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Direct development of the brittle star *Amphiodia occidentalis* (Ophiuroidea, Amphiuridae) from the northeastern Pacific Ocean

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Abstract. The highly modified development of the brittle star *Amphiodia occidentalis* is described from post-fertilization to the juvenile stage. Fertilized eggs are negatively buoyant, ~190 μm in diameter, surrounded by a thick hyaline layer and a tough fertilization envelope. After gastrulation, embryos flatten into a bilaterally symmetrical disk with a U-shaped ridge surrounding an indented stomodeum on the oral surface. Internally, a ring of ~22 calcitic ossicles grows at the edge of the disk. Vestigial ophiopluteal structures such as a ciliated band, paired larval spicules, or larval arms are not expressed during development. Although the fertilization envelope disintegrates on day 3, developmental stages remain immotile for five more days until they move with podia. At hatching, five hydrocoelic lobes are evident on the left side of the post-gastrula, and these migrate clockwise around the stomodeum, establishing pentamerous radial symmetry. Central and radial plates originate on the right side and migrate to a dorsocentral location as pentamerous symmetry is established. Development of the juvenile oral skeletal frame follows closely that described by Hendler (1978) for *Amphioplus abditus* except that *A. occidentalis* did not form buccal scales. The juvenile mouth opened by day 12. Fifty-five days after fertilization, juveniles had not added their first arm segments, although the first lateral arm plates had appeared. Developmental stages identical to those described here have been found in plankton tows taken in Oregon usually after storms that bring high waves. The unusual development of this species probably occurs in both benthic and pelagic environments.

Additonal key words: lecithotrophy, skeleton, ontogeny, juvenile, echinoderm

There was a novel form called a mesogen
Dubbed by McEdward it wasn't a whim for him
Storms stir this star and up stages go again
There goes a larval mesogen, begin again …[1]

Development of marine invertebrates varies greatly within and across taxa (Thorson 1950; Strathmann 1985, 1993; Young et al. 2002). Comparison of ontogenies among related species is the primary way to interpret evolution of development at the organismal level. Sometimes larval or post-embryonic forms differ from known patterns so greatly that interpreting them in terms of comparative anatomy is a challenge. The present study describes the highly modified and unusual development of the brittle star *Amphiodia occidentalis* Lyman 1860.

Echinoderms are one of the best-studied groups in terms of their comparative development, which can be indirect via a feeding larva or abbreviated with partial to complete loss of the feeding larval form (Strathmann 1974; Hendler 1975; Emlet et al. 1987; McEdward & Miner 2001). Direct development is a category often used for developmental patterns that have lost virtually all features found in feeding larvae, and is here considered an extreme form of abbreviated larval development. After hatching from the fertilization envelope, planktonic stages swim and often feed with cilia (e.g., Strathmann 1971; Kelman & Emlet 1999); nonplanktonic development occurs

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This paper is dedicated to the memory of the "larval marvel" Larry McEdward, a dedicated scientist, advisor, and scholar.

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[1] Words by R. Emlet to the tune of the children's song "Michael Finnigan."

on the benthos in tough envelopes (e.g., Fell 1941; Hendler 1977; Byrne 1995) or more often under (or inside) the protection of a parent (e.g., Chia 1968; David & Mooi 1990; Hendler 1991; Byrne 1996). When fertilization occurs in the water column, embryos develop rapidly and hatch as functional swimming forms (Staver & Strathmann 2002). When development occurs on the benthos or internally, newly released stages are motile for the environment that they enter. The present study describes the development of an ophiuroid whose embryonic stages, initially encapsulated in tough envelopes, lack cilia for locomotion, and are found in the plankton.

The Ophiuroidea with ~2000 species is the largest among the five living classes of echinoderms. This taxon has species with planktotrophic larvae (called ophioplutei), pelagic-nonfeeding larvae (called vitellariae), brooded embryos, and benthic encapsulated development (Hendler 1975, 1991; McEdward & Miner 2001). McEdward & Miner (2001) mapped and analyzed developmental patterns of 67 species (~3.4% of the class) with the phylogenetic hypothesis of family relationships reported by Smith et al. (1995). Like others (e.g., Strathmann 1974, 1978; Emlet 1990; Wray 1995; McEdward & Janies 1997) they assumed a planktotrophic larval ancestor, and they estimated that nonfeeding developmental stages have evolved ≥ 10 times, benthic (brooded?) stages at least eight times, and direct development two times. As with the other three classes of echinoderms (asteroids, echinoids, and holothuroids) with feeding larval development, loss of planktotrophy has occurred repeatedly.

Amphiodia occidentalis occurs intertidally and subtidally in the northeastern Pacific Ocean from Kodiak, AK to San Diego, CA (Austin & Hadfield 1980). Adult specimens of *A. occidentalis* with disks ≤ 1.5 cm in diameter and arms >15 cm long live in consolidated sediments and extend their arm tips into the water column to suspension feed (R.B. Emlet, unpubl. data). I obtained fertilized eggs of *A. occidentalis* in the San Juan Islands, WA in June 1991, and followed development through metamorphosis. Subsequently and repeatedly, I found similar, encapsulated developmental stages in plankton tows taken in coastal waters of Coos Bay, OR. These observations suggest that development may be both pelagic and benthic over the ~8-d period from fertilization to onset of motility of juvenile stages. The novel pattern of development in *A. occidentalis* increases the minimum number of times that nonfeeding development has evolved within the Amphiuridae and is an additional case of direct development for ophiuroids.

Methods

Collection of developmental stages

Fertilized eggs were obtained twice from adult specimens of *Amphiodia occidentalis* collected at an intertidal site (~−0.3 to −0.5 m below chart datum) south of Buck Bay on Orcas Island, WA (48°36.16′N, 122°49.83′W). Adults were located under emergent, large cobbles and small boulders embedded in a beach made up of a sand–shell–mud matrix. The first collection yielding many viable embryos occurred on June 28, 1991 and a second collection occurred on April 27, 1998. For each of these collections and numerous others, adult brittle stars with well-developed gonads were put in buckets with seawater and taken by boat to the Friday Harbor Laboratories on San Juan Island, WA within 1.5 h of collection. On both occasions, upon return to the laboratory, eggs with fertilization envelopes were found in the buckets with adults. Eggs were subsequently sorted into fingerbowls containing seawater filtered through a mesh bag (~1 μm pore size) and maintained at ambient seawater temperatures of 12°–14°C for the duration of the study. The spawning in 1998 yielded few oocytes and only one fertilized embryo. This individual did not survive to the juvenile stage, but did provide opportunities to see stages not observed in the culture from 1991.

In the years that followed the initial embryonic culture, I collected gravid adults of *A. occidentalis* in the San Juan Islands, WA and at Cape Arago, OR numerous times and attempted to induce spawning. Methods such as temperature shocking the adults in dishes by repeated changes with cold fresh seawater, shining bright light on the individuals, and incubating females in dishes with a dilute suspension of sperm all failed to induce shedding of gametes. Furthermore, dissected ovaries and oocytes incubated in 1-methyl adenine failed to undergo germinal vesicle breakdown and oocytes failed to fertilize.

Planktonic developmental stages

In March 1994, B.A. Miller collected plankton samples off the coast of Sunset Bay, OR (43°20.1′N, 124°22.8′W), and in sorting through the fixed samples, we identified eight specimens of what appeared to be hatched developmental stages of *A. occidentalis*. On other occasions between 2000 and 2003 (see Table 1), plankton tows (135-μm-mesh net) were taken at the entrance to the Coos Bay, OR (43°21.2′N, 124°20′W). Upon return to the laboratory, the live plankton was examined. On several occasions the plankton tows contained eggs or

Table 1. Dates and locations of collections of developmental stages of *Amphiodia occidentalis*.

1991	June 28	Orcas Island, WA	Fertilized eggs in bucket
1998	April 27	Orcas Island, WA	One fertilized and six oocytes
1994	March 26	Plankton[a]	Eight individuals (hatched, ~8 d)
2000	December 15	Plankton[b]	4–16 cell stages
2002	December 16	Plankton[b]	Morulae to "3-day-olds"
2003	February 21	Plankton[b]	Fertilized eggs
2003	March 4	Plankton[b]	Multicellular to morulae

[a] Off Sunset Bay, near Cape Arago, OR.
[b] In entrance to Coos Bay, OR.

developmental stages that appeared to be the same as or very similar to those of *A. occidentalis*. The stages were sorted from the plankton samples and maintained in the culture dishes at 8°–10°C in the laboratory for observations on subsequent development. Adult specimens of *A. occidentalis* are common in several open coast intertidal locations near Coos Bay, OR (R.B. Emlet, unpubl. data).

Treatment of material for observation

Observations were made on living and fixed material. The diameters of live oocytes were measured to the nearest 4 μm using a compound microscope. Embryos were fixed with 2% formaldehyde in seawater buffered with sodium borate, dehydrated through an ethanol series, and stored in 70% ethanol buffered with sodium glycerophosphate (Turner 1976). Some of the fixed embryonic and juvenile stages were dehydrated through an ethanol series and cleared in a solution of benzyl alcohol and benzyl benzoate. The fertilization envelope was mechanically removed from these stages before clearing. When viewed with differential interference contrast (DIC) optics using Olympus BH-2 (Olympus America, Melville, NY) or Zeiss Universal (Carl Zeiss, Inc., Thornwood, NY) microscopes, these preparations revealed some of the internal organization of the embryos. When viewed with cross-polarized light, these preparations revealed calcite skeletal elements. Images were recorded with digital and film photomicrography and videomicrography. Hendler's (1978) description of the development of the juvenile skeleton of *Amphioplus abditus* was the source of nomenclature used for the juvenile skeletal elements described in the present study.

Several of the fixed stages were also prepared for laser scanning confocal microscopy (using a Zeiss LSM 310) by staining material with propidium iodide and then clearing as described above. Confocal images at ~2–3 μm intervals were converted to stacks and three-dimensional (3D) projections with ImageJ software (ver. 1.32j, National Institutes of Health, Bethesda, MD). Stereo-pairs were made by aligning two of the same projections side by side and offsetting their *y*-axis rotations by 10°. Several of the fixed stages were also prepared for scanning electron microscopy (SEM) by critical point drying and coating with gold palladium alloy before viewing on a JEOL JSM-35 scanning electron microscope (JEOL, Ltd., Tokyo, Japan).

