of Ischnochitonina and Acanthochitonina are hypothesized as united by their shared-derived reduction in acrosomal morphology as well as the accompanying anterior nuclear extension (Eernisse, unpublished data). Sperm variation among the latter two suborders of chitons is



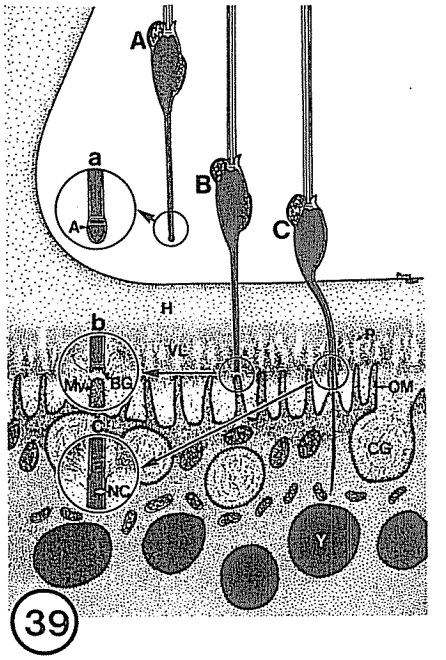



Fig. 39. Diagrammatic representation of the major events involved in sperm–egg fusion in *Tonicella lineata*. Three sperm are depicted at the base of a cupule in different stages of penetration. Sperm A approaches the hull, with acrosome intact (see circle **a**). Sperm B has penetrated the hull and located a pore in the vitelline layer; the acrosome has fired, the apical granule has been exhausted, and the basal granule is being used up as the tip of the anterior filament approaches an egg microvillus (see circle **b**). Sperm C has fused with an egg microvillus, creating a membranous tube through which the sperm chromatin is being injected into the egg cytoplasm, devoid of nuclear membrane (see circle **c**). A, Acrosome; BG, basal granule; CG, cortical granule; H, hull; Mv, egg microvillus; NC, nuclear chromatin; OM, oocyte membrane; P, pore through vitelline layer; VL, vitelline layer; Y, yolk granule. (From Buckland-Nicks et al., 1988b.)

also of phylogenetic and functional interest. According to Hodgson et al. (1988), the sperm of members of Callochitonidae have a rather conventional midpiece, similar to that of *Leptochiton asellus* sperm, with a ring of five or six spherical mitochondria located behind the nucleus. All other nonlepidopleurid chitons thus far examined have a basal asymmetrical placement of midpiece mitochondria, and some also have one or more laterally placed mitochondria. The function of these lateral mitochondria is not yet known. One possibility is that they somehow are involved in helping the nucleus penetrate the egg surface during fertilization, perhaps in a manner similar to the ascidian (Chordata) sperm–chorion-binding reaction process observed and studied experimentally by Lambert and Epel (1979). Another possibility is that these mitochondria do not aid sperm movement or fertilization but instead contribute paternal mitochondrial DNA to the zygote. Paternal contribution of mitochondria has been suggested for certain bivalve molluscs based on fertilization studies (Longo and Anderson, 1969, 1970; Longo, 1973) and mitochondrial DNA heteroplasmy estimates (Hoeh et al., 1991). If the fertilization model of Buckland-Nicks et al. (1988b) is correct, however, then there would be little opportunity for a similar paternal contribution of mitochondrial DNA in chitons. Buckland-Nicks and coworkers postulated that only nuclear chromatin penetrates the egg during fertilization.

## CONCLUSIONS

The genealogical relationships of chitons to other molluscs remain controversial. Polyplacophora is generally considered to be sister taxon to a clade, Conchifera, that encompasses all molluscs except chitons and aplacophorans (Götting, 1974, 1980; Lauterbach, 1983; Wingstrand, 1985; Salvini-Plawen, 1985; Scheltema, 1988; Eernisse et al., 1992). By this view, only one (Aplacophora with subgroups Neomeniomorpha and Chaetodermomorpha) or two (Caudofoveata and Solenogastres) lineages of "aplacophorans"

are placed outside the chiton–conchiferan clade (Testaria). Alternatively, Simroth (1892–1894), Garstang (1896), Hoffman (1949), Boettger (1955), Salvini-Plawen (1972, 1980), and Scheltema (1993) are among those who have advocated a grouping of chitons and aplacophorans, Amphineura (or Aculifera), which they regard as sister taxon to Conchifera. Supporting the former chiton–conchiferan clade are many similarities of a detailed nature, but these could be viewed either as derived in "testarians" or as plesiomorphic, depending on whether one advocates derivation of all molluscs from a simple worm-like ancester or views the testarian similarities as shared from a more ancient ancestor, with large-scale simplification having occurred in aplacophorans due to their vermiform life style (Wingstrand, 1985).

Proponents of the latter chiton–aplacophoran grouping have emphasized shared attributes argued to be derived, especially the calcareous spicules or spines embedded in the cuticular mantle of both chitons and aplacophorans. The issue of whether these spicules are primitive or derived, like many other issues faced in this chapter, is confounded by the lack of living nonmolluscan outgroups with an even remotely similar body plan and an imprecise knowledge of molluscan relationships to surviving spiralian lineages.

