Aesthete Canal Morphology in Twelve Species of Chiton (Polyplacophora)

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Abstract. Epoxy casts were made of the aesthete canal system in chiton valves (shell plates) from twelve species, representing four families and the three major modern suborders of the Polyplacophora. In this study, Mopaliidae was represented by *Mopalia muscosa, Mopalia acuta, Nuttallochiton hyadesi,* and *Placiphorella velata*; Tonicellidae by *Lepidochitona hartwegii* and *Nuttallina californica*; Ischnochitonidae by *Lepidozona cooperi, Lepidozona mertensii, Lepidozona pectinulata, Ischnochiton textilis,* and *Ischnochiton variegatus*; and Lepidopleuridae by *Lepidopleurus cajetanus.* The casts reveal a diversity of large and small-scale canal forms in the chitons studied. However, members of each suborder and family share fundamental features of the aesthete canal system, which suggests that epoxy casting of the aesthete canals provides a set of characters useful in future taxonomic and phylogenetic studies of chitons. The casts also reveal a greater connectivity in the total aesthete canal system than is widely realized. For instance, canals in the apical area connect to those in the slit rays, the ventral area below the jugum, and the dorsal surface of the valve. Canal morphology also seems to be influenced by the shell layer in which canals occur. For example, those canals that exist within the articulamentum are much more flattened in cross section than those that occur in the tegmentum.

INTRODUCTION

Chiton valves (shell plates) consist of a thin outermost organic periostracum layer and three underlying aragonitic layers: tegmentum (upper), articulamentum (middle), and hypostracum (lowermost). The principal shell layers are the tegmentum, which bears the shell sculpture, and the underlying denser articulamentum, whose marginal projections form the insertion plates and sutural laminae (Baxter & Jones, 1981). Marshall (1869) was the first to describe the tissue-filled canal system that penetrates the chiton tegmentum, pointing out that fine vertical canals at the surface connect to bulbous cavities that in turn lead to horizontal canals that run at the interface between the tegmentum and articulamentum. The tegmentum is penetrated by

canals through much of its volume, and the distribution and nature of the canal elements varies between the valve areas in at least some chitons (Fischer & Renner, 1979). Although most discussion of aesthete canals in chitons has focused on the tegmentum layer where the canals are densest, the presence of pores in the jugal sinus and slit ray regions of the ventral surface of the valves indicates that the aesthete canal system infiltrates the articulamentum layer in certain areas (Baxter & Jones, 1981).

Moseley (1884, 1885) was one of the first to describe aesthete tissues in fine histological detail, noting the occurrence of two distinct size classes: he named the larger ones megalaesthetes and the smaller ones micraesthetes. In addition, he discovered "eyes," or ocelli, characterized by a refractive lens and the presence of pigmentous cells seen in much larger chambers within the valves of certain chiton species (Moseley, 1885), and Boyle (1969) nearly a century later confirmed the presence of photoreceptors within these "eyes."

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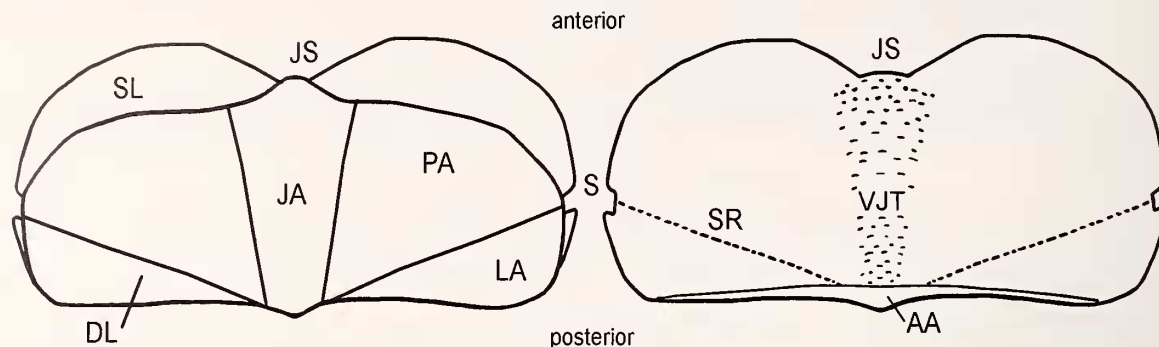
Figure 1.   Drawing of chiton valve showing terminology (dorsal valve surface on left; ventral on right). Key: JS = jugal sinus; SL = sutural laminae; DL = diagonal line; S = slit; SR = slit ray; JA = jugal area; PA = pleural area; LA = lateral area; AA = apical area; VJT = ventral jugal triangle.

Micraesthetes typically consist of a single cell with its nucleus lodged within the megalaesthete chamber. Megalaesthetes are larger, multicellular organs generally composed of several secretory. cells, microvillous cells (central cells), one or more photoreceptor cells, and peripheral cells (Fischer, 1988; Eernisse & Reynolds, 1994). However, megalaesthete cellular composition varies between species and at least one species, *Tonicella marmorea*, lacks photoreceptor cells altogether (Baxter et al., 1987). Ocelli, which occur in much larger spaces that are thought to be modified megalaesthete chambers, have so far been found in just a few chiton species and are sparsely distributed on the valve surface in those species (Moseley, 1885; Boyle, 1969; Boyle, 1976).

The function of the typical (non-ocelli) aesthetes been debated, with proposals including chemoreception (Fischer, 1988); mechanoreception (Moseley, 1885); periostracum replenishment and secretion (Boyle, 1974; Baxter et al., 1990); secretions serving protective functions (Fischer, 1988); and photoreception (Moseley, 1885; Blumrich, 1891; Omelich, 1967; Boyle, 1972; Fischer, 1978, 1988). An early electron microscopy study (Omelich, 1967) and a more recent immunocytochemical study (Reindl et al., 1997) have shown that aesthetes clearly contain neuronal structures, suggesting a sensory function. The presence of photoreceptive cells and ocelli in larger aesthete complexes, along with the fact that many chitons have been shown to be positively or negatively phototactic (e.g., Crozier, 1920; Omelich, 1967; Boyle, 1972; Fischer, 1988; Currie, 1989), has led many to view photoreception as one of the primary functions of aesthetes, although it seems likely that aesthetes serve multiple roles (Haas and Kriesten, 1978; Fischer, 1979, 1988).

Baxter & Jones (1981, 1984) described three main types of aesthete canal (channel) systems that occur in distinct areas of the valves of *Lepidochitona cinereus* and *Callochiton achatinus*: multiple branch channels, found in the lateral and pleural areas (see Figure 1 for an illustration of chiton valve terminology); jugal area channels; and slit ray channels. Multiple branch channels open along the anterior margin of the tegmentum. These branch repeatedly along their horizontal pathway through the tegmentum, with branches subsequently leading up to megalaesthete and micraesthete canals just below the valve surface. Slit ray channels open along the lines of pores (slit ray) on the ventral surface of valves that have slits in the insertion plates, as occurs in most chiton species. Baxter and Jones (1981) argued that these channels pass dorsally and posteriorly from the ventral openings through all shell layers to open at the dorsal valve surface. Jugal area channels form a triangular area of pores on the ventral valve surface and are similar to slit ray channels because they were also thought to pass through all valve areas to open on the dorsal surface (Baxter and Jones, 1981).

Moseley (1885) proposed that the structure and arrangement of the "eyes" in those chitons that have them could be useful in phylogeny. More broadly, Leloup (e.g., 1934, 1936, 1937, 1940a, 1940b, 1942, 1948, 1952) incorporated many drawings of the patterns of micraesthetes and megalaesthete chambers in his taxonomic descriptions of numerous chiton species. More recent workers (e.g., Boyle, 1974; Baxter & Jones, 1981, 1984; Baxter et al., 1987; Fischer, 1988; Currie, 1989; Baxter et al., 1990; Currie, 1992; Sturrock & Baxter, 1993; Reindl et al., 1997) have also documented aesthete and aesthete canal morphology in different chiton species. These studies have shown that the fine scale form of the aesthetes and the canals that house them vary between species and so represent characters that could be used in phylogenetic analyses. In fact, aesthete pore densities and arrangements on the valve surface have already been used in phylogenetic studies (e.g., O'Neill, 1985; Bullock, 1985). However, it still seems true that, as Currie (1992:3) wrote, "The paucity of information on aesthete morphology and distribution in a range of chiton species/families, and indeed habitats, clearly remains a hindrance to our understanding of aesthete function and evolution."

Epoxy casts have been used to infer the shape of tunnels in shells with endolith borings (e.g., Golubic et al., 1970; Vogel & Marincovich, 2004) and shell pores in limpets (Reindl and Haszprunar, 1994). In addition, Haas & Kriesten (1978) used this method to obtain details of the aesthete canal system in *Chiton albolineatus*, revealing short micraesthete canals that feed into elongate megalaesthete chambers that connect to a small number of large horizontal canals. Vendrasco et al. (2004) used the epoxy casts of a chiton valve to compare with the tunnels in valves of multiplacophorans, an unusual type of Paleozoic chiton.

The purpose of this study was to provide more information about: (1) the morphology of the total aesthete canal system; (2) how aesthete canal patterns differ between a set of chiton species; and (3) whether aesthete canal patterns correlate best with taxonomic relationships or environmental factors.