Results

Observations on spawning

Fertilized eggs and embryos that were definitively from adults of *Amphiodia occidentalis* were obtained only twice in the San Juan Islands, WA in late June 1991 and late April 1998. After each of these collections of adult brittle stars, the boat trips back to the laboratory were notably rough and may have triggered spawning. On five occasions (March 1994, December 2000, December 2002, February 2003, and March 2003) embryos or hatched juveniles were collected from the plankton near Coos Bay, OR. These specimens matched the size, color, and morphology of the developmental stages of *A. occidentalis* reared in 1991 and 1998. The material collected in 1994 was from preserved plankton samples and contained the most advanced stages, consisting of hatched juveniles that had tips of arms extending just beyond the peripheral ossicles and adoral shield spines but no dental plates or teeth. These (fixed) individuals were similar to 8-d-old stages of *A. occidentalis* raised at 12°–14°C. The other planktonic collections contained fertilized eggs (February 21, 2003), early cleavage stages (December 15, 2000), solid multi-cellular morulae (March 4, 2003), and morulae to ~3d post-fertilization (December 16, 2002). Many of the plankton samples with developing stages similar to *A. occidentalis* were made following storms that caused high waves and turbulence that may have induced spawning or mixed developmental stages into the water column.

Early development

Oocytes and fertilized eggs were spherical, pink or rose colored, and negatively buoyant. Two females, collected with the animals that spawned in June 1991, had very large and well-developed gonads, and the largest oocytes removed from their ovaries had mean diameters of 183 μm (±6.7 SD, $n=15$) and 191 μm (±5.4 SD, $n=15$). Two additional females collected in May 1993 from the same locality had mean diameters for large oocytes of 236 μm (±11.4 SD, $n=15$) and 249 μm (±11.2 SD, $n=9$). For comparison, the diameters of two fertilized eggs, collected from the plankton in Oregon, were 182 and 186 μm. Fertilized eggs were surrounded by a thick hyaline layer (>10 μm) and by an expanded, translucent, tough, and sticky envelope ~300–350 μm in diameter (Fig. 1A).

For collections in Washington that yielded embryos, the exact time of fertilization was unknown, because there was a 3-h period between the time of collection of adults and first observations in the laboratory. The time of spawning and fertilization was assumed to be the time at return to the laboratory, and thus may be in error by 1–2 h of the actual time.

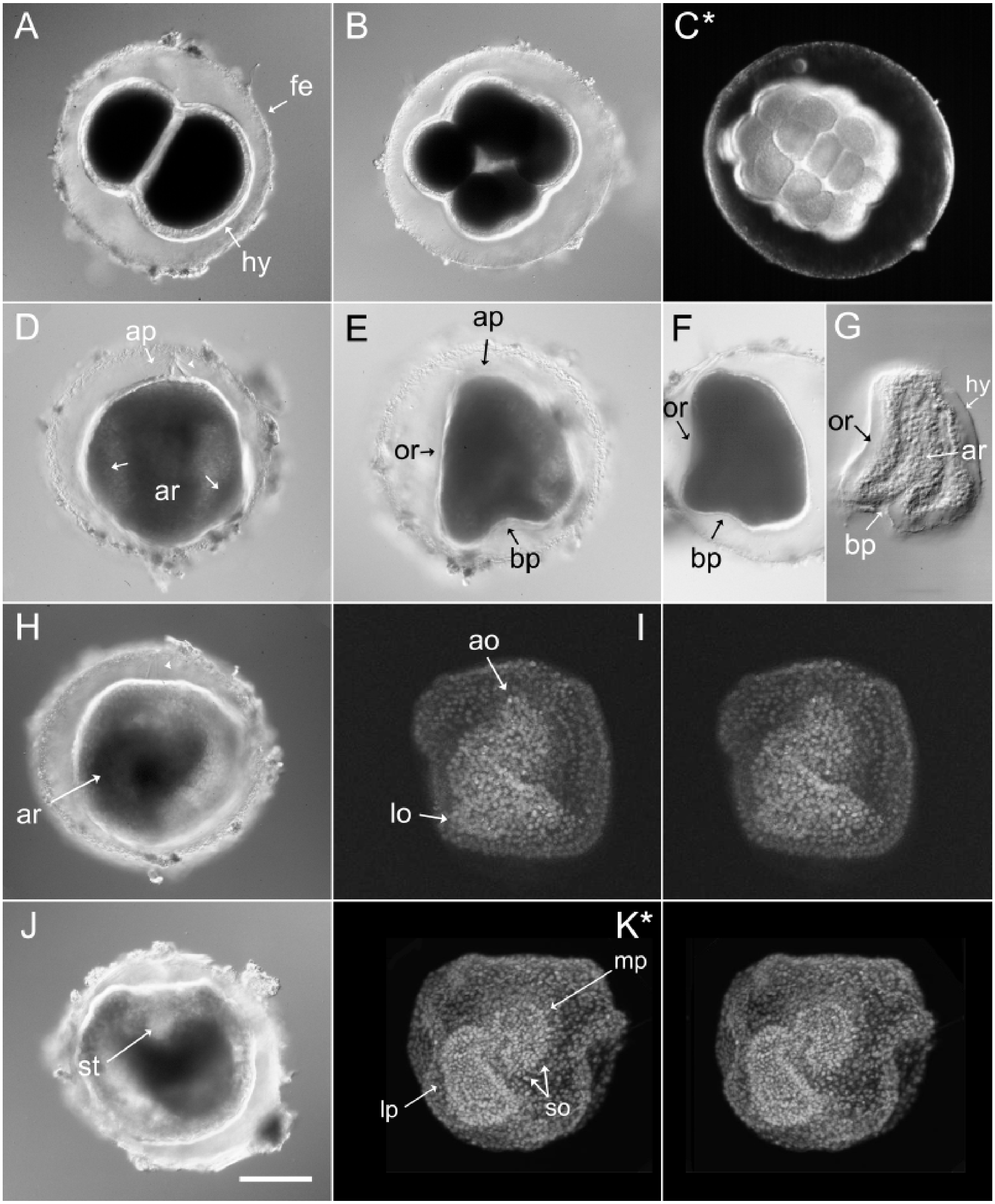
Cleavage began within 3 h of return to the laboratory. Initial cleavages were complete and approximately equal. Time of fertilization or developmental rates apparently varied in the June 1991 culture, because 7.5 h after returning to the laboratory most embryos were at the four- to eight-cell stage (Fig. 1B) while a few were still at the two-cell stage (e.g., Fig. 1A). An hour later most embryos were eight-cell stages with approximately equal-sized blastomeres, and a few embryos were undergoing their fourth cleavage. Arrangements of the eight blastomeres were highly variable, with only a few showing radial cleavage (two tiers of four cells). Several embryos collected from the plankton in December 2000 were at the 32-cell stage and had an obvious radial cleavage pattern (Fig. 1C).

At 21 h after fertilization, blastulae were spherical and partially hollow as judged by their translucent centers. Blastulae were not obviously ciliated and did not rotate within the fertilization envelope. In both the 1991 and 1998 cultures, embryos began gastrulation ~29 h after fertilization. At this time they were radially symmetrical along the animal–vegetal (A–V) axis, and the flattened vegetal plate was concave in its central region. At 45 h, late gastrulae were approximately bilaterally symmetrical about a plane passing through the A–V axis. The landmarks of symmetry included the lumen of the archenteron, along the A–V axis, and a pair of angular and opaque regions at the lateral edges of each individual, in a plane orthogonal to that of bilateral symmetry (see Fig. 1D). Cleared specimens contained groups of mesenchyme cells at these lateral locations. At the animal pole or apical end of the gastrula, several translucent radial projections were visible in the gap between the gastrula and the fertilization envelope (Fig. 1D). These structures were sometimes present in later stages and were useful for orienting the unhatched, developing individuals (e.g., Fig. 1H). In lateral view the late gastrula was roughly triangular in outline with an indentation at the site of the blastopore. Internally the archenteron was noticeably closer to one surface that eventually became the oral surface of the juvenile (Fig. 1E).

In slightly older individuals (46.5 h), hereafter called post-gastrulae, the oral surface became concave marking the location where the stomodeum will form, and the aboral surface (opposite the oral surface) appeared to collapse onto the oral surface so that the blastopore was shifted to the oral side of the flattening gastrula opposite the animal pole (Fig. 1F,G). At 49.5 h, the archenteron was transforming into one or more coelomic precursors (Fig. 1H). From an aboral view, the archenteron arced to the left from the site of the blastopore (now apparently closed) and formed one outpocketing at a left posterior location and another at the anterior end of the curved archenteron in an oral, medial location (Fig. 1I). By 53 h, the post-gastrula had a flat anterior end compared with the otherwise circular outline of the now disk-shaped embryo (Fig. 1J,K). A well-defined stomodeal invagination was present on the oral surface, located along the midline and offset toward the anterior end of the embryo (Fig. 1J). This stage's interior was opaque on its left side and central region (when viewed from an aboral perspective) because of the development of two distinct pouches—one on the left and one medial—produced by constriction of the archenteron (Fig. 1K). The medial pouch partially overlapped the stomodeal cavity and had two small outpocketings on its posterior side (Fig. 1K). The lower right side of the disk contained only blastocoel and was notably translucent. At no time were cilia or movements that might have been caused by ciliary activity observed in the encapsulated gastrulae or post-gastrulae.

Early skeleton formation

Numerous calcite crystals began to form in the blastocoel along the periphery of the flattening post-gastrula by 46.5 h after fertilization (Fig. 2A). The ossicles grew into short rods, arranged in a sinuous circle (Fig. 2A–C). The number of rodlike elements was usually 22 (range 19–24) among the individuals observed. At no time was a bilateral pair of spicules that could be interpreted as a reduced larval skeleton observed in association with or separate from this ring of rods. At 53 h, ossicles at the anterior end were arranged in an arc convex toward the aboral surface (see Fig. 3B for arc shape) and appeared as a straight line when viewed aborally (Fig. 2C). The three or



four ossicles on each side of this arc were also forming arcs convex toward the oral surface, and these arcs appeared to be straight lines when viewed from the aboral or oral sides (Fig. 2C). The remaining ossicles formed a semicircle including the lateral and posterior edges of the post-gastrula (Fig. 2C). By 57 h, ossicle arcs became more obvious. When the embryo was oriented with the anterior end toward 12 o'clock, there were four aboral arcs at locations corresponding to 12, 3, 6, and 9 o'clock and four oral arcs corresponding to 1:30; 4:30, 7:30, and 10:30 on a clock face. The sinuous circle of ossicles was positioned in the plane of bilateral symmetry so that oral arcs occurred in the upper left and right regions and lower left and right regions of the embryo (Fig. 2D). By 75 h, the peripheral rods had elongated and contacted adjacent rods, and each rod began to grow radial spurs at its middle and ends (Fig. 2E, inset).