Within chitons, lepidopleurids are typically asserted to have retained the more primitive (i.e., plesiomorphic) polyplacophoran condition, partly because small, nondescript, and deepwater attributes are assumed primitive and partly because a simple to complex progressive grade of shell organization continues to dominate chiton systematics. Even if their generally more poorly developed shell features are indeed more like ancestral chitons, as fossil evidence would suggest, there is little *a priori* reason to assume that any other given lepidopleurid character state is plesiomorphic. Outgroup comparisons are a more reliable means to establish character polarization. For example, the lepidopleurid sperm condition was above argued to be likely plesiomorphic based on outgroup comparison with aplacophorans and other marine molluscs. A similar argument could be made for their posterior gill arrangement, based on outgroup comparison with admittedly highly divergent but posterior gill-bearing *Chaetoderma* (Eernisse, unpublished manuscript). Regarding posterior gill placement as plesiomorphic in ancient molluscs is counter to many recent reconstructions of molluscan "archetypes," which feature one or more laterally placed gill pairs.

Eernisse (1984) noticed the coincidental occurrence of an abanal gill type and cup- or modified cup-like hulls. This observation suggests that members of Acanthochitonina may have closer affinities with those members of Ischnochitonina with abanal gills and cup-like hulls than they have with the remaining members of Ischnochitonina, which have spiny hulls and adanal gills (the latter also observed in lepidopleurids). Eernisse (1984) cautioned that the polarity of ad- or abanal gill and cup-like or spiny hull conditions within nonlepidopleurid chitons was difficult to determine due to a lack of suitable outgroups and conflicting evidence among the few available characters. Comparative sperm ultrastructural studies in recent years have added corroborating evidence for viewing abanal gills, hull cups, and certain sperm characters as derived (Eernisse, unpublished manuscript), thus suggesting that Acanthochitonina might be better viewed as a family-level subgrouping of Ischnochitonina, with particular affinities to members of Lepidochitonidae and Mopaliidae. SEM and TEM observations are clearly providing excellent new sources of data for establishment of phylogenetic relationships. A better understanding of phylogeny goes hand in hand with interpreting the functional significance of microscopic details of anatomy.

The invariant nature of chiton anatomical, shell, and radular morphology has often been noted, giving many the impression that chitons are a homogeneous "living fossil" group. Elements of this view may be true, making chitons of great utility as outgroups for the reconstruction of early molluscan evolution. Any serious discussion of the anatomy of the presumed common ancestor for all molluscs will likely involve careful examination of the condition and extent of anatomical variation found in chitons, since chitons are appropriate outgroups for phylogenetic analysis of virtually any other ancient molluscan or spiralian lineage. Yet chitons also have many remarkable features, with the shell and girdle sensory organs, the mineralization of radular teeth, the elaborate egg hull ornamentation, and the unusual sperm morphology being a few of the examples emphasized here. Although not exhaustive, it is our hope that this review will have a role in stimulating further investigation into the many remaining questions.



## NOTE ADDED IN PROOF

In a recent publication Currie (1992b) described a previously unknown type of sensory organ that he discovered in the valves of *Cryptoplax mystica* (Acanthochitonidae). Curry argued that the features of these organs were consistent with a photoreceptive or balance function, but that their anatomy was unlike that of the ocelli (Chitonidae: Acanthopleurinae; Schizochitonidae) or intrapigmented bodies (Callochitonidae) already known for chitons.

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## Chapter 4

# Gastropoda: Prosobranchia

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### INTRODUCTION

The subclass Prosobranchia (originally *Prosobranches*) was first defined by Milne Edwards (1848) as a subdivision of *Gastéropodes branchifères*, the other subdivision being *Opisthobranches*. It consists of approximately 20,000 species (Boss, 1971) in about 4,500 genera (Peel, 1987). Although it contains the majority of marine gastropods, there is no uniquely derived character that defines the group (Hickman, 1988). In fact, it is now generally recognized as a paraphyletic group (Bieler, 1992).

Prosobranchs are one of the few groups of animals to inhabit marine, freshwater, and terrestrial environments. They are found from the depths of the oceans to mountain streams, from Antarctic waters to continental deserts. They crawl, float, or swim through the water. They burrow in sand, rock, shell, and wood. They include suspension feeders, detritivores, herbivores, carnivores, scavengers, and parasites. They range in size from a few millimeters to over 70 cm in shell length (Kohn, 1985) and may live for 20 years or more (P.W. Frank, 1969; Heller, 1990).

The body typically consists of a muscular foot bearing an operculum, a differentiated head with one pair of tentacles, the viscera, and a double epithelium called a mantle that forms a forward-opening cavity surrounding the respiratory organs and that posteriorly surrounds the viscera. Many organs are quite glandular, and mucus is essential to almost every aspect of their biology. Muscles are generally embedded in a connective tissue matrix that gives them their characteristic tough, rubbery texture. The shell is of calcium carbonate in a proteinaceous matrix and is secreted by the edge of the mantle. The gut is U-shaped. The mouth leads to a chamber containing a characteristic radula, or tongue-like ribbon covered with rows of teeth. The gut loops through the body and turns back on itself so that the anal opening is usually above and to the right of the head. A single or paired gill, or ctenidium, is the most common respiratory organ. The circulatory system spans the range from an open to an all-but-closed system. There may be one or two auricles anterior to a single ventricle. The excretory system consists of one or two kidneys, one of which may be dedicated to the discharge of