MATERIALS AND METHODS

Individuals of *Mopalia muscosa* (Gould, 1846) (Santa Barbara Museum of Natural History (SBMNH) 83143 and 83144), *Lepidochitona hartwegii* (Carpenter, 1855) (SBMNH 83146 and 83147), and *Nuttallina californica* (southern form; previously *Nuttallina fluxa*) (SBMNH 83148–83149) were collected by CZF and MJV in the middle to lower intertidal of Palos Verdes, California on the surface of rocks. Individuals of *Lepidozona pectinulata* (Carpenter in Pilsbry, 1893) (SBMNH 83152 and 83153) were also collected from Palos Verdes, from under submerged stones in the lower intertidal. Individuals of *Lepidozona cooperi* (Dall, 1879) (SBMNH 83150 and 83151), one specimen of *Nuttallina californica* (Nuttall MS, Reeve, 1847) (SBMNH 83156), and one specimen of *Lepidozona mertensii* (von Middendorff, 1847) (SBMNH 83145) were collected by MJV from the rocky intertidal of Cambria, California. Specimens of *Mopalia acuta*, *Placiphorella velata*, *Lepidozona merteusii*, *Ischnochiton textilis*, and *Ischnochiton variegatus* were obtained from the SBMNH and isolated valves of *Nuttallochiton hyadesi* were provided by the Los Angeles County Museum (LACM). All other chitons were obtained from shell dealers.

One specimen of *Ischonochiton textilis* (Gray, 1828) (SBMNH 83158) was collected from intertidal rocks in Chelsea Point, Port Elizabeth in the Eastern Cape, South Africa; the other (SBMNH 369435) is from the George Hanselman collection, also collected from Port Elizabeth, South Africa at 0–2 m. One specimen of *Ischnochiton variegatus* (H. Adams and Angas, 1864) (SBMNH 83159) was collected under rocks in the intertidal zone in DeMole Point, South Australia; the other (SBMNH 369437) was collected from Port MacDonnell in South Australia, under small rocks

with sand in the intertidal. One individual of *Mopalia acuta* (Carpenter, 1855) (SBMNH 83160) was collected half buried in sand on the side of a rock in Doheny Beach, Orange County, California; the other (SBMNH 369432) was collected from Oceanside, near Camp Pendleton, on cobble reef on the underside of a rock in the intertidal zone during a −0.37 m tide. One individual of *Placiphorella velata* Carpenter MS, Dall, 1879 (SBMNH 83161) was collected from the intertidal zone in Pacific Grove, Monterey Peninsula, California; the other (SBMNH 369440) is from the Spencer Thorpe collection, found in Timber Cove, Sonoma County, California on a rock in the intertidal zone. *Nuttallochiton hyadesi* (de Rochebrune, 1889) (SBMNH 83157) had been dredged from a depth of 384–494 m at 56°06′S, 66°19′W off the coast of Tierra del Fuego. One specimen of *Lepidozona mertensii* (SBMNH 369438) was collected from Port Gamble, Washington, USA in the intertidal. One specimen of *Lepidopleurus cajetanus* (SBMNH 83154) was collected at 2.5–4 m depth under stones in Galeria, Corsica, Mediterranean; the other individual (SBMNH 83155) on the coast of Croatia under rocks on sand in the lower intertidal. All chitons used in this analysis were adults.

Valves from at least two individuals from each species were used, except for the rare *Nuttallochiton hyadesi*. Both the northern and southern form (the latter was previously named *Nuttallina fluxa*) of *Nuttallina californica* were used. Valves from only one individual of the northern form was examined, although two individuals of the southern form were processed. One to three intermediate valves of each individual were embedded and examined.

Whole specimens were boiled so the valves could be removed from the flesh. Even isolated shell plates were boiled in order to clean them. The isolated intermediate valves of all species were soaked in bleach for up to 24 hr and placed in a sonicating bath for 25 min at room temperature to dislodge remnant organic material and other debris. Valves were dehydrated through an ethanol series and then embedded in epoxy using a method modeled after (Golubic et al., 1970). A low viscosity embedding medium was created using the Embed 812 kit from Electron Microscopy Sciences. The Embed 812 kit consisted of Embed 812 embedding resin, Dodecenyl Succinic Anhydride (DDSA), Nadic Methyl Anhydride (NMA), and Benzyldimethylamine (BDMA). They were combined in the following proportions: 44.1% Embed 812, 35.3% DDSA, 17.6% NMA, and 2.9% BDMA. The valves were submerged in resin and placed under a vacuum in a desiccating chamber for 24 hr and then cured in an oven at 60°C for 24 hr. The cured blocks were trimmed using a Buehler low speed saw or with a dremel tool using a thin-bladed saw. Cuts were made around the edges of the valves, making sure to intersect the valve on all

sides. The blocks were placed in 10% HCl for another 24 hr or until all of the calcium carbonate dissolved away, then rinsed with distilled water, cleaned with bleach, and split apart into a dorsal and ventral cast. The resultant casts were gold sputtered and most were examined under SEM using a LEO 1430 with an accelerating voltage of 10–15 kV.

The cladistic analysis using aesthete characters was performed using PAUP 4.0b10 (Swofford, 2002). An exhaustive search was completed using maximum parsimony; all characters were unweighted and all character states were unordered.

Assignments to chiton orders and families were based on Sirenko (1997), although alternative taxonomic schemes are mentioned in the Discussion of this paper.

Isolated valves and epoxy casts of each species in the analysis were deposited in the chiton collections of the Santa Barbara Museum of Natural History.

RESULTS

In this section, the trend of the canal system is described in a manner consistent with the direction of valve growth (see Baxter & Jones, 1981, 1984) and the flow of sensory information. The openings of the canals on the dorsal surface of the valve are taken to be the origin and the places where the canals enter the body of the chiton (e.g., at the anterior and lateral eaves) are described as the exit. The portions of the valve surface referred to in the text are shown in Figure 1. All directional indicators (anterior, posterior, dorsal, ventral) are meant with respect to the valve in life position. The two pieces of the aesthete canal cast are referred to as dorsal and ventral, also defined based on life position.

Lepidopleurus cajetanus
(Lepidopleurina: Lepidopleuridae)

The dorsal casts (Figures 5f–h) reveal micraesthetes (~4–6 μm diameter) that feed into a bulbous megalaesthete chamber (~30–60 μm diameter), that then connects to a single, narrow (~10 μm diameter) horizontal canal that leads to the anterior or lateral margin. Megalaesthetes are arranged in anterior-posterior rows along the ridges of the central area. The megalaesthetes are much denser, and less organized, in the ridged lateral areas. The megalaesthete bulbs typically have a sub-cylindrical shape in the central area, often with a constriction at the base where they attach to, or become, the horizontal canal. In some cases the bulbs have a more expanded tent-like shape and have a greater number of micraesthetes (>10 vs. ~7) feeding into them. There is a distinct spacing of ~10 μm between adjacent aesthete complexes in the central area, leading to a clustered appearance of

aesthete pores on the dorsal valve surface. The canals have a meandering, somewhat interweaving pathway from megalaesthete bulb to valve margin.

The ventral casts show that a large number of aesthete canals terminate on the ventral surface of the shell plate, even though *Lepidopleurus* lacks the slit rays and associated ventral slit ray canals. There is an abundance of canals in the ventral jugal triangle, at and near the apical area, and in the region underneath the lateral areas.

Mopalia muscosa (Acanthochitonina: Mopaliidae)

The dorsal casts (Figures 2a–c) reveal nearly straight, primary horizontal canals (~20–35 μm in diameter) that run from the posterior to the anterior margin through all valve areas. The canals are closely spaced (~12–20 μm between primary canals; ~20 canals per mm along the horizontal plane) and in some cases adjacent horizontal canals merge with each other. At least two layers of primary horizontal canals can be seen as they near the anterior margin of the valve. There are also many smaller-diameter (~10–20 μm), short, connecting canals, above and sub-parallel to primary horizontal canals, that merge with the primary canals at regular intervals. The connecting canals, and in some instances the primary horizontal canals, connect to the dorsal valve surface via a megalaesthete chamber fed into by a megalaesthete or micraesthete canal. Micraesthete canals are ~2–3 μm in diameter. Megalaesthete canals begin as a short length of canal with a diameter of ~6–7 μm, before gently flaring out to a diameter of ~15–25 μm as they continue down towards the horizontal canals. At the top of the casts of the micraesthete canals and megalaesthete chambers are cup-shaped protuberances that appear to be filled-in subsidiary (~6–8 μm diameter) and apical (~10–11 μm) caps, respectively.

In the jugal area, some horizontal canals have a more flattened appearance and turn down towards the ventral valve surface. On the ventral casts (Figures 2d–f), the corresponding area (herein referred to as the ventral jugal triangle) has short lengths of similarly flattened canals. These ventral canals occur along valve growth lines; ~2–5 canals per growth line can be seen on the cast. A number of canals pass from the apical area into the ventral jugal triangle.

Some canals (visible on the dorsal casts) near the boundary of the pleural and lateral areas, along the diagonal line, are turned downwards. The canals in this region originate dorsally and merge with each other (on average, 2–3 canals merge) as they near the ventral valve surface. On the ventral cast, a single line of horizontal canals is present parallel to the slit ray; on *Mopalia muscosa* valves, slit rays correspond exactly to the diagonal line.