Additional sites of calcification were first noticed by 75 h (Fig. 2E). At 91 h, the calcification sites on the right side corresponded with what would become central and radial plates of the dorsal surface (Fig. 2F). These ossicles were distributed at varying depths inside the developing juvenile, as they were not all in the same focal plane. Spread across the post-gastrula from left to right and posterior to the stomodeum, there were also ossicles that would become associated with rays (Fig. 2F). The radial spurs of the peripheral rods continued to elongate to become almost as long as the rods themselves.

Intermediate development

The post-gastrular stage continued with further demarcation of the anterior end (57 h) and with complete separation of the left and medial internal pouches (Fig. 3A). The leftmost pouch, presumably the left coelom, developed undulations that would become hydrocoelic lobes. An extension off the lower right side of the left coelom was also present. The medial pouch separated into at least the two pouches at the left and posterior sides of the stomodeal invagination (Fig. 3A). From the eight specimens at this stage that were examined with the confocal microscope, I could not tell if a third pouch located aboral but separate from the stomodeum also remained.

Externally a U-shaped ridge formed on the oral surface around the stomodeum (Fig. 3C). This raised region bordered the vegetal and lateral sides of the concave oral surface and merged with the disk-shaped region at the anterior end, in part because of the aboral arc that was present in this region of the disk (Fig. 3B,C). The U-shaped ridge was made of tightly packed cells and was three or four cells wide. At 67 h in uncleared specimens, large numbers of cells were distributed internally throughout the left side of the organism, fewer cells were on the right side, but no obvious coelomic cavities were visible (Fig. 3D). Individuals from the 1991 culture began hatching out of their fertilization envelopes by 70 h and $<5\%$ remained unhatched by 76 h. Hatching involved disintegration of the fertilization envelope. Hatchlings retained their thick hyaline layer, showed no evidence of ciliary movement, and were nonmotile. The one developing individual from the 1998 culture never hatched, but the embryos collected in the plankton off Oregon hatched at approximately the same time and at the same stage as those of the 1991 culture.

By 75 h, six cell-lined cavities were present on the left, posterior, and right posterior sides of the

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Fig. 1. Photomicrographs of cleavage to the post-gastrula stages of *Amphiodia occidentalis*. **A.** Two-celled embryo with a thick hyaline layer (hy) and a fertilization envelope (fe) with attached debris. **B.** Four-celled stage in the process of cleaving to eight cells. **C.** Embryo at the 32-cell stage collected from the plankton in December 2000. D–K are all oriented with the apical end toward the top of the plate. **D.** A 45-h gastrula viewed from the aboral side. The apical end (ap) of the embryo has distinctive translucent projections (arrowhead) and the archenteron (ar) can be seen along the central axis. Arrows indicate lateral regions with slight angularity and associated blastocoelic regions that are opaque. **E.** A 45-h gastrula in lateral view with oral side (or) and blastopore (bp) labeled. **F.** A 46.5-h post-gastrula in lateral view. **G.** A cleared, 47-h post-gastrula in lateral view showing concave oral side (or), archenteron (ar), and blastopore (bp). The hyaline layer (hy) is swollen on this specimen because of the effects of the clearing solution. **H.** Aboral view of a 49.5-h post-gastrula with a curved archenteron (ar) that gives rise to coelomic pouches. Arrowhead indicates radial projections at the apical end. **I.** Stereopair, confocal projection of a post-gastrula, viewed aborally. The archenteron originates in the lower, vegetal region, arcs left, gives rise to a left posterior outpocketing (lo), and then turns right terminating as an anterior outpocketing (ao) over the region where the stomodeum will form. **J.** Oral view of a 53-h post-gastrula with a flattened apical end, a stomodeum (st), and further development of internal pouches. **K.** Stereopair, confocal projection of a stage similar to J, viewed aborally, with the archentron giving rise to a left pouch (lp) and a medial pouch (mp) over the stomodeum. The medial pouch has two small outpocketings (so) along its posterior side. A–K are the same magnification. Scale bar = 100 µm. Photos marked with * are of cultured specimens collected as embryonic stages in the plankton off Coos Bay, OR.

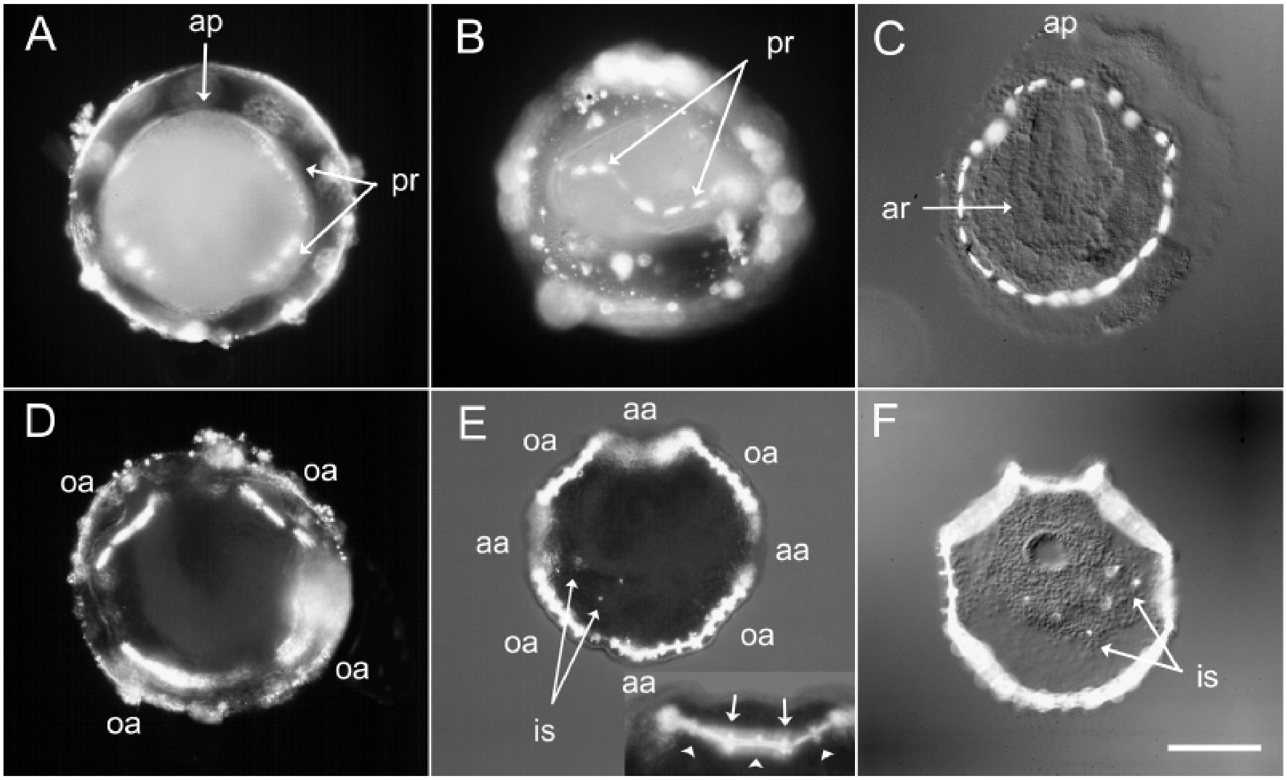


Fig. 2. Photomicrographs using cross-polarized light of early skeletal development of *Amphiodia occidentalis*. All images except B are oriented with the apical end (ap) toward the top of the page. **A.** Aboral view of a 46.5-h post-gastrula shows calcite crystals of the peripheral rods (pr). **B.** Side view of a 53-h individual showing the sinuous arrangement of the ring of peripheral rods (pr). The out-of-focus, increased birefringence is debris adhering to the fertilization envelope. **C.** A 53-h post-gastrula with 23 short calcite rods around its periphery and the archenteron (ar) displaced to the left side of the plane of bilateral symmetry. **D.** Oral view of a 57-h post-gastrula; the sinuous ring has four regions (oa) arcing toward the oral side of the post-gastrula at 1:30; 4:30, 7:30, and 10:30. The peripheral rods of the intervening regions are not birefringent because their *c*-axes are aligned with the polarizing filters. **E.** Oral view of a 75-h post-gastrula with oral arcs (oa) and aboral arcs (aa) of peripheral rods marked. The inset is a magnified part of the apical region of this individual showing three peripheral rods with radial projections beginning at the middles (arrowheads) and ends of rods (arrows). Internal skeletal elements (is) have also begun to form. **F.** Aboral view of a 91-h post-gastrula. Internal calcite crystals (is) become part of the adult skeleton. A–F are the same magnification. Scale bar = 100 µm.

stomodeum (Fig. 3E–G). By following the apparent fates of these cavities, I have assigned names. Because of a lack of specimens fixed at intervening stages at ~57–75 h, the origin of some of these cavities remains uncertain. On the left side, the largest cavity was the hydrocoel recognized by its five slender lobes (Fig. 3E–H). A cavity present at the posterior end, flattened in the aboral–oral direction, represented the (left) somatocoel (Fig. 3E–G). The hydrocoel and left somatocoel arose from the anterior and posterior regions of the left coelom, respectively.

Three cavities arranged in a row from left to right could be seen posterior to the stomodeum (Fig. 3E). The leftmost one of these will develop a tubular extension (hydroporic canal or stone canal) down to the hydrocoel, and was here identified as the left axocoel. It was apparently derived from the medial pouch that first formed over the stomodeum. The middle of these three cavities, apparently also derived from the medial pouch, will form the anterior region of the gut (Fig. 3E,F). The rightmost cavity represented all or part of the right coelom, and may also have come from the medial pouch, but this is uncertain (Fig. 3E,F). One final cavity located in the right posterior region was the posterior gut, because it fused with the anterior gut cavity at a later time (Fig. 3E,F). This posterior gut cavity may be the extension from the left coelom identified in Fig. 3A.

