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</tr>
<tr>
<td></td>
<td>Bishop, Cory; St Francis-Xavier University, Biology</td>
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Ossicle development of the crinoid *Florometra serratissima* through larval stages

Ariane Comeau

Email: ariane.comeau@umontreal.ca

Cory D Bishop

Email: cbishop@stfx.ca

Christopher B Cameron

Email: c.cameron@umontreal.ca

1 Département de Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-ville, Montréal, Québec, Canada H3C 3J7

2Department of Biology, St Francis-Xavier University, 2320 Notre Dame Avenue, Antigonish, Nova Scotia, Canada B2G 2W5

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Abstract

Crinoids are the oldest living class of echinoderm and sister group to the remaining Eleutherozoan clade and so are key to discussions on the evolution and development of the echinoderm skeleton. Here we present the intraspecific variation of ossicle development of the feather star *Florometra serratissima* (Clark, 1907) during its three larval stages: doliolaria, cystidean and early pentacrinoid. To induce settlement larvae were cultured on a sea table in glass bowls containing coralline algae. The soft tissues of sixty larvae were dissolved to isolate and observe the ossicles with compound and scanning electron microscopy. From the late doliolaria stage to 56-day old pentacrinoids, a total of four types of ossicle developed: oral plates, basal plates, columnar stalk ossicles and an attachment disk. Occasionally an additional plate was found under the basal plates, that may represent a vestigial infrabasal plate. The shape of the attachment disk was plastic to accommodate the substrate. Crinoid ossicle development is variable in size, shape and number, and the timing of development is asynchronous, traits that may have contributed to the early rapid radiation and phenotypic disparity of echinoderms.

Keywords: Crinoid, feather star, comatulid, *Florometra serratissima*, skeleton, ossicle, larva, doliolaria, cystidean, pentacrinoid, evolvability
Résumé


Mots clé : Crinoïde, comatule, *Florometra serratissima*, squelette, ossicule, larve, doliolaria, cystidienne, pentacrinoïde, évolvabilité
**Introduction**

Among extant echinoderms, crinoids are the sister group to the remaining echinoderm classes, the Eleutherozoa (Smith 1997). The subclass Articulata are the only living crinoids and include the comatulids, also called feather stars, and the stalked sea lily forms (Cohen et al. 2004). The pentacrinoid stage of comatulids is stalked, reminiscent of the sea lilies, but the stalk is abandoned in the transition to the mobile juvenile stage. Morphological phylogenies have placed feather stars and sea lilies as monophyletic sister taxa. Molecular studies on the other hand show that some sea lilies have evolved from feather stars, making the sea lilies a polyphyletic group, and suggesting that these sea lilies are the paedomorphic pentacrinoid form of a feather star (Rouse et al. 2013). Relationships among the comatulids are also in contest as members of the family Antedonidae, including *Florometra* and *Antedon*, may be para- or polyphyletic (Hemery et al. 2013). What little information is available on the development of early fossil taxa suggests that ancient taxa follow similar developmental trajectories to extant taxa (Brower 1974). Thereby the study of skeleton development in early stages of extant crinoids is expected to shed light on the primitive mode of crinoid skeletal development.

Skeletal terminology and interpretations of crinoid thecal plate homologies have been largely established by palaeontologists and thecal plate homology between monocyclic and dicyclic forms is the main point of dispute (Ubaghs 1969; Moore and Teichert 1978; Simms 1993; Ausich 1996; Guensburg and Sprinkle 2003). Monocyclic and dicyclic forms are defined by the number of circlets of thecal plates. The classic terminology (Ubaghs 1969; Moore and Teichert 1978), established that in monocyclic forms the first circlet at the oral axis are the oral plates, followed by a second circlet of radial plates and a third circlet of basal plates (Figure 1). The dicyclic forms possess an additional circlet of plates below the basal plates called the infrabasal plates. Other authors suggest that the additional circlet found in the dicyclic form might not be
the bottom circlet of the calyx and therefore the homology of the plates between monocyclic and dicyclic forms would differ from the classic scheme (Simms 1993; Guensburg and Sprinkle 2003). In our work, we adopt the classic terminology to identify plates in *Florometra serratissima* (Clark, 1907) because it is the most widely accepted scheme of plate identification. We use the term spicule for the early ontogenetic stage of ossicle development, and the term ossicle as a later developmental stage when a stereom, with at least one complete stroma, has formed. We use the term plate ossicles for the ossicles of the calyx and columnal ossicles for the ossicles of the stalk. The adult crinoid calyx or theca develops from the larval calyx plates.