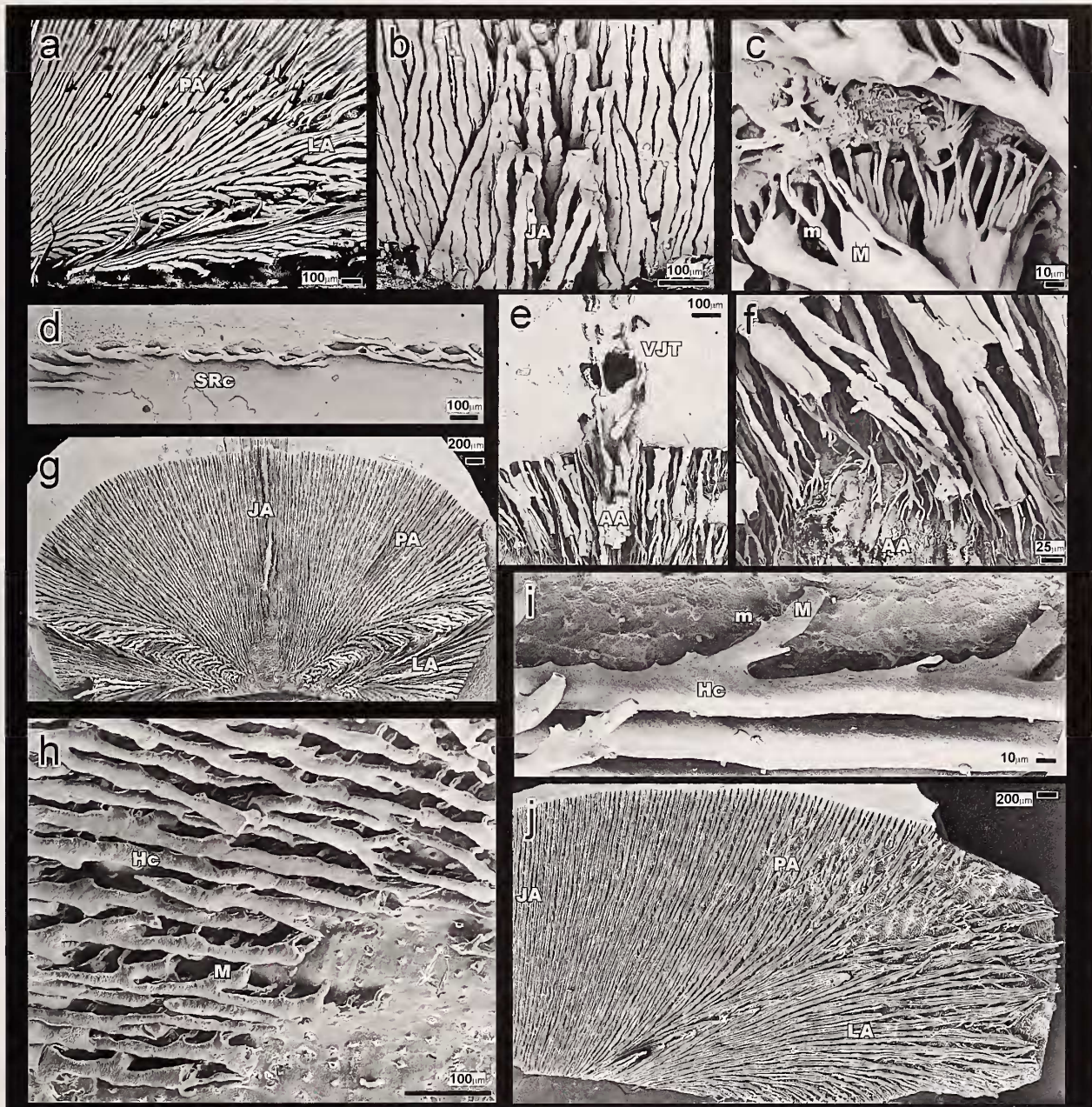The ventral casts show the apical area canal system

Figure 2. SEM images of casts of aesthete canal systems of *Mopalia muscosa* (SBMNH 83143) (a–f), *Nuttallochiton hyadesi* (SBMNH 83157) (g, h), and *Mopalia acuta* (i, j). Images d–f are of a ventral cast. All other images are of dorsal casts. a. Horizontal canals in pleural area and part of lateral area. b. Close-up of the jugal area showing flattened canals. c. Close-up of megalaesthete chambers and micraesthete canals. d. Slit ray canals. e. Apical area and ventral jugal triangle showing canals passing from the apical area into the ventral jugal triangle. Canals exiting at the lip of the apical area can also be seen. f. View of apical area showing micraesthete canals that originate at the apical area. g. Overview of valve cast showing little differentiation of valve areas. h. Close-up showing megalaesthete chambers. i View of horizontal canals showing megalaesthete chambers and incompletely cast micraesthete canals (SBMNH 83160). j. Overview of half of valve cast (SBMNH 369432). Key: M = megalaesthete chamber; m = micraesthete canal; SRc = slit ray canals; all others abbreviations are shown in Figure 1.
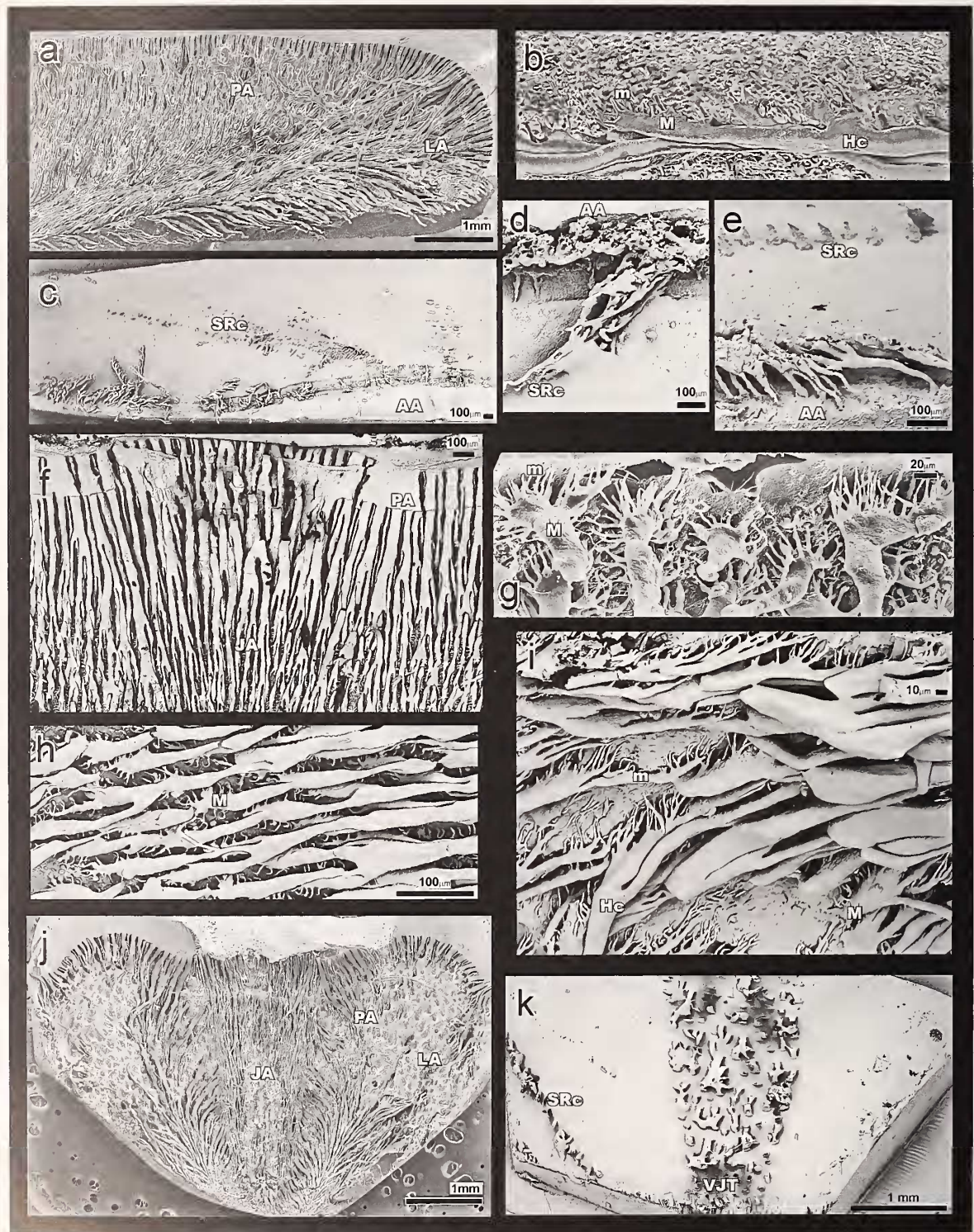
Figure 3. SEM images of casts of aesthete canal systems of *Placiphorella velata* (a–e), *Lepidochitona hartwegii* (f–h), and *Nuttallina californica* (i–k). Images c–e, k are of a ventral cast. All other images are of dorsal casts. a. Overview of half of cast showing little differentiation of valve areas (SBMNH 369440). b. Close-up of horizontal canals on same specimen, showing megalaesthete chambers and micraesthete canals. c. Overview of half of cast showing canals at slit ray region and apical area and partial canals at the ventral jugal triangle (SBMNH 83161). d. Magnification of canals at apical area passing into slit ray region.

in great detail. Numerous micraesthete-sized canals originate at the dorsal valve surface, merging with a larger subsidiary canal or with each other then with a subsidiary canal before then merging with a primary horizontal canal that exits at the anterior lip of the apical area. A few canals can be seen crossing from the apical area into the slit ray region.

Mopalia acuta (Acanthochitonina: Mopaliidae)

The dorsal casts (Figures 2i, j) reveal primary horizontal canals (~25–35 µm diameter) that run from the anterior to the posterior margin through all valve areas. There is a single principal layer of primary canals, with a density of ~19 canals per mm and a spacing of ~8–17 µm between the canals within this layer. Indistinct, elongate megalaesthete chambers (~8–17 µm diameter) feed into the main horizontal canals after a short length. The chambers merge along the length of the primary canals at regular intervals. Numerous micraesthetes (~1–3 µm in diameter) feed into each megalaesthete chamber all along its length.