By 80 h, the hydrocoel began its rotation around the stomodeum as signified by the anterior extension of the first hydrocoelic lobe relative to the stomodeum (Fig. 3H). At 91 h, the hydrocoel formed a C-shape around the stomodeum, with the first hydrocoelic lobe located at 1 o'clock and the fifth or most posterior hydrocoelic lobe in its original position at ∼7–8 o'clock (Fig. 3I,J). Confocal images of this stage showed that the left somatocoel was elongated perhaps by movement of the hydrocoel, but the other cavities remained approximately in their earlier locations (e.g., Fig. 3J). The stone canal was visible as a circular tube extending aborally and located between the fourth and fifth hydrocoelic lobes (Fig. 3J). Also at this time the anterior gut cavity was fusing with the stomodeum (Fig. 3J), and in some individuals the anterior and posterior gut cavities were closely appressed (not pictured).

Through a clockwise rotation (when viewed from the aboral surface), the hydrocoel completely encircled the stomodeum by 98 h (Fig. 4A). At this time the stone canal was located at 11 o'clock, having migrated anteriorly from 8 o'clock (Fig. 4B). Because it was still directed aborally and perpendicular to the hydrocoelic ring, it apparently moved with the rotation of the hydrocoel. On the basis of its original position between the fourth and fifth hydrocoelic lobes, the anterior region of the hydrocoel underwent extensive rotation such that the first lobe was now located at 7 o'clock relative to the plane of bilateral symmetry. The fifth lobe moved to a position at 9–10 o'clock. The gut, now an elongated sac, ran from the stomodeal cavity to the right side of the post-gastrula ending blindly at ∼2–3 o'clock, indicating it rotated in a direction opposite that of the hydrocoel (Fig. 4B, inset). Because of the flattened nature of the specimens at this stage, it was not possible to follow the changes in the left somatocoel or right coelom.

During the movement of the hydrocoel, the raised U-shaped oral surface became circular, and the once concave oral surface filled in around the stomodeum becoming level with the raised border. The stomodeum also migrated from an anteromedial position in the post-gastrula to a central position in the juvenile (Figs. 3H–J,4A). After 98 h, the locations of the ambulacra were fixed with respect to the plane of bilateral symmetry. There was an anterior ambulacrum and a pair of ambulacra in the upper left and right sides and a pair in the lower left and right sides of the anterior–posterior embryonic axis (Fig. 4A,B). The hydrocoelic lobes, now radial canals, were each differentiating pairs of podial buds laterally (Fig. 4B).

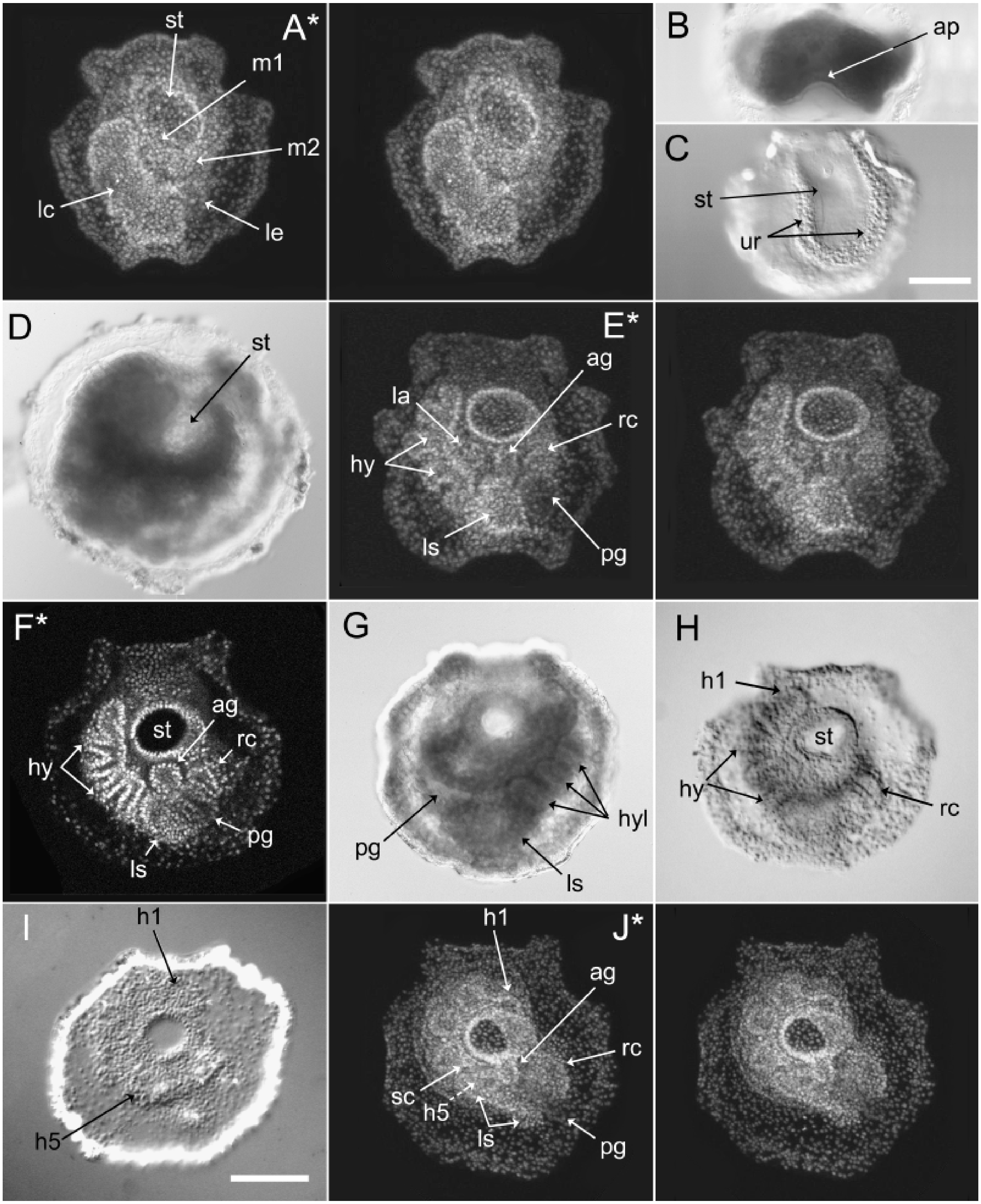
As development of the juvenile skeleton continued, by 98 h the radial and central plates were clearly evident in a single focal plane on the right dorsal surface (Fig. 4C, see also 4B). Two or three triradiate ossicles were associated with each of five hydrocoelic lobes of the radial canals (Fig. 4C). When two ossicles were present, they were located lateral to the hydocoelic lobe and were the primordia of the adoral shields (AS). When a third ossicle was present, it was located over a hydrocoelic lobe and was the primordium of the terminal arm plate (TP). By 104 h, the radial and central plates had migrated to the center of the juvenile aboral surface, and pentamerous radial symmetry of the ambulacral plates was clearly evident (Fig. 4E). Also by 104 h, another pair of ossicles formed proximally in each ambulacrum, these were primordia of the proximal oral plates (POP). After the radial and central plates on the aboral side moved to their central location and began to grow and branch (Fig. 4F), their birefringence in cross-polarized light was greatly reduced when viewed from the aboral side. This observation indicated that each of these calcite plates had a *c*-axis that was close to perpendicular with the plate and with the plane of the aboral surface.

Development of the juvenile form

By 104 h, the five, radially symmetric ambulacral regions were each comprised of three distal lobes (Fig. 4D). The central lobe was the bud of the terminal unpaired podium and the two outer lobes were the buds of the initial pair of podia. Most individuals also showed a second pair of lobes proximal to the first three lobes in some ambulacra (Fig. 4D). At this stage, the peripheral oral ectoderm formed a distinct ring around the ambulacra. By 116 h, the ambulacral system increased in diameter relative to the whole juvenile (compare Fig. 4D,E with G,H). The AS ossicles had elongated, and the five ossicles associated with each ambulacrum formed a pentagon with the terminal plate ossicle at the distal end of the ambulacrum (Fig. 4H). The peripheral oral ectoderm appeared to ingress centrally at interambulacral locations and to surround the distal pair of podial buds in each ray (see Fig. 4G).

At 116 h, the second, more proximal pair of podial buds was evident in each ambulacrum and these buds were smaller in diameter than the more distal pair (Fig. 4G). The proximal pair projected medially, and by 143 h was covered by oral ectoderm (Fig. 4J). These podia developed into buccal podia and were not seen in whole-mounted specimens until 12 d after fertilization when one or two podia could be seen between the jaws of some specimens. Their presence as paired buccal podia was confirmed in decalcified specimens viewed with cross-polarized light that revealed the birefringent muscles of the podial walls.

The next plates to develop were present by 130 h and were paired ossicles that formed between the POP in each ray (see Fig. 4K). These new ossicles developed into the distal oral plates (DOP). They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formed and grew at a more aboral position within each ray than did the POP. These DOP eventually joined with adjacent POP to comprise the primary jaw plates. By 139 h, a single plate, identified as interadial-1, was noted in the interambulacrum that was to the immediate left of the anteriormost ambulacrum when viewed from the aboral side (Fig. 5A). This plate maintained its position and grew in size. By 27 d, similar plates had formed in the two adjacent interambulacra. By 42 d, interradial-1 was present in all five interambulacra.

As the juveniles developed, interambulacral regions became more evident. For example, by 143 h, the region between rays was more pronounced than at 116 h (Fig. 4G,J). The anteriormost arm tip extended beyond the aboral disk beginning around 5.5 d and all arms extended beyond the aboral disk after 7 d. When viewed from the oral side, the tips of the anteriormost arm and the one to its immediate left grew out on the oral side of the peripheral ring of ossicles (Fig. 5B). The other three arm tips all grew out on the aboral side of the peripheral rods. Also by day 7 the mouth had changed from circular to star shaped in cross section, with the rays of the star aligned with the ambulacra (Fig. 5B). The POP and AS were elongated and had numerous outgrowths perpendicular to their long axes (Fig. 5C).

The first movement of podia and jaws was noted in videotapes made on day 7 (164 and 176 h). Initially the podial buds in each ambulacrum moved very slightly relative to one another. Later, twitching of the distal oral plates was observed from the aboral surface, and sometimes the radial plates moved relative to one another. On day 8 (196 h), the podia were highly motile on individuals that were placed on their aboral surface. Slight lateral movement was also discernable in juveniles resting on their oral surfaces.