During post-embryonic development, feather stars, including *F. serratissima*, pass through doliolaria, cystidean and pentacrinoid larval stages. Doliolaria larvae swim with ciliated bands, settle, and metamorphose into a club shaped, non-feeding cystidean (Mladenov and Chia 1983). The cystidean loses the cilia, the stalk begins to elongate, and the calyx rotates away from the stalk to an upward facing position. After about one month the feeding podia emerge from the calyx, and the pentacrinoid stage begins to feed. Pentacrinoids of *F. serratissima* abandon the stalk to become a free moving juvenile months later (Mladenov and Chia 1983).

Our objectives are to document the order, position and characteristics of ossicle development, through the doliolaria, cystidean and pentacrinoid stages of the comatulid *F. serratissima*. We compare its skeletal ontogeny to those published for the comatulids *Aporometra wilsoni* (Bell, 1888) (Haig and Rouse 2008), *Antedon bifida* (Pennant, 1777) (Clark 1921; Lahaye and Jangoux 1987), *Oxycomanthus japonicus* (Müller, 1841) (Shibata et al. 2008) and the stalked sea lily *Metacrinus rotundus* (Carpenter, 1885) (Nakano et al. 2003; Amemiya et al. 2016). This comparative approach suggests that dicyclic development may be the ancestral crinoid condition and that variation in the form and number of ossicles, as well as an asynchronous
timing of development among individual larvae may be ancient crinoid, if not echinoderm, traits.

Materials and Methods

Manipulations of living *F. serratissima* took place at the Bamfield Marine Sciences Centre (BMSC), Vancouver Island, B.C. from mid April to early July 2013. Individual *F. serratissima* were collected in 30 metres depth of water in Bamfield Inlet (Latitude: 48.835 Longitude: -125.137) by SCUBA and transferred in plastic bags to the BMSC where they were kept in the dark in seawater tables, which are uncovered basins through which natural seawater flows unidirectionally. The depth of water (~30 cm) was regulated by placing a stand pipe in the drain and water flow rates were regulated to ensure that the temperature remained consistent with ambient seawater temperatures. Crinoids are gonochoristic. To determine the sex of an individual, a pinnule, which houses the tubular gonad, was removed from an arm and dissected to liberate oocytes or sperm. To reduce the risk of natural spawning, males and females were kept in separate tables. Within an individual gonad gametogenesis is non-synchronous. Pinnules from each of the females were removed daily and checked for mature oocytes. Mature oocytes, which are round and non-sticky were separated from the immature oocytes which adhere into clusters (Mladenov and Chia 1983). Sperm, also taken from pinnules by dissection, was in most cases, mature (mobile) and ready for fertilization. When mature oocytes were obtained, fertilization was conducted in small glass bowls with a drop of sperm diluted in sea water, immediately followed by several rinses of fresh seawater using a pipette. In preliminary experiments with rearing cultures in filtered seawater, artificial seawater (Jamarin-U, Jamarin Co.) and unfiltered seawater we observed that development was asynchronous and variable. The results reported
here were obtained from embryos cultured in 50 µm filtered seawater, changed daily. Embryos and larval stages were reared in glass bowls. Individual cultures were kept at approximately 8 °C by placing the bowls partially submerged on a shallow sea table. A developmental timetable of the species is provided\(^1\). In an effort to enhance the settlement of doliolariae a few pieces of branched coralline algae were added to some cultures. After about two weeks of development, feeding pentacrinoids were fed a mixture of *Rhodomonas*, *Isochrysis* and *Chlorella* every other day. The oldest larvae cultured were 56-day old pentacrinoids; the earliest pentacrinoids initiated metamorphosis at 30 days.