On the ventral casts, growth lines as well as a few incomplete canals can be seen in the ventral jugal triangle. In one specimen, there is a line of partially preserved horizontal canals that parallel the slit ray, in addition to the apical area canals.

Nuttallochiton hyadesi (Acanthochitonina: Mopaliidae)

The dorsal casts (Figure 2g, h) reveal primary horizontal canals (~30–50 µm in diameter) that are preserved throughout the entire shell. There is a spacing of ~20–50 µm between canals, with ~18 canals per mm along the horizontal plane. Micraesthetes (1–3 µm diameter) feed into the elongate, indistinct megalaesthete chambers that feed directly, after a short distance, into the primary horizontal canals. This canal pattern is similar to that seen in the Mopalia spp. The megalaesthete chambers have a diameter of ~10–12 µm before widening to the same diameter as the connecting canals (~17–20 µm). At the region above the slit ray, the horizontal canals curve towards each other, merge, and proceed downward towards the slit rays.

The ventral casts reveal a narrow line of slit ray canals, apical area canals, but very few ventral jugal triangle canals.

Placiphorella velata (Acanthochitonina: Mopaliidae)

The dorsal casts (Figures 3a, b) reveal primary horizontal canals (~21–34 µm in diameter) that run from the posterior to the anterior margin through all valve areas. There is significant spacing between horizontal canals through much of the valve interior, but at the anterior margin, there are ~24 canals per mm with a spacing of ~22–40 µm between canals along the horizontal plane. At least two layers of primary canals can be seen. At regular intervals along the length of primary canals, there are junctions where short canals (~20–25 µm in diameter) from the megalaesthete chambers merge with the primary canal in a manner similar to that seen in Mopalia acuta and the other mopaliids. Numerous micraesthetes (~3–4 µm in diameter) feed into each elongate, indistinct megalaesthete chamber along its length. No obvious differences in aesthete canal morphology in the different valve areas can be detected in the casts.

On the ventral casts (Figures 3c–e), there are a few partial canals preserved at the ventral jugal triangle. There are also a number of slightly flattened canals scattered along the lines parallel to the slit rays. Canals are also preserved along the entire apical area. The canals turn upwards towards the dorsal valve surface and are oriented parallel to the direction of valve growth. They appear to originate as megalaesthete chambers close to the dorsal valve surface with two or more of these chambers merging into a single horizontal canal which exits at the apical area.

Lepidochitona hartwegii
(Acanthochitonina: Tonicellidae)

The dorsal casts (Figures 3f–h) reveal primary horizontal canals (~35–45 µm in diameter) that run from the posterior to the anterior margin through all valve areas. There is a spacing of ~15–35 µm between the primary canals, with ~20 canals per mm along the horizontal plane. The primary horizontal canals vary in diameter as they traverse the valve. At the posterior valve margin, many short lengths of subsidiary canals can be seen merging with each other to form a larger primary canal. As these primary canals continue to course towards the anterior margin, subsidiary canals merge into them at regular intervals. Megalaesthete

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e. Close-up of apical area on same specimen showing canals turned up towards dorsal valve surface. A line of canals present at slit ray region can be seen behind the apical area. f. Overview of cast of *Lepidochitona hartwegii* (SBMNH 83146). g. Magnification of megalaesthete chambers and micraesthete canals (SBMNH 83147). h. Horizontal canals at posterior showing merging of megalaesthete chambers (SBMNH 83146). i. View of horizontal canals in same specimen, showing megalaesthete chambers and micraesthete canals. j. Overview of cast of *Nuttallina californica* (southern form; previously *N. fluxa*) (SBMNH 83149). k. Overview of cast of *Nuttallina californica* (SBMNH 83156) showing flattened canals at the ventral jugal triangle, slit ray region, and apical area. Key: Same as in Figures 1 & 2.

chambers that are typically elongate and indistinct, but occasionally with a slightly pear-shaped appearance, originate at the dorsal valve surface with a diameter of ~10–14 μm, then widen to a diameter of ~25–30 μm, before tapering off to ~9.5–14 μm in diameter. Numerous micraesthete canals with a diameter of 6–9 μm merge into the megalaesthete chambers. Each micraesthete canal originates at a sub-spherical subsidiary cap. The megalaesthetes fuse into the horizontal canals after a short length.

There is some variation in canal morphology in the different valve areas. In the lateral and pleural areas, there is a shorter distance between megalaesthete chamber and primary canal than in the jugal area, where there is a longer distance and where many of the primary canals turn down towards the ventral valve surface.

The ventral casts show portions of flattened canals in the ventral jugal triangle and a few partial canals along the lines parallel to the slit rays, as well as apical area canals.

Nuttallina californica (Acanthochitonina: Tonicellidae)

The dorsal casts (Figures 3i, j) show an irregular spacing (~25–40 μm between canals) of primary horizontal canals (~20–40 μm diameter) packed at ~24 canals per mm along the horizontal plane. The primary canals run in a posterior-anterior direction at the jugal area and fan out diagonally at the pleural and lateral valve areas. There are some canals near the posterior margin of the valve that flank the jugal area, coursing inwards to form a v-shaped pattern. The most conspicuous primary canals are flattened with many tubular subsidiary canals (~12–15 μm in diameter) that feed into them. The subsidiary canals run above and subparallel to the primary canals. They begin at the dorsal valve surface as a megalaesthete chamber with a diameter of ~6–10 μm and branch and widen to a range of diameters as they run into the primary canals. There are also numerous micraesthete canals ~2.5–3.5 μm in diameter that merge all along the branching subsidiary canals. The megalaesthete chambers are not well defined, making it difficult to define where the megalaesthete chamber ends and the subsidiary canal begins.

At the jugal area, flattened horizontal canals appear to turn downwards towards the ventral valve surface. These canals appear to correlate with flattened, upward oriented canals found on the ventral jugal triangle of the ventral cast.

On the ventral casts (Figure 3k), a layer of large, flat canals can be seen overlying a lower layer of smaller, branching subsidiary canals in the apical area. Some of the large, flat canals extend from the apical area into the ventral jugal triangle. Numerous micraesthetes that originate at the apical area feed into subsidiary canals. There are also smaller and less flattened canals along

the lines parallel to the slit rays, some of which are turned upwards towards the dorsal valve surface, and others downwards towards the ventral valve surface.

There does not seem to be any significant difference in the aesthete canal systems between the southern form of this species (previously *N. fluxa*) and the northern form.

In addition to the aesthete canal system, the dorsal cast of one individual shows numerous borings produced by endolithic microorganisms. The clusters of borings are scattered among the aesthete canals located near the posterior end of the valve.

Lepidozona mertensii (Chitonina: Ischnochitonidae)

The aesthete canal morphology seen in the dorsal casts (Figures 4a–d) correlates with the dorsal valve sculpture. Canals are arranged in discrete zones divided by linear regions that correlate with ridges on the valve surface. There is a v-shaped pattern of aesthete canals in the jugal area, a more parallel set of canals in the pleural area, and some canals that run diagonally from the slits to the apex of the valve in the lateral areas.

There is a single layer of primary horizontal canals close to the articulamentum layer. These primary canals vary in size (~15–30 μm in width) and shape, ranging from thin and round to thick and flattened. There are ~27 canals per mm along the horizontal axis, with a spacing of ~20–40 μm between canals at the anterior margin. These primary horizontal canals connect the large, bulbous megalaesthete chambers. Each separated longitudinal zone of bulbous megalaesthete chambers in the lateral and pleural areas is approximately 5–7 chambers wide. The ovoid megalaesthete chambers are oriented sub-parallel (angled slightly upwards) to the dorsal surface, with a maximum width of ~35–45 μm, tapering at each end to ~7–10 μm in diameter. The chambers seem to either connect to a primary horizontal canal or to the body of a megalaesthete chamber adjacent to it. A number of micraesthete canals that start off at the valve surface at 5–7 μm in diameter and taper to 2–3 μm in diameter are located directly above the megalaesthete chamber and surround the megalaesthete canal that originates at the dorsal valve surface.

The ridges on the valve surface correspond with symmetrical pairs of megalaesthete chambers that have numerous micraesthetes feeding into them. The megalaesthete chambers in these areas are slightly larger than those in other regions of the valve.