By 195 h, the adoral shield spines were present on some of the rays. By 196 h (8 d, 4 h), dental plates had begun forming proximally over the ipsilateral POP of adjacent rays (see, e.g., Fig. 5D). By 244 h (10 d, 4 h), many individuals showed gaps in their rings of peripheral rods, indicating that resorption of these rods had begun. By 12.3 d, only short regions of peripheral rods was seen in some individuals, and on day 15 the last remnant of a portion of the peripheral rods was seen in a single individual (Fig. 5F). By 12.3 d, tooth plates were noted in the mouth and adjacent to the dental plates (Fig. 5E). Initially some of the tooth plates, when viewed with cross-polarized light, were birefringent while adjacent dental plates were not. As tooth plates developed, they had the same *c*-axis orientation as the dental plates, because both types of ossicles showed similar patterns of birefringence and extinction when rotated on a microscope stage with cross-polarized light. By 12.3 d a new single ossicle began to appear in the distal oral region between the podia in each arm ray, and by 15 d the ventral arm plate-1 was present in each arm (Fig. 5F,G).

SEMs of juveniles showed the mouth was open by 12.3 d (Fig. 5H). By 19 d, juveniles were feeding. Their stomachs were tinted brown, and several preserved specimens of this age had pennate diatoms inside their stomachs. Juveniles walked with the single (distal) pairs of podia on each ray (Fig. 5I). The oldest stages observed were 55 d post-fertilization (Fig. 5J), and several of the individuals examined showed initiation development of the first lateral arm plates.

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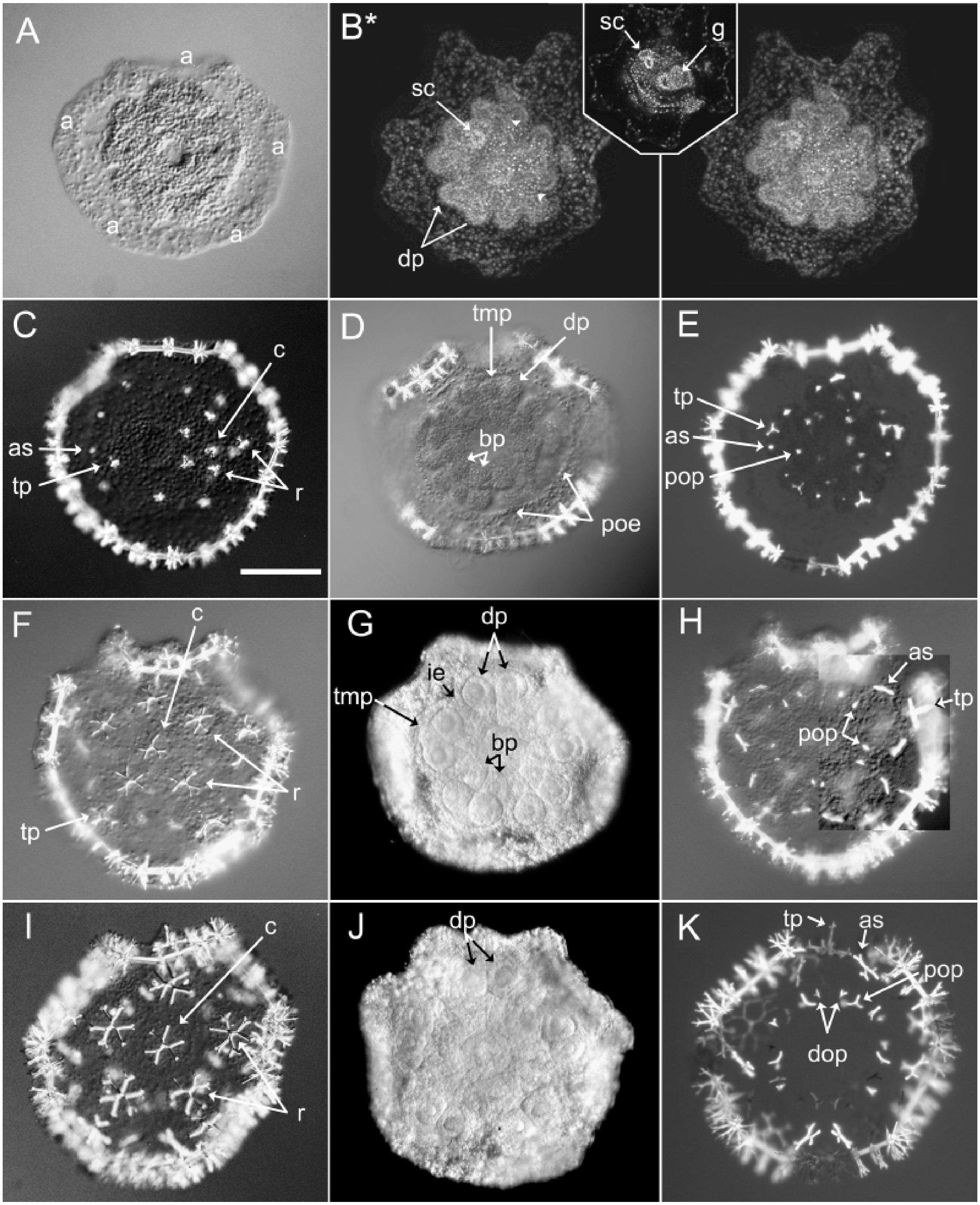
Fig. 3. Photomicrographs of intermediate developmental stages and coelom formation of *Amphiodia occidentalis*. All images except B are oriented with the apical end toward the top of the page. **A.** Stereopair, confocal projection, of stage with further separation of the coelomic pouches. The undulations on the outer periphery of the left coelom (lc) will become hydrocoelic lobes. An extension (le) off the lower right side of the left coelom is present. The medial pouch has separated into two sacs on the left (m1) and posterior side (m2) of the stomodeal invagination (st). **B.** Lateral view of a 66-h juvenile oriented with the embryonic apical end (ap) in the center of the image. Note the sinuous edge of the flattened dorsal region. **C.** Oral view of a 67-h juvenile removed from its fertilization envelope. The U-shaped ridge (ur) passes laterally and posterior to the indented stomodeum (st) covered by a hyaline layer. **D.** Aboral view of a 67-h juvenile with further development of internal tissues on the left side. **E.** Stereopair, confocal projection of a stage with six cavities defined: left hydrocoel (hy), left somatocoel (ls), left axocoel (la), anterior gut (ag), right coelom (rc), and posterior gut (pg). **F.** A confocal section from a stage similar to E, with cavities labeled as above. **G.** Oral view of a 75-h juvenile with hydrocoelic lobes (hyl), left somatocoel (ls), and posterior gut partially visible. **H.** Aboral view of an 80-h juvenile shows the hydrocoel as its migration around the stomodeum begins. The anteriormost hydrocoelic lobe (h1) is at 11o'clock. **I.** Aboral view of a 91-h juvenile with hydrocoel partway around the stomodeum. The anterior lobe (h1) has moved clockwise to a position of 1 o'clock, while the fifth lobe (h5) remains. **J.** Stereopair, confocal projection of a stage similar to I. The left somatocoel (ls) has elongated and covers the fifth hydrocoelic lobe (h5). The stone canal (sc) is present and the anterior gut cavity (ag) is fusing with the stomodeum. A and D–J are the same scale; B–C are a 0.8 scale of other images. Scale bars = 100 μm. Photos marked with * are of cultured specimens collected as embryonic stages in the plankton off Coos Bay, OR.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Discussion

The development of *Amphiodia occidentalis* is unlike that previously described for any brittle star. Developing embryos and juveniles lack characteristic structures found in ophiopluteus larvae, including larval arms, paired larval spicules, and ciliated bands. They also lack ciliated bands found in vitellariae. In fact, developmental stages lack cilia altogether. Hatchlings emerge immotile from their fertilization envelopes after 3 d (at 12°C), and remain thus for another 5 d before they can use podia for movement. The post-gastrula forms a unique peripheral ring of >20 calcareous ossicles that are resorbed after the juvenile becomes motile. Despite the loss of larval structures and the occurrence of novel ones, there are still a number of traits shared with some or most other developing ophiuroids, including several traits that suggest a feeding larval ancestor.

An MS thesis by Rumrill (1982; see also Strathmann & Rumrill 1987) reported that eggs of *A. occidentalis* in Monterey Bay were yellow green, 90–106 μm in diameter, and that development was pelagic. I was able to obtain fertilized eggs of *A. occidentalis* in the San Juan Islands, WA in June 1991, and follow development through metamorphosis. Eggs were pink, around 190 μm in diameter, and development was essentially direct. The species I collected was confirmed to be *A. occidentalis* by G. Hendler (LA County Museum of Natural History).

The fertilized eggs and embryos of *A. occidentalis* are negatively buoyant and encased individually in large, tough, sticky fertilization envelopes. Hendler (1977) reported that *Amphioplus abditus* spawns large yolky eggs that become surrounded by tough, expanded, and sticky fertilization envelopes that adhere to the substrate and to a culture dish. The fertilization envelope of *A. occidentalis* was also sticky as debris readily adhered to it; however, it did not stick to culture dishes. Another brittle star known as Kirk's ophiuroid deposits clusters of eggs individually encased in transparent, tough envelopes that Fell (1941:392) described as "chitinous." In all of these cases, the envelopes presumably act to protect the early stages, and for at least two species to attach them to the substratum. Eggs and embryos of *A. occidentalis* may also be demersal, but they can be suspended in the water column as documented by collections off Oregon of eggs and developmental stages believed to be *A. occidentalis*.

While inside the fertilization envelope, the egg, cleavage stages, and even post-gastrulae of *A. occidentalis* are covered in a thick hyaline layer. The hyaline layer is also notably well developed in fertilized eggs and embryos of several ophiuroids, including *Amphipholis kochii* (Yamashita 1984) and *Ophiura albida* (Olsen 1942). In *A. occidentalis* this layer persisted after juveniles hatched and may serve to protect the noncilated, nonmotile stages from physical disruption and pathogens. The juveniles

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Fig. 4. Photomicrographs show the formation of the pentaradial juvenile of *Amphiodia occidentalis*. All images are oriented with the embryonic apical end toward the top of the page. **A.** Aboral view of a cleared 98-h juvenile shows the central stomodeum and the surrounding pentaradial hydrocoel. The locations of future ambulacra are noted (a). **B.** Stereopair, confocal projection of a stage similar to **A**, in aboral view, shows that the stone canal (sc) has migrated to 11 o'clock and the hydrocoelic lobes are forming paired podial buds (dp). The cells associated with central and radial plates can also be seen (between arrowheads) and obscure the underlying gut. The inset is a confocal section through the same specimen at the level of the gut (g). **C.** Aboral view of a cleared 98 h juvenile shows the radial (r) and central (c) plates on the right side and two or three arm plates associated with rays. as, adoral shield; tp, terminal plate. **D.** Oral view of a 104-h juvenile shows the three distinct podial buds in each ambulacrum (tmp, terminal podial bud; dp, distal podium). Another pair of podial buds (bp, buccal podia) has also begun to form in several ambulacra and are most obvious in the ambulacrum located at 7 o'clock. The peripheral oral ectoderm (poe) has formed a distinct ring surrounding the developing ambulacra. **E.** In this aboral view of a 104-h cleared juvenile, the radial and central plates (out of focus) have migrated to the center of the juvenile and clear pentamerous radial symmetry is shown by the rays. Each ray has five ossicles: one terminal plate (tp), flanked by two adoral shields (as), and two proximal oral plates (pop). **F.** Aboral view of a cleared 115 h juvenile shows the central and radial plates in focus. Terminal arm plates can also be seen at 12, 2, 5, 8, and 10 o'clock. **G.** This oral view of a 116-h juvenile reveals a pair of circular distal podial buds (dp) associated with each ray. Between each pair is a small unpaired, terminal podium (see tmp in the ray at 10 o'clock) that is later covered with oral epithelium. Buccal podial buds (bp) can also be seen proximal to the distal pair. Ingressing ectoderm (ie). **H.** Oral view of a cleared 115-h juvenile shows further development of the five ossicles associated with each arm. The inlay is of the same individual but a slightly different focal plane. **I.** Aboral view of a cleared 129-h juvenile shows further growth of the central and radial plates. **J.** Oral view of a 5-d (143-h) juvenile with the distal pair of podial buds, the only ones seen on the surface. **K.** Oral view of a cleared 139-h juvenile shows the ossicles associated with the arms including newly formed distal oral plates (dop). All images are the same magnification. Scale bar = 100 μm.

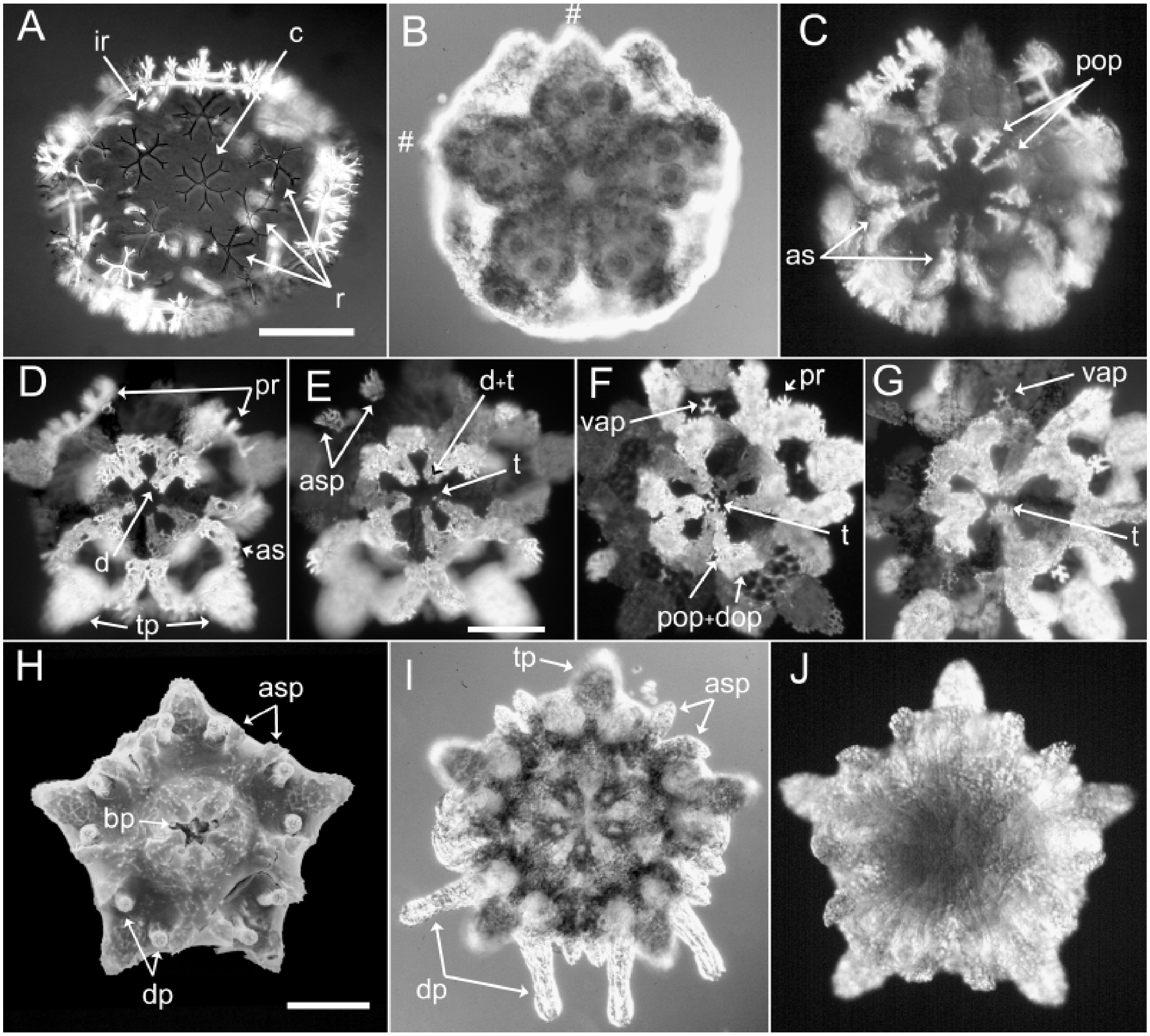

Fig. 5. Photomicrographs showing further development of the juvenile including jaws and the emergence of the rays of *Amphiodia occidentalis*. **A.** Aboral view with polarized light of a cleared 139-h juvenile showing further branching of central and radial plates and the occurrence of an interradial plate 1 (ir). This particular juvenile has an extra radial plate (the middle one of the three labeled) that is not centered over a ray. **B.** Oral view of a 7-d juvenile with rays that are beginning to extend beyond the circumference of the disk. The two marked rays (#) grow out on the oral side of the ring of peripheral rods whereas the other three rays grow out on the aboral side of this ring. **C.** Oral view of a 7-d juvenile viewed with cross-polarized light. The pairs of elongated central ossicles are the proximal oral plates (pop). These pairs lead distally into the adoral shields (as). **D.** A 10-d juvenile with dental plates (d) at the central edges of the proximal oral plates. The ring of peripheral rods (pr) is beginning to be resorbed. tp, terminal plate; as, adoral shield. **E.** A 12-d juvenile with tooth plates (t) central to the dental plates. Adoral shield spines (asp) are also present. **F.** A 15-d juvenile with larger, pointed tooth plates and ventral arm plates (vap) in several rays. This individual has a small remnant of the peripheral rods. dop, distal oral plate. **G.** A 19-d juvenile with well-developed teeth. **H.** A scanning electron micrograph of the oral side of a 12.3-d juvenile. The mouth is open and buccal podia (bp) are visible. Distal podia (dp) and adoral shield spines (asp) are present. **I.** A 19-d juvenile with podia (dp) projecting from two rays. **J.** An 8-wk juvenile in oral view. A–C are all the same magnification; D–G are all the same magnification; H–J are all the same magnification. Scale bars = 100 μm.

hatched on day 3, but motility did not occur until day 8 (at 12°C). Why an ontogeny would include a hatched stage that lacks movement by cilia or muscles for 5 d remains unclear. The post-gastrulae of the basket star *Gorgonocephalus eucnemis* have also been reported to be immotile (Patent 1970).

As the embryo of *A. occidentalis* began to flatten after gastrulation, it developed a bilateral symmetry that corresponds topologically with the bilateral symmetry of an ophiopluteus larva. The archenteron was bisected along its length by the symmetry plane that ran from the anterior or apical end of the embryo to the opposite end. As in gastrulae of feeding larvae, the blastopore moved to a subterminal location on the oral side of the flattened post-gastrula. In ophioplutei, the blastopore becomes the larval anus and is located on the oral side of the larvae in the aboral field. Upon flattening, the ectoderm formed slight but distinct angular, opaque regions at the lateral edges that were in locations equivalent to where the posterolateral arms first form in ophioplutei. However, larval spicules were not associated with these locations. The sinuous circular region of *A. occidentalis* that eventually became the juvenile disk was bilaterally symmetrical as was the U-shaped region around the stomodeum. Finally, the hydrocoel lobes developed on the left, and the radial plates formed on the right side of the symmetry plane.

Bilateral symmetry in development of *A. occidentalis* appears to be conserved from development of feeding larvae; however, more specific assignment of regions of the post-gastrula of *A. occidentalis* to features of ophioplutei is not possible. The oral and aboral arcs at the edge of the circular post-gastrula do not clearly correspond to arm or interarm regions of the ophiopluteus. The raised U-shaped region may correspond to the suboral pocket or the thickenings associated with this pit in some ophioplutei nearing metamorphosis (Mortensen 1921, 1931; Hendler 1991). This possibility is supported by the observations that the juvenile ambulacral structures appear to form from it. The U-shaped region may also correspond to a region of the ciliated band located posterior to the mouth in ophioplutei (see, e.g., Strathmann 1971). In ophioplutei the cells in the ciliated band are columnar. No cilia were present on this ridge and cell shape and packing were not determined in this study. The U-shaped region may also be a novel feature.

Reduction or loss of larval skeletal elements is a common feature of ophiuroids with abbreviated or direct development, but a gain of new skeletal elements has not been reported until now. A pair of reduced larval spicules is found in several nonfeeding larvae and some brooded developmental stages of ophiuroids (tabulated in Selvakumaraswamy & Byrne 2000). Nonfeeding larvae may be reduced ophioplutei or vitellaria, and the latter sometimes lack even reduced larval spicules. The brooded embryos with larval spicules are more or less reduced forms of feeding larvae, but many brooded embryos apparently lack larval structures entirely (Selvakumaraswamy & Byrne 2000). Whether these modified stages possess or lack larval spicules, the next ossicles to form are destined to be permanent parts of the juvenile and adult skeleton, usually the radial and terminal plates. *Amphiodia occidentalis* is unique in that it lacks a pair of larval spicules but develops a transient peripheral ring of numerous calcified ossicles. These begin to develop before the adult skeleton and are resorbed soon after the juvenile becomes motile and has an open mouth. This ring of mineralized ossicles probably increases the specific gravity of developmental stages, and may help them resist suspension in the water column as well as increase their sinking rate, speeding their return to the benthos.