The lateral areas lack the clearly defined longitudinal zones of megalaesthete chambers seen in the jugal and pleural areas. Instead, there are closely spaced megalaesthete chambers surrounding rows of pits that house large mound-shaped structures (assumed to be an extremely large megalaesthete chamber) with numerous micraesthete canals feeding into them. These pits correlate with the enlarged granules arranged in rows
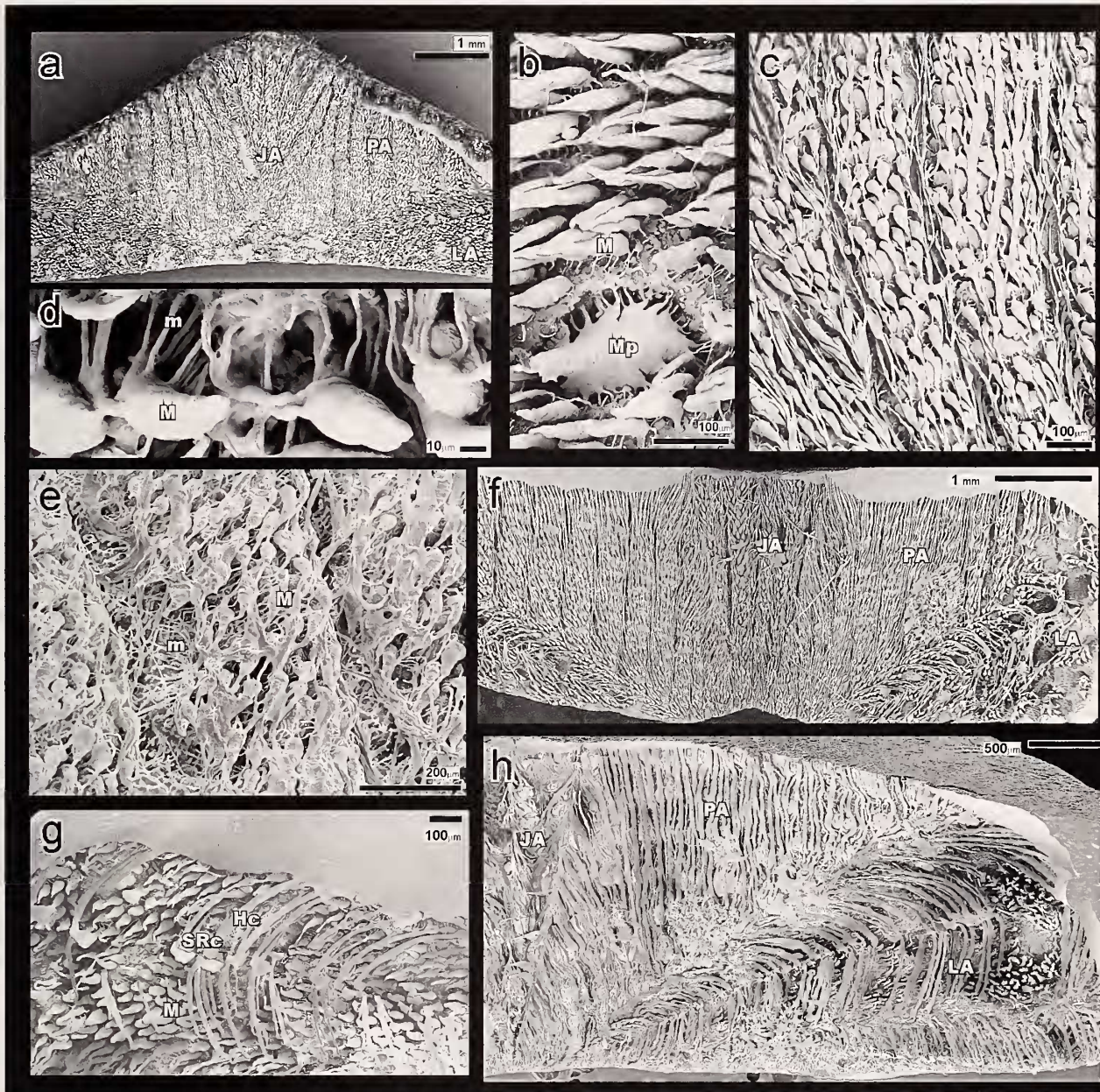
Figure 4. SEM images of casts of aesthete canal systems of *Lepidozona mertensii* (a–d), *Lepidozona cooperi* (e, f), and *Lepidozona pectinulata* (g, h). All images are of dorsal casts. a. Overview of cast of *Lepidozona mertensii* (SBMNH 83145) showing differentiated valve areas which correlate with valve sculpture. b. Megalaesthete chambers and megalaesthete pit located in lateral area in same specimen. c. Longitudinal columns of megalaesthetes with some flattened primary horizontal canals in same specimen. d. Veiw of micraesthete canals and megalaesthete chambers in same specimen. e. View of *Lepidozona cooperi* (SBMNH 83150) longitudinal columns of megalaesthetes with micraesthete canals. f. Overview of cast (SBMNH 83151) showing differentiated valve areas. g. View of *Lepidozona pecinulata* (SBMNH 83152) showing lateral area with horizontal canals converging into slit ray canals. h. Overview of cast (SBMNH 83153) showing differentiated valve areas. Key: Mp = megalaesthete pit; all others same as in Figures 1 & 2.

on the dorsal valve surface. The megalaesthete chambers are oriented more perpendicular to the valve surface than their counterparts in the jugal and pleural areas.

On the ventral cast, canals are evident at the ventral jugal triangle, slit rays, and apical area. The small portions of canals in the ventral jugal triangle are flattened and periodically turn up towards the dorsal valve surface. Canals at the slit rays seem to be thin and diagonally oriented towards the valve surface. Canals at the apical area are present along the entire width of

the posterior valve margin. Flat horizontal canals appear to exit at the anterior lip of the apical area and have many micreasthete canals which originate at the apical area feeding into them.

The slit ray canals consist of multiple rows that expand outward from the apex to the anterolateral margin. The jugal area canals are not abundant on the ventral casts.

Lepidozona cooperi (Chitonina: Ischnochitonidae)

The aesthete canal morphology of this species (Figures 4e, f) is similar to that of *Lepidozona mertensii*, except *L. cooperi* has a slightly greater proportion of horizontal canals. These canals are most noticeable along the anterior margin, and in the lateral areas. The thin, meandering horizontal canals curve towards each other in the lateral areas.

The ventral casts show a greater extent of jugal area canals preserved than in *L. mertensii*.

Lepidozona pectinulata (Chitonina: Ischnochitonidae)

The aesthete canal morphology of this species (Figures 4g, h) bears similarities to that of the other members of *Lepidozona* in this study. However, this species differs fundamentally from the other two species in that it lacks the huge esthete chambers in granule spaces and, more noticeably, has an extensive complement of large horizonatal canals throughout much of the tegmentum interior. The curvature of these canals in the lateral area is more regular in *L. pectinulata* than in *L. cooperi*.

Ischnochiton textilis (Chitonina: Ischnochitonidae)

The dorsal casts (Figures 5a, b) reveal a canal system similar to that of *Lepidozona cooperi* and *Ledidozona pectinulata* in having horizontal canals that converge at the diagonal line and distinct, tear-drop shaped megalaesthete bulbs that feed into horizontal canals. There are ~29 primary horizontal canals (~9–23 μm in diameter) per mm along the horizontal plane at the anterior margin. Primary horizontal canals are spaced ~15–27 μm apart. These canals thin out towards the posterior margin, revealing rows of bulbous, sub-conical megalaesthetes above them. Micraesthete canals (~1–4 μm in diameter) that originate at the dorsal surface merge into the ends of the megalaesthete chambers. The horizontal canals show a strong curvature in the pleural and lateral areas, and are straight in the jugal area.

The ventral casts reveal a high density of flattened canals present at the ventral jugal triangle and at the slit rays. Canals in the apical area are sparsely scattered along the posterior valve margin.

In one individual (SBMNH 83158), an unusual, probably secondary (see Discussion) pattern of large, bulbous cavities oriented sub-perpendicular to the shell surface occur in the uppermost tegmentum layer. These cavities connect to tiny elongate canals that form a horizontal web just below the shell surface. Four way intersections of these tiny tunnels are present, although it is not clear if the intersections connect the large cavities to each other. The bulbous chambers (~40–50 μm in diameter at origin, widening to ~53–60 μm in diameter) have many small canals merging into them all along their height. The valves of this individual have occasional large openings on the dorsal surface that correspond to the bulbous cavities.

Ischnochiton variegatus (Chitonina: Ischnochitonidae)

On the dorsal casts (Figures 5c–e), there is evidence of two or more layers of primary horizontal canals. The top row of horizontal canals (~9–13 μm in diameter), spaced approximately ~12–22 μm apart from each other, can be clearly seen at the anterior valve margin. There are ~34 canals per mm along the horizontal plane at the margin. The dorsal casts reveal that micraesthete canals feed into teardrop-shaped mega-laesthete chambers that taper down to connect to the primary horizontal canals. The primary canals are relatively thin, with low density, through much of the valve interior. The horizontal canals appear to be straight in the jugal area, curve slightly in the pleural areas (though to a lesser degree than in *I. textilis*), and have strong curvature in the lateral areas.

Both individuals examined showed the unusual, probably secondary (see Discussion) bulb and tunnel system in the uppermost portion of the tegmentum as described for the one individual of *I. textilis*. Above the primary horizontal canals, there is an interconnecting web of tiny, criss-crossing tunnels. Periodically, the small tunnels connect to the large, bulb-shaped cavities (~70–80 μm in diameter at the origin, widening to ~80–120 μm in diameter). There are approximately 15 bulbs per mm^2.

On the ventral cast, the ventral jugal triangle and lines parallel to the slit rays contain broken off canals. These partial canals are flattened and some are angled upwards towards the dorsal valve surface. There are also a few small diameter canals present in some regions of the apical area. Some canals in the apical area pass into the ventral jugal triangle and slit ray regions.