Unlike other feeding and nonfeeding larvae of ophiuroids, the tube-shaped archenteron of *A. occidentalis* did not persist during the process of coelom formation. In feeding and nonfeeding larvae that have been described, the archenteron produces one or more coelomic cavities by outpocketing (e.g., Grave 1900; Hendler 1991, Selvakumaraswamy & Byrne 1999). In *A. occidentalis* the archenteron transformed into two pouches, and each gave rise to coelomic cavities and part of the gut. The original medial pouch produced a cavity that became the anterior of the gut and the left pouch apparently produced the posterior part of the gut.

The hydrocoel and left somatocoel of *A. occidentalis* arise from a common precursor, and are thus similar to that reported for feeding ophioplutei of *Ophiopholis aculeata* (Olsen 1942) and *A. kochii* (Yamashita 1984), and vitellariae of *Ophioderma brevispinum* (Grave 1900). Also similar among these species is that the stone canal connects to the hydrocoel between the fourth and fifth hydrocoelic lobes (as numbered 1–5 from anterior to posterior of the larva). Because there is uncertainty in the origin and fate of the right coelom (and any of its components) and the fate of the left somatocoel in *A. occidentalis,* detailed comparisons must await further study.

Despite the great reduction of larval features, the post-gastrula of *A. occidentalis* undergoes hydrocoel growth and rotation similarly to that reported for species with feeding larvae (e.g., Olsen 1942). After the five hydrocoelic buds formed on the left side of the flattened post-gastrula, they moved clockwise

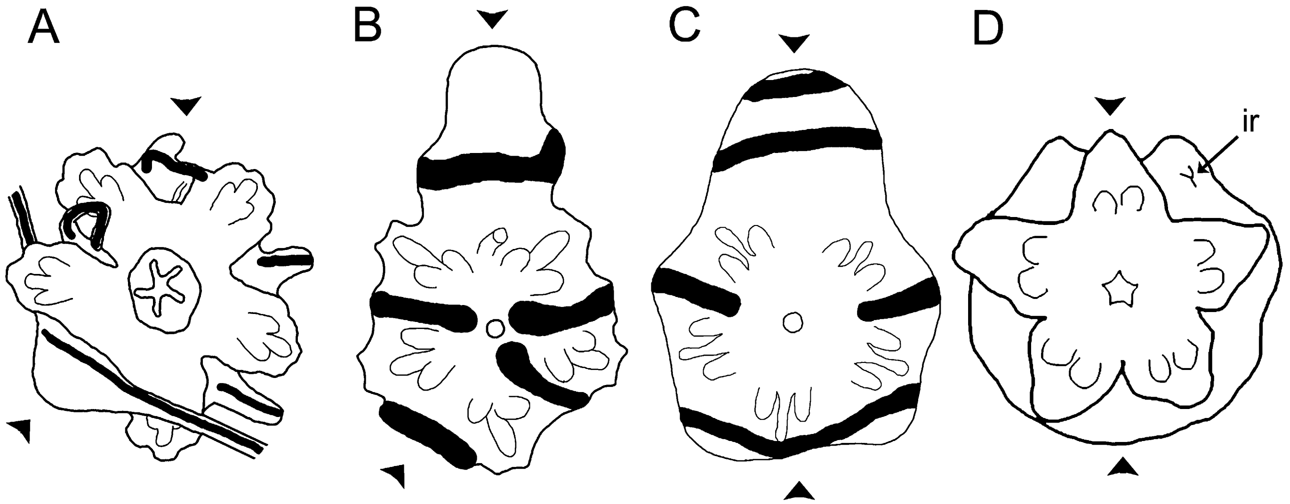

Fig. 6. Line drawings showing the orientation of the pentamerous juvenile on bilateral larval forms of various ophiuroids. In all species the larval anterior end is oriented at the top of the page and the plane of bilateral larval symmetry is marked with arrowheads. **A.** Ophiopluteus of *Ophiopholis aculeata* with arms resorbed except that posterolaterals are cut off in the drawing (redrawn from Olsen 1942). **B.** Vitellaria of *Ophionereis squamulosa* (redrawn from Mortensen 1921). **C.** Vitellaria of *Ophioderma brevispinum* (redrawn after Brooks & Grave 1899). **D.** A 7-d juvenile of *Amphiodia occidentalis* (drawn from Fig. 4K). The first interradial-1 ossicle (ir) is located on the right side when viewed from the oral surface.

(when viewed from the aboral surface) around the anterior side of the stomodeum. Followed by the other hydrocoelic lobes, the most anterior lobe migrated around the stomodeum from ~11 o'clock and ended up at 7 o'clock. The fifth lobe moved anteriorly from 7–8 o'clock to 9–10 o'clock, and the fourth lobe was at 12 o'clock. The stone canal located between the fourth and fifth lobes served as a marker to identify lobes, and ended up at 11 o'clock, to the left of the midline.

Olsen (1942) described a very similar migration of the hydrocoelic lobes in the ophiopluteus of *O. aculeata,* although he observed the rotation to continue until the first lobe was back at its original position and the fifth lobe was at the anterior end of the larva. Olsen (1942) also noted that the stone canal was to the right of the larval dorsal midline. In *A. occidentalis* this rotation moved the stomodeum posteriorly to a more central location. Just as the rotation was completed, the central and radial plates that originated on the right side of the post-gastrula moved to a more central location.

Presumably most vitellariae undergo hydrocoel rotation. Grave (1900) described extensive rotation of the left hydrocoel of the vitellaria larva of *O. brevispinum* that brought the stone canal to the right of the midline. Grave also noted that the esophagus moved to a more posterior location during hydrocoel rotation. Mortensen studied the vitellaria of *Ophionereis squamulosa* and figured a stage with the hydrocoel on one side of the archenteron and a subsequent stage with a five-lobed hydrocoel extending anteriorly and obliquely over the midline and the anterior end of the archenteron (Mortensen 1921: pl. 31, figs. 5 and 6). Fenaux (1969) showed a similar, obliquely oriented, five-lobed hydrocoel in *Ophioderma longicauda.*

The orientation of the pentamerous juvenile on the bilateral larval axis is different in *A. occidentalis* than in species with ophioplutei and vitellaria larvae (Fig. 6). *Amphiodia occidentalis* has an anteriormost arm ray that is aligned with the larval axis of bilateral symmetry and the other four radii occur in twos on either side of this axis. Late in development in species with feeding larvae and most of those with vitellariae, the larval axis of bilateral symmetry becomes bent concave to the left when viewed from the juvenile oral surface (Fig. 6A,B). Two juvenile radii project to the left of the larval axis and three radii project to the right of the larval axis when viewed from the juvenile oral side. In ophioplutei, the preoral arms are resorbed and the remnant of the preoral hood is positioned between two juvenile arm radii (see, e.g., Olsen 1942: figs. 47 and 56; Mladenov 1985: figs. 1 and 2). In *O. aculeata,* the hydropore is located in this interradius (Olsen 1942). The juvenile radius opposite this interradius projects posteriorly to the larval right, and the posterior end of the larva is located to the left of this ray when viewed from the oral side. All vitellariae described to date also have an anterior lobe located between two radii, and in vitellariae of

Table 2. Chronology of skeleton formation in *Amphiodia occidentalis.*

Time	Description
46 h	22± peripheral rods present
75–80 h	Adult radial plates and central plate present
91 h	Adoral shields and terminal plates in some rays
98 h	All adoral shields and terminal plates present
104 h	Proximal oral plates present
130 h	Distal oral plates initiated
139 h	Interradial-1 present in one ray
195 h	Adoral shield spines present on some rays
196 h	Dental plates beginning on some POPs
9.3 d	All five dental plates on proximal sides of POPs
10 d	Peripheral rods beginning to be resorbed
12.3 d	Peripheral rods almost completely resorbed.
12.3 d	Tooth plates present
15 d	Ventral arm plate-1 in all five rays, cannot distinguish dental and tooth plates, as they have the same *c*-axes
27 d	Other interradial-1s forming
41 d	All interradial-1s present
55 d	Several specimens forming first lateral arm plates

O. brevispinum and *O. squamulosa* this interradius is also the location of the hydropore (Grave 1900; Mortensen 1921). All vitellariae except that of *O. brevispinum* have an enlarged, posterior, ciliated region on the left side of the radius that is opposite the anterior lobe when these larvae are viewed from their oral side (Fig. 6B). Vestigial larval spicules are present in some vitellariae, and when they are posteriorly located they are associated with these posterior lobes (*Ophiolepis cincta* Mortensen 1938; *Ophionereis annulata* Hendler 1982). *Ophioderma brevispinum* lacks the remnant of the larval posterior and has a single radius oriented along the larval axis (Grave 1900).

The differing orientations of the juvenile relative to the larval axis of bilateral symmetry for the various ophiuroids may be related in part to the amount of rotation that occurs as the hydrocoelic lobes move around the stomodeum. The location of the precocial intertial-1 ossicle may be a key to the amount of rotation. Hendler (1978) described the occurrence of a precocial interradial-1 ossicle in the abbreviated development of *A. abditus,* and he noted that this was the same structure that Murakami (1940) identified as the madreporite in *Amphipholis squamata*. In his discussion, Hendler (1978) implied that this ossicle was associated with the interradius where the hydropore was located. In the ophiopluteus of *O. aculeata* and vitellariae of *O. brevispinum* and *O. squamulosa,* the pore canal is located in the interradius associated with the larval anterior (Grave 1900; Mortensen 1921; Olsen 1942). In *A. occidentalis,* the single interradial-1 ossicle that develops before the others is located to the right of the larval axis when viewed from the juvenile oral surface (Fig. 6). If this interradial-1 ossicle of *A. occidentalis* is also associated with the hydropore, then that interradius has rotated less far around the stomodeum in *A. occidentalis* than in other species.

Development of the juvenile skeleton of *A. occidentalis* was very similar to that of *A. abditus* as described by Hendler (1978). With a single exception, the order of skeletal element addition was essentially the same for *A. occidentalis* and *A. abditus* but the rate of skeletal development of the former was slower up to the time of tooth formation (compare Table 2 in this manuscript with table 1 of Hendler 1978). *Amphiodia occidentalis* was raised at 12°C while *A. abditus* was raised at 21°C, so differences in rate include effects of temperature. By day 55, some juveniles of *A. occidentalis* had begun to develop some of their first lateral arm plates, and this was the same time for *A. abditus* (Hendler 1978).