DISCUSSION

The casts reveal a remarkable degree of variation in morphology of the aesthete canals between species, families, and suborders of the Polyplacophora. This method of epoxy casting provides a unique way to observe the full morphology of the aesthete canal system in chitons. Moreover, the results of a cladistic analysis using only aesthete canal characters suggest that the aesthete canal system provides a suite of characters
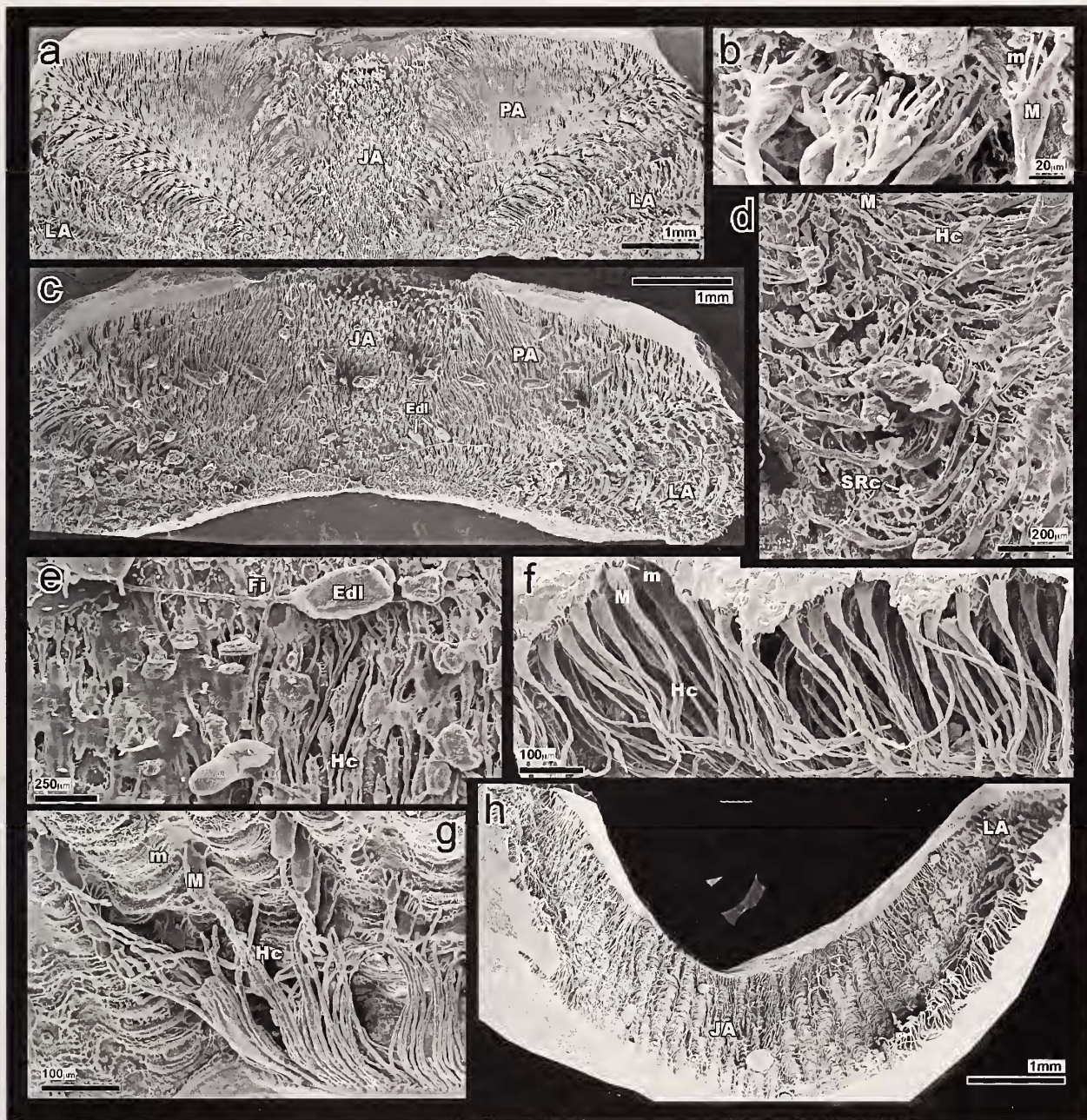
Figure 5. SEM images of casts of aesthete canal systems of *Ischnochiton textilis* (a, b), *Ischnochiton variegatus* (c–e), and *Lepidopleurus cajetanus* (f–h). All images are of dorsal casts. a. Overview of cast of *Ischnochiton textilis* (SBMNH 369435) showing some differentiation of pleural and lateral arcas. b. Close-up of megalaesthete chambers surrounded by micraesthetes in same specimen. c. Overview of cast of *Ischnochiton variegatus* (SBMNH 369437) showing some differentiation of pleural and lateral areas. d. View of lateral area showing slit ray canals in same specimen. e. Close-up view of pleural area in same specimen showing endolithic borings. A four-way endolith tunnel intersection can be seen. f. View of *Lepidopleurus cajetanus* (SBMNH 83154) showing megalaesthete chambers at posterior margin. g. Magnificd view of micraesthete canals and megalaesthete chambers in same specimen; note the single rows of megalaesthete chambers. h. Overview of cast of same specimen. Key: Edl = Endolith boring; Fi = four way intersection; all others same as in Figures 1 & 2.

useful in future taxonomic and phylogenetic analyses of chitons, confirming preliminary conclusions to this effect by Fernandez et al. (1999), which were also reported in Vendrasco et al. (2006).

The proportion of the entire aesthete canal system replicated in the casts appears to have varied slightly from species to species and to a lesser extent from individual to individual. In a few cases, large portions of canals in certain regions are missing. However, we examined at least two valves of at least two individuals of each species except for *Nuttallochiton*, and observed consistent canal infiltration patterns. Therefore, it seems most likely that the apparent incomplete cases of canal replication are due in part to an original low density or a natural closure of portions of the canal system during the life of the chiton. Also, some canal casts may have broken off during processing of the specimens (e.g., splitting dorsal from ventral cast). Regardless, the valve casts are complete enough to allow an accurate reconstruction of the entire aesthete canal system.

The casts reinforce many of the observations that Leloup made about the aesthete canals at the shell surface of numerous chiton species. For example, Leloup (1934) described that the aesthetes in *Lepidopleurus cajetanus* are clustered on the valve surface, with each megalaesthete associated with ~7–10 micraesthetes, and with the megalaesthete more centrally located in each cluster in the lateral areas compared to the central area. These results are consistent with what we found. In addition, for *Mopalia muscosa*, the flared-end, trumpet tube shape of the megalaesthetes, the size proportions of micraesthetes and megalaesthetes, and elongate nature of near-surface canals that the micraesthetes and megalaesthetes feed into, as revealed by these casts, are similar to what Leloup (1940) drew for this species. In *Mopalia acuta*, the relative sizes and shapes of the micraesthetes and megalaesthetes revealed by these casts are also similar to what Leloup (1942) illustrated for this species. In *Lepidochitona hartwegii*, the relative width of the megalaesthete chambers, the large number of micraesthetes per megalaesthete, and the lack of much separation between micraesthetes from adjacent chambers as revealed by these casts and valve surface observations are all similar to what Leloup (1940) illustrated for this species. In *Nuttallina californica*, the disparity in sizes between megalaesthetes and micraesthetes, the large number of micraesthetes, and the relative spacing between aesthetes, as revealed by the casts and surface observations, are similar to what Leloup (1940) described for this species, although in his drawings the micraesthetes are a bit less regularly spaced, and he drew bulbous megalaesthete chambers, not the unusual elongated near-surface canals that our casts reveal. In *Lepidozona mertensii*, the shape and orientation of the

megalaesthetes and associated micraesthetes as revealed by these casts are similar to what Leloup (1940) illustrated for this species. In addition, the huge megalaesthete chambers associated with granules in *L. mertensii* were also illustrated by Leloup (1940). However, in *Placiphorella velata*, the megalapores are only slightly larger than the micropores, as seen in an examination of the shell surface and in the dorsal casts of the canal system. This contrasts with the drawing of the pore system of *P. velata* in Leloup (1942), which shows distinctly larger megalapores.

Phylogenetic Utility of Aesthete Canal Characters

Figure 6 shows how the results of a cladistic analysis based only on aesthete characters matches a recent hypothesis of chiton phylogeny, with members of the same family (based on the taxonomy presented in Sirenko, 1993 and 1997) grouping together in the resultant cladogram. Sirenko's taxonomy was used because it is largely supported by phylogenetic analyses using morphology (Buckland-Nicks, 1995) and molecular data (Okusu et al., 2003). Figure 6 reveals that there are clear differences between the aesthete canal systems of chitons of the sub-order Chitonina and those of the Acanthochitonina. Moreover, members of each family share derived aesthete canal characters in common.

In addition to the results of this cladistic analysis, the canal system of *Lepidochitona cinerea*, as drawn by Knorre (1925), is similar in many respects to that of *Lepidochitona hartwegii* as revealed in this study. Both show the uniquely-shaped, elongate megalaesthete chambers that have numerous micraesthetes feeding into them along their length, and that feed directly, after a short length, into the main horizontal canals at regular intervals. Moreover, Knorre (1925) drew a small portion of the aesthete canal system in *Ischnochiton herdmani*, which Kaas & Van Belle (1994) later synonymized with *Stenoplax alata*, and the presence of megalaesthete bulbs evenly spaced that feed via long stalks into thin, widely-spaced horizontal canals is quite similar to that of the ischnochitonids in this study. In addition, the aesthete canal system revealed for *Chiton albolineatus* by Haas & Kriesten (1978) differs substantially from that of any of the taxa in this study, none of which is thought to be in the same family as *Chiton*.