At no time were the ossicles that become buccal scales observed in *A. occidentalis*. In *A. abditus,* these form early, after the proximal oral plates, around the same time as and medially to the distal oral plates. The relative growth and fusion of the proximal oral plates and distal oral plates create an ambulacral gap where the buccal scales are located between the interambulacral jaws. These gaps formed in *A. occidentalis,* but no calcified elements (e.g., buccal scales) were seen in this species for the duration of observations. Hendler (1988) analyzed the ontogenies and homologies of the skeletal plates of three groups of amphiurid brittle stars, including members of what he identified as the *Amphiodia* group (*Ophiophragmus filograneus* and *Ophiocnida scabriuscula*). He reported that *O. filograneus* initially had buccal scales but lost their buccal scales when they had >35 arm segments. This pattern of loss of buccal scales was also seen in the *Amphipholis* group but buccal scales were retained in the *Amphiura* group. The absence of buccal scales in *A. occidentalis* may represent a second type of transition series (complete loss of buccal scales) within the *Amphiodia* group or may be associated with the direct development of *A. occidentalis*. It is also possible that they have yet to form (and be subsequently lost). Studies of skeletal development in other species of *Amphiodia* with different types of development should help clarify this matter.

Development is known or can be inferred for at least four species of *Amphiodia*. In addition to the direct, nonbrooded development described here for *A. occidentalis,* Hendler & Bundrick (2001) recently described a new species *A. akosmos* that broods its young. Another NE Pacific species, *A. urtica* with small eggs, ~100 μm in diameter (Schiff & Bergen 1996), also has planktotrophic development (G. Hendler, unpubl. data). Hendler & Littman (1986) reported a small egg, 65 μm in diameter, and probable planktotrophic development for the Caribbean species *A. pulchella*. The occurrence of planktotrophic, nonfeeding, and/or brooded development in the genus *Amphiodia* is also found in two other amphiurid genera, *Amphiura* and *Amphipholis*. The incidence of multiple types of development within each of these genera implies that loss of feeding larval development has occurred at least three times within the Amphiuridae.

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References

Austin WC & Hadfield MG 1980. Ophiuroidea: the brittle stars. In: Intertidal Invertebrates of California. Morris RH, Abbott DP, & Haderlie EC, eds., pp. 146–159. Stanford University Press, Stanford, CA.

Byrne M 1995. Changes in larval morphology in the evolution of benthic development by *Patiriella exigua* (Asteroidea: Asterinidae), a comparison with the larvae of *Patiriella* species with planktonic development. Biol. Bull. 188: 293–305.

——— 1996. Viviparity and intragonadal cannibalism in the diminutive sea stars *Patiriella vivipara* and *P. parvivipara* (family Asterinidae). Mar. Biol. (Berlin) 125: 551–567.

Chia F-S 1968. The embryology of a brooding starfish, *Leptasterias hexactis* (Stimpson). Acta Zool. 49: 1–44.

David B & Mooi R 1990. An echinoid that "gives birth": morphology and systematics of a new Antarctic species, *Urechinus mortenseni* (Echinodermata, Holasteroida). Zoomorphology 110: 75–89.

Emlet RB 1990. World patterns of developmental mode in echinoid echinoderms. In: Advances in Invertebrate Reproduction, Vol. 5. Hoshi M & Yamashita O, eds., pp. 329–334. Elsevier Press, Amsterdam.

Emlet RB, McEdward LR, & Strathmann RR 1987. Echinoderm larval ecology viewed from the egg. In: Echinoderm Studies, Vol. 2. Jangoux M & Lawrence JM, eds., pp. 55–136. Balkema Press, Rotterdam.

Fell HB 1941. The direct development of a New Zealand ophiuroid. Quart. J. Microsc. Sci. 82: 377–441.

Fenaux L 1969. Le developpement larvaire chez *Ophioderma longicauda* (Retzius). Cah. Biol. Mar. 10: 59–62.

Grave C 1900. *Ophiura brevispina*. Mem. Biol. Lab. Johns Hopkins Univ. 4: 83–100.

Hendler G 1975. Adaptational significance of the patterns of ophiuroid development. Am. Zool. 15: 691–715.

——— 1977. Development of *Amphioplus abditus* (Verrill) (Echinodermata: Ophiuroidea): I. Larval biology. Biol. Bull. 152: 51–63.

——— 1978. Development of *Amphioplus abditus* (Verrill) (Echinodermata: Ophiuroidea). II. Description and discussion of ophiuroid skeletal ontogeny and homologies. Biol. Bull. 154: 79–95.

——— 1982. An echinoderm vitellaria with a bilateral larval skeleton: evidence for the evolution of ophiuroid vitellariae from ophioplutei. Biol. Bull. 163: 431–437.

——— 1988. Ophiuroid skeleton ontogeny reveals homologies among skeletal plates of adults: a study of *Amphiura filiformis, Amphiura stimpsonii* and *Ophiophragmus filograneus* (Echinodermata). Biol. Bull. 174: 20–29.

——— 1991. Echinodermata: Ophiuroidea. In: Reproduction of Marine Invertebrates, Vol. 6, Echinoderms and Lophophorates. Giese AC, Pearse JS, & Pearse VB, eds., pp. 355–511. Boxwood Press, Pacific Grove, CA.

Hendler G & Bundrick CJ 2001. A new brooding brittle star from California (Echinodermata: Ophiuroidea: Amphiuridae). Contr. Sci. (Los Angeles) 486: 1–11.

Hendler G & Littman BS 1986. The ploys of sex: relationships among the mode of reproduction, body size and habitats of coral-reef brittlestars. Coral Reefs 5: 31–42.

Kelman D & Emlet RB 1999. Swimming and buoyancy in ontogenetic stages of the cushion star *Pteraster tesselatus* (Echinodermata: Asteroidea) and their implications for distribution and movement. Biol. Bull. 197: 309–314.

MacBride EW 1907. The development of *Ophiothrix fragilis*. Quart. J. Microsc. Sci. 51: 557–606.

McEdward LR & Janies DA 1997. Relationships among development, ecology, and morphology in the evolution of echinoderm larvae and life cycles. Biol. J. Linn. Soc. 60: 381–400.

McEdward LR & Miner BG 2001. Larval and life-cycle patterns in echinoderms. Can. J. Zool. 79: 1125–1170.

Mladenov PV 1985. Development and metamorphosis of the brittle star *Ophiocoma pumila*: evolutionary and ecological implications. Biol. Bull. 168: 285–295.

Mortensen T 1921. Studies of the Development and Larval Forms of Echinoderms. G.E.C. Gad, Copenhagen. 261 pp.

——— 1931. Contributions to the study of the development and larval forms of echinoderms I–II. K. Dan. Vidensk. Selsk. Skr. Naturvidensk. Mat. Afd. Ser. 9 4: 1–39.

——— 1938. Contributions to the study of the development and larval forms of echinoderms IV. K. Dan. Vidensk. Selsk. Skr. Naturvidensk. Mat. Afd. Ser. 9 7: 1–59.

Murakami S 1940. On the development of the calcareous plates of an ophiuran, *Amphipholis japonica* Matsumato. Jpn. J. Zool. 9: 19–33.

Narasimhamurti N 1933. The development of *Ophiocoma nigra*. Quart. J. Microsc. Sci. 76: 63–88.

Olsen H 1942. The development of the brittlestar *Ophiopholis aculeata* (O. Fr. Muller), with a short report on the outer hyaline layer. Bergens Museum Aarbok, Natur. 6: 1–107.

Patent DH 1970. The early embryology of the basket star *Gorgonocephalus caryi* (Echinodermata, Ophuroidea). Mar. Biol. 6: 262–267.

Rumrill SS 1982. Contrasting reproductive patterns among ophiuroids (Echinodermata) from southern Monterey Bay, USA. MS Thesis, University of California, Santa Cruz. 260 pp.

Schiff KC & Bergen M 1996. Impact of wastewater on reproduction of *Amphiodia urtica*. South. Calif. Coast. Water Res. Proj. Annu. Rep. 1994-1995: 78–84.

Selvakumaraswamy P & Byrne M 1999. Early events of coelomogenesis in a lecithotrophic ophiuroid larva. In: Echinoderm Research 1998. Candia Carnivali MD & Bonasoro F, eds., pp. 227–228. Balkema, Rotterdam.

——— 2000. Vestigial ophiopluteal structures in the lecithotrophic larvae of *Ophionereis schayeri* (Ophiuroidea). Biol. Bull. 198: 379–386.

Smith AB, Paterson GLJ, & Lafay B 1995. Ophiuroid phylogeny and higher taxonomy: morphological, molecular and palaeontological perspectives. Zool. J. Linn. Soc. 114: 213–243.

Staver JM & Strathmann RR 2002. Evolution of fast development of planktonic embryos to early swimming. Biol. Bull. 203: 58–69.

Strathmann RR 1971. The feeding behavior of planktotrophic echinoderm larvae: mechanisms, regulation and rates of suspension-feeding. J. Exp. Mar. Biol. Ecol. 6: 109–160.

——— 1974. Introduction to function and adaptation in echinoderm larvae. Thal. Jugo. 10: 321–339.

——— 1975. Larval feeding in echinoderms. Am. Zool. 15: 717–730.

——— 1978. The evolution and loss of feeding larval stages of marine invertebrates. Evolution 32: 894–906.

——— 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annu. Rev. Ecol. Syst. 16: 339–361.

——— 1993. Hypotheses on the origins of marine larvae. Annu. Rev. Ecol. Syst. 24: 89–117.

Strathmann MF & Rumrill SS 1987. Phylum Echinodermata, class Ophiuroidea. In: Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast. Strathmann MF, ed., pp. 556–573. University of Washington Press, Seattle, WA.

Thorson G 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1–45.

Turner RD 1976. Fixation and preservation of molluscan zooplankton. In: Zooplankton Fixation and Preservation. Steedman HF, ed., pp. 290–300. Unesco Press, Paris.

Wray GA 1995. Evolution of larvae and developmental modes. In: Ecology of Marine Invertebrate Larvae. McEdward L, ed., pp. 413–447. CRC Press, Boca Raton, FL.

Yamashita M 1984. Electron microscopic observations on the cortical reaction of the brittle-star *Amphipholis kochii* Lutken, with special reference to its vitelline coat modification as revealed by the surface replica method. Dev. Growth Differ. 26: 177–189.

Young CM, Sewell MA, & Rice ME, eds. 2002. Atlas of Marine Invertebrate Larvae. Academic Press, London. 626 pp.