There is a difference of opinion, however, on assignments to families between Sirenko (1997) and Kaas & Van Belle (1994), and those suggested from the results of (Okusu et al., 2003). In particular, the assignment of *Nuttallochiton* to the Mopaliidae is not supported by (Okusu et al., 2003) or Kaas & Van Belle (1994). Also, Thiele (1931) argued that *Nuttallochiton* belongs in the family Lepidochitonidae, but he also

Lepidopleurus cajetanus (Lepidopleurina: Lepidopleuridae)

62

Ischnochiton variegatus (Chitonina: Ischnochitonidae)

Ischnochiton textilis (Chitonina: Ischnochitonidae)

100

69

Lepidozona pectinulata (Chitonina: Ischnochitonidae)

100

Lepidozona mertensii (Chitonina: Ischnochitonidae)

Lepidozona cooperi (Chitonina: Ischnochitonidae)

71

Lepidochitona hartwegii (Acanthochitonina: Tonicellidae)

Nuttallina californica (Acanthochitonina: Tonicellidae)

93

Mopalia acuta (Acanthochitonina: Mopaliidae)

57

Nuttalochiton hyadesi (Acanthochitonina: Mopaliidae)

Mopalia muscosa (Acanthochitonina: Mopaliidae)

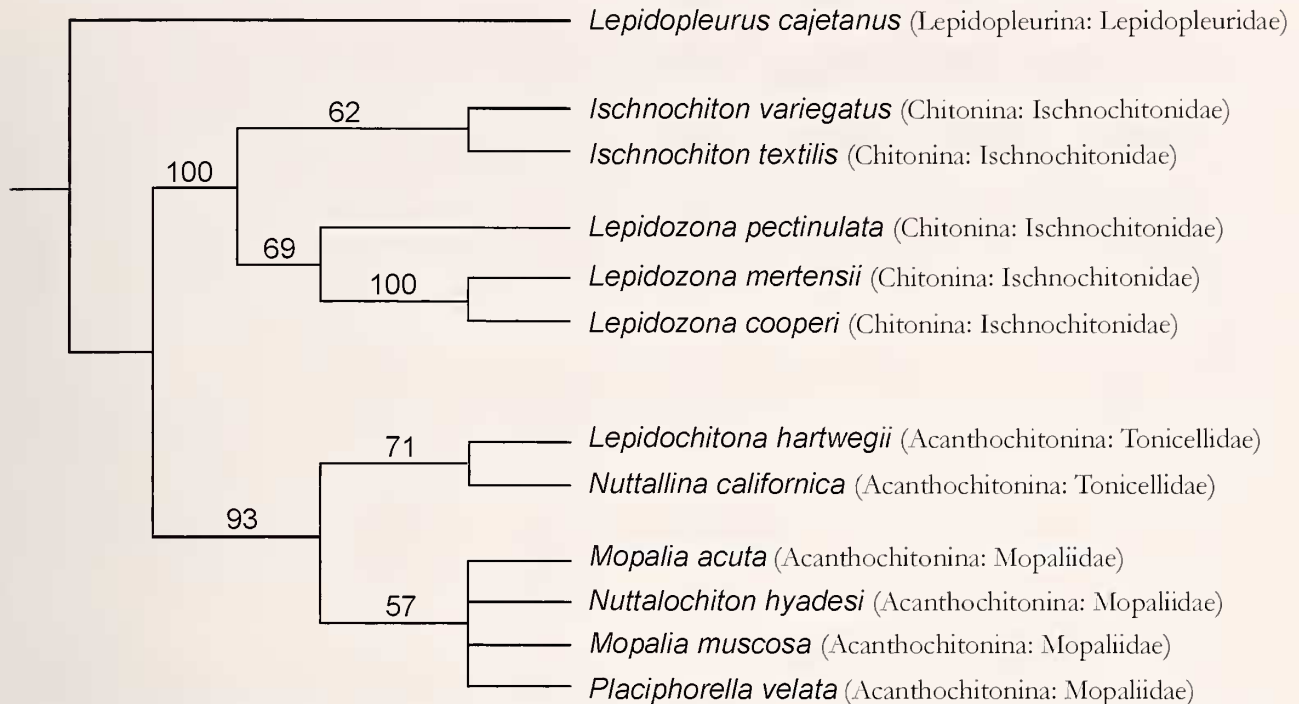Placiphorella velata (Acanthochitonina: Mopaliidae)

Figure 6. Majority rule consensus of the 42 most parsimonious trees. Numbers indicate the frequency of occurrence of the branch in the 42 trees. Assignments to chiton suborders and families (in parentheses) are based on Sirenko (1997). The data matrix for the analysis is shown in Table 1 and the characters and character states are listed in Table 2.

argued that *Lepidopleurus* belongs in this family so his concept of the Lepidochitonidae was quite broad. Our results are in line with Sirenko's (1997) hypothesis.

Nuttallina and *Lepidochitona* were placed in the family Tonicellidae by Sirenko (1997). Oldroyd (1927), Thiele (1931); E. P. Chace (1940), and Ferreira (1982) had previously placed both genera in the family Lepidochitonidae. However, the results of Okusu et al. (2003) suggest a close relationship between *Lepidochitona* with members of the Mopaliidae, although they did not include *Nuttallina* in their analysis. A. Myra Keen (1963) placed *Lepidochitona* in the Ischnochitonidae, united with *Lepidozona*, *Ischnochiton*, and others, and she placed *Nuttallina* in the separate Family Callistoplacidae. Kaas & Van Belle (1994) also placed *Nuttallina* and *Lepidochitona* in the Ischnochitonidae. The data from the aesthete canal system in this paper support the linkage of these two genera, and are consistent with a close relationship with the mopaliids, but do not support the inclusion of *Lepidochitona* and *Nuttallina* in the family Ischnochitonidae.

The aesthete canal system of *Lepidopleurus cajetanus* is unique in a few ways compared to the other chitons in this study: it has rows of megalaesthetes, one or two wide, in the central area, and each megalaesthete feeds directly into a thin, meandering canal that interweaves

with others on the way towards the anterior or lateral margin.

The ischnochitonids, even though found on separate continents and with different valve sculptures (*Ischnochiton textilis*, Africa; *Ischnochiton variegatus*, Australia; *Lepidozona* sp., North America), have similar canal systems that are largely different from those seen in the other species in this study. They each possess sub-conical megalaesthete chambers and a distinctly different aesthete canal form in the lateral area compared to central area. We interpret the huge bulbs distributed throughout the tegmentum of both individuals of *I. variegatus* and one individual of *I. textilis* are interpreted not as ocelli-bearing cavities, but as having been produced by an endolithic microorganism, because of their unusual shape, the lack of any connection to a primary horizontal canal, their connection to a web of tiny, intersecting horizontal tunnels that do not look like aesthete canals, and their absence in one individual of *I. textilis*.

The mopaliids, by contrast, all have large, closely spaced primary horizontal canals that exist within much of the valve interior as well as regular merging of subsidiary branches with the primary canals. Both tonicellids have a greater frequency of merging of subsidiary canals in the posterior portion of the valve

Table 1

Discrete aesthete characters used in the PAUP analysis. Descriptions of characters and character states provided in Table 2.

	1	2	3	4	5	6	7	8	9	10	11
Species	agc	are	blb	mcr	mhc	deh	hmc	hcc	apz	mgc	doc
Lepidopleurus cajetanus	1	0	1	0	0	0	0	0	1	2	0
Mopalia muscosa	0	0	0	1	1	2	0	0	0	0	2
Mopalia acuta	0	0	0	1	1	2	0	0	0	0	2
Placiphorella velata	0	0	0	1	1	1	0	0	0	0	2
Nuttalochiton hyadesi	0	0	0	1	1	2	0	0	0	0	1
Lepidochitona hartwegii	1	0	0	2	0	2	0	0	0	0	?
Nuttalina californica	1	0	0	2	0	2	0	0	0	0	2
Lepidozona mertensii	0	1	1	0	0	1	1	?	1	1	?
Lepidozona pectinulata	0	1	1	0	0	1	0	1	1	1	1
Lepidozona cooperi	0	1	1	0	0	1	1	1	1	1	1
Ischnochiton variegatus	0	1	1	0	0	1	0	1	0	1	1
Ischnochiton textilis	0	1	1	0	0	1	0	1	0	1	1

to form the primary horizontal canals, and a correlation between the megalapores and granules.

The results suggest that many aesthete canal characters may be useful for future phylogenetic studies. However, some other aesthete characters seem to be less phylogenetically significant. For example, Currie (1989) argued that average pore density on the upper valve surface, as well as the micropore/megalapore ratio, did not show tight correlation with phylogeny in the four Australian species that he examined. Future comparisons of aesthete canal character state distributions in a wider range of taxa will better determine the phylogenetic utility of such characters.

Environmental Influence on Aesthete Canal Patterns

Currie (1992) argued that the diversity of aesthete complexity suggests the capacity to perform a variety of functions. However, he found that the fine structural detail of different aesthete classes showed no significant differences between chiton taxa, leading him to argue that aesthete canal differentiation is of little importance in affording a range of functions.

Some of the aspects of the biology of the chiton species in this study are shown in Table 3. There seems to be little correlation between aspects of ecology and

Table 2

Aesthete characters and character states for the PAUP analysis.

#	Code	Description of character	0	1	2
1	agc	aesthete/granule correlation	none apparent	one megalapore per granule	
2	are	megalaesthete canal morphology/pattern differ by valve area	absent	present	
3	blb	megalaesthete bulbs in central area	indistinct	distinct	
4	mcr	micraesthetes feeding into subsidiary canals along their length	absent	present	only near "megalaesthete"
5	mhc	connection between surface and main horizontal canals	long canal	short canal	
6	deh	density of horizontal canals in middle to posterior	very low (much visible space between canals)	low (some visible space between canals)	high (little visible space)
7	hmc	huge aesthete chambers in large granules	absent	present	
8	hcc	degree of horizontal canal curvature towards diagonal line	none to low	medium	high
9	apz	canals differentiated into anterior-posterior columns	indistinct	distinct	
10	mgc	typical megalaesthete chamber shape	non-differentiated from canal	bulbous teardrop	bulbous sausage
11	doc	direction of convergence of horizontal canals in lateral area	no clear convergence	converge towards anterior	converge towards posterior

Table 3

Information on ecology for the twelve species used in this analysis.

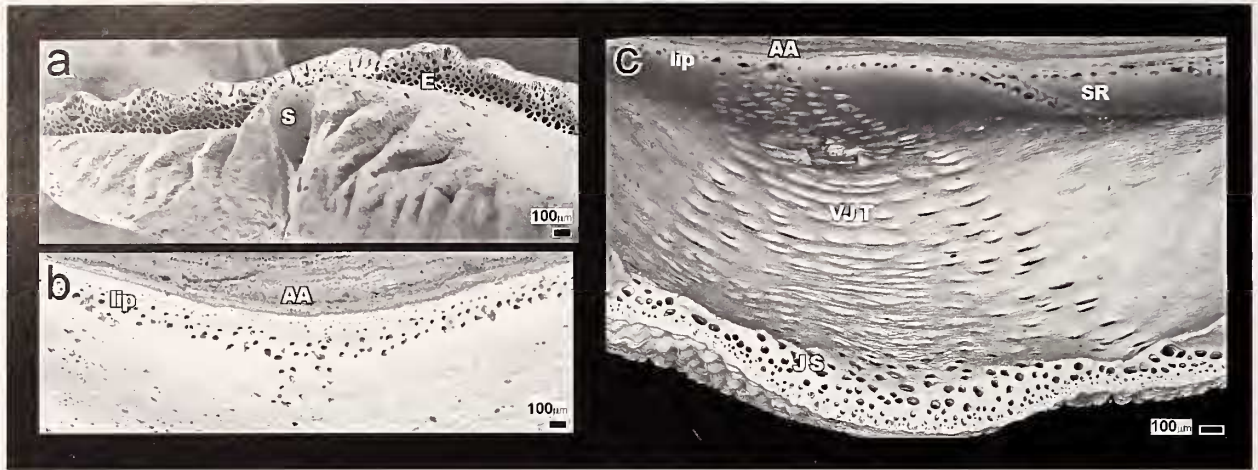| Species	Depth	Habitat	Other comments	References
Mopalia muscosa	Intertidal zone.	Wet, protected large crevices; high protected tidepools; usually in direct contact with moisture. Also exposed on surfaces of rocks in high energy environments.	Valves often covered; active at night; negatively phototactic when young.	Andrus & Legard, 1975; Kaas & Van Belle, 1994; Fitzgerald, Jr., 1975; Smith, 1975; Liff-Grieff, 2006; personal observation.
Mopalia acuta	Intertidal to 40 m.	Under rocks on cobble reef; in cracks and crevices protected from the sun.	—	Collection data, 1970; Kaas & Van Belle, 1994; Liff-Grieff, 2006;.
Placiphorella velata	Shallow subtidal to 20 m; occasionally intertidal.	Flat sides of movable rocks or upside down under boulders; in crevices or walls of deep tidepools; in sea urchin excavations in bedrock.	Carnivorous; avoids light; always in shade; shell plates often covered with organisms.	Andrus & Legard, 1975; Mclean, 1961; Clark, 1991, 1994; Kaas & Van Belle, 1994.
Nuttallochiton hyadesi	20 m +	In deep water (up to at least 400 m).	—	Kaas & Van Belle, 1994.
Lepidochitona hartwegii	Upper intertidal (usually) to 17 m.	Under *Silvetia compressa* fronds or less commonly under *Fuchus distichus*; also occur in small, wet crevices or depressions in the upper intertidal, or in tidepools in the upper intertidal.	Negatively phototactic; most active at night while dry or in swash. Valves often eroded.	Lyman, 1975; Andrus & Legard, 1975; Eernisse, 1984, 1986; Kaas & Van Belle, 1994; Liff-Grieff, 2006; personal observation.
Nuttallina californica	Upper and middle intertidal zone.	In exposed habitat on top of rocks—wedged in small crevices or grooves in high shallow tidepools; squeezed between bases of *Tetraclita squamosa reubescens*, *Pollicipes polymerus*, and *Mytilus californicus*.	At rest during low tide; grazing during high tide; lives in oval pits created by radular scraping. Valves often eroded.	Andrus & Legard, 1975; Piper, 1984; Kaas & Van Belle, 1994; Liff-Grieff, 2006; personal observation.
Lepidozona mertensii	Intertidal to 100 m.	Under rocks.	Negatively phototactic.	Ferreira, 1978; Ricketts et al., 1985; Kaas & Van Belle, 1994; personal observation.
Lepidozona cooperi	Intertidal to 20 m.	Under rocks.	Negatively phototactic.	Ferreira, 1978; personal observation.
Lepidozona pectinulata	Intertidal to 20 m.	Under rocks.	Negatively phototactic.	Ferreira, 1978; personal observation.
Ischnochiton variegatus	Intertidal to shallow subtidal zone.	Under small rocks with sand.	—	Collection data, 1972; Kaas & Van Belle, 1994.
Ischnochiton textilis	Intertidal to shallow subtidal zone.	Under rocks.	—	Kaas & Van Belle, 1994; Slieker, 2000.
Lepidopleurus cajetanus	Typically in intertidal zone; ranges to 40 m.	On stones, rocks, and old shells	Unslit insertion plates.	Smith, 1960; Kaas & Van Belle, 1994.

Figure 7.   SEM images of valves. a. Eaves of *Mopalia muscosa*. b. Ventral valve surface showing apical area of *Mopalia muscosa* showing pores at lip. c. Ventral valve surface of *Nuttallina californica* showing pores at jugal sinus, ventral jugal area, and lip of apical area. Key: E = eaves; all others same as in Figure 1.

those of the aesthete canal morphology revealed in this study. Many of the species in this study share similar habitats and depth preferences (e.g., many are intertidal or have a wide depth range) but have different aesthete canal patterns. Likewise, the mopaliids in this study occupy different environments (*Mopalia muscosa* and *Mopalia acuta* in the intertidal; *Placiphorella* in the shallow subtidal; and *Nuttallochiton* in deep water) and yet show striking similarities in aesthete canal morphology.

Other Influences on Aesthete Canal Patterns

Some aspects of the form of the aesthete canal system are likely to be influenced by the shell layers in which they occur. The slit ray and ventral jugal triangle canals have a flattened appearance in all casts where they were preserved. Perhaps this is due to the fact that these canals pass through the denser articulamentum. Interestingly, at the jugal sinus, pores with a circular cross-section can clearly be seen (Figure 7c); these connect to the flattened canals in the valve interior.

Also noticeable is a correlation between the thickness of the tegmentum and the number of levels of the canal system. This is evidenced by the multiple rows visible at valve eaves (Figure 7a), as well as the multiple levels seen most clearly along the anterior margin of the casts. For example, the *Ischnochiton* species in this study have a thin tegmentum and so have only two levels of horizontal canals while the *Nuttallina californica* and *Mopalia muscosa*, which both have a thicker tegmentum, have more than two layers of horizontal canals.

Currie (1992) argued that aesthete canal differentiation in the three Australian species he examined may represent inherent differences in shell growth associated with the formation of major valve sculptures. Similarly, *Lepidozona mertensii*, *Lepidozona cooperi*, and *Lepidopleurus cajetanus* show a strong connection between the aesthete canal system and valve sculpture (Figure 4a), with spaces in the canal system corresponding to ridges and granules on the dorsal valve surface. However, *Ischnochiton variegatus* has a similar valve shape and sculpture to that of *L. mertensii*, but lacks the corresponding spaces within its aesthete canal system. Along these same lines, there is a diversity of valve sculptures within the mopaliid and *Ischnochiton* species in this study, but the mopaliids each have a strongly similar aesthete canal morphology to each other, as do the *Ischnochiton* spp.

Connectivity of the Aesthete Canal System in the Apical Area

Many slit ray and ventral jugal triangle canals seem to connect to those in the apical area (seen in the casts of *Mopalia muscosa* and *Placiphorella velata*) and there is evidence in these regions for a direct connection between upper and lower valve surfaces (seen in the casts of *Mopalia muscosa* and *Nuttallina californica*), as postulated by Baxter & Jones (1981). Canals in the apical area seem to be more complicated than was previously appreciated. For example, canals seem to originate at the apical area itself rather than just from the dorsal valve surface above the apical area. Additionally, in some species, canals exit at the lip of the apical area, evidenced by the canals in the cast (seen in *Nuttallina californica* and *Mopalia muscosa*) and the presence of pores in this area of the valves (Figure 7b).

Conclusions

This method of embedding chiton valves has proven remarkably effective at revealing the three-dimensional morphology of the aesthete canal system. Inferences from these casts allow detailed comparisons between the twelve taxa in this study. The casts reveal that: (1) both large and small-scale canal morphologies are highly variable between the nine species examined; (2) the variation shows a much stronger correlation with phylogenetic relationships than with ecological factors; (3) there is a high degree of connectivity in the total aesthete canal system; and (4) canal morphology seems also to be strongly influenced by the shell layer and region in which the canals occur, confirming results by previous authors.

The results suggest that the aesthete canal system provides a suite of characters that are useful in phylogenetic and taxonomic studies of chitons at the species level (note in particular the differences between members of *Lepidozona*) and even more useful at higher taxonomic levels. Future embedding of valves of species within other chiton families will allow a better assessment of the extent of this utility throughout the Polyplacophora.

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