Some larvae were fixed at specific stages to observe their ossicles later on during the season. These larvae were collected and fixed in cold 2 % formaldehyde (formulated by dissolving 2 g of paraformaldehyde in 100 mL in Millipore\textsuperscript{®} filtered seawater) for 1 hour and then washed with Millipore\textsuperscript{®} filtered seawater. To preserve larvae for future examination they were stored in 5.0 g/L sodium glycerophosphate powder in 70 % ethanol, or 4 % formaldehyde made in seawater and neutralized with sodium borate, at 4°C. Ossicles were isolated from either living or fixed doliolaria, cystidean and pentacrinoid larvae by dissolving the tissues with one drop of 6 % bleach added to a drop of water that was pipetted with the larva directly onto a slide for microscopic observation, then rinsed with distilled water. The ossicles of twenty doliolariae, twenty cystideans and twenty pentacrinoids were isolated for microscopy. Observations were made with an Olympus FluoView\textsuperscript{TM} 300 compound microscope equipped with polarizing filters and photographs were taken with a CoolSnap ProCF camera (Roper Scientific Photometrics, Arizona, USA). The skeletal elements of these larvae were only observed once. Scanning electron microscopy (SEM) was performed on pentacrinoid ossicles at St. Francis Xavier

\(^1\) see Supplementary Files Table S1: Timetable of developmental events of the feather star *Florometra serratissima*, from fertilization to pentacrinoid
University. Ossicles were cleaned in 6 % bleach for two minutes on a slide until the soft tissues were dissolved. Ossicles were washed several times with distilled water, dried in 95 % ethanol and transferred to an aluminum SEM stubs. Ossicles were carbon coated using a Devon D205A sputter coater, analyzed and imaged using a JEOL JSM-6010LA SEM (JEOL, Massachusetts, USA).

Results

The first spicules appeared in the doliolaria at 4 days post-fertilization. There were ten or eleven columnar ossicles including the attachment disk (mean = 10.4, \(n = 7\)) and the number increased with time. At that early stage, the attachment disk was not always morphologically differentiated from the other columnal ossicles. Ten to thirteen (mean = 11.1, \(n = 10\)) and eleven to fourteen (mean = 12.1, \(n = 10\)) columnar ossicles were present in cystideans and pentacrinoids respectively. From the doliolaria to the early pentacrinoid stage, there were five basal plates and five oral plates in fifty-one individuals and their appearance was contemporaneous with that of the first columnar ossicles. Nine individuals had an eleventh calyx plate ossicle, which we further discuss below. To complement micrographs, we provide a diagram of ossicle development during larval development (Figure 1).

Presumptive plate ossicles first appear in 4 day doliolaria as tri-radiate (Figure 2A), tetra-radiate or penta-radiate spicules (Figure 2B). Upon further growth they became plate-shaped and developed stroma, but, with the exception of their position, the oral calyx ossicles were indistinguishable from the basal calyx ossicles from the doliolaria to the mid-cystidean stages (Figure 2C to E). The oral and basal plates were located in the postero-ventral region of the doliolaria (Figure 2F), the region fated to be the calyx in the cystidean stage (Figure 2G). No two calyx plate ossicles were identical at these development stages: their size, shape and number of
stroma varied (Figure 2H to Q). The timing of the first appearance of stroma varied among doliolaria. Observations of hundreds of stromata suggest that they developed by the fusion of paired extensions to form the characteristic circular holes of the stereom (Figure 3A-B).

Columnar stalk ossicles began development as ellipsoidal granules (Figure 4A), and subsequently took a semicircle form (Figure 4A-B) before opposing ends fused, resulting in an O-shape (Figure 4C to E). The columnar ossicles develop along the dorsal midline of the doliolaria (Figure 4F) and the most distal columnar ossicle developed where the antero-ventral adhesive pit of the doliolaria is located. In other words, the oral-aboral axis of the cystidean is anti-parallel to the anterior-posterior axis of the doliolaria (Figure 4F-G). The antero-ventral pit of the doliolaria is the attachment site of the settled doliolaria, and the location of the most aboral adhesive plate of the cystidean (the attachment disk) (Figure 4H). Following settlement the cystidean stalk straightened and consequently the calyx rotated from its position against the stalk to an erect, upright position (Figure 4G to I). From doliolaria to early cystidean, the columnar ossicles were wider than long (Figure 4E), but lengthened with cystidean maturation (Figure 4I). In advanced cystideans, new ossicles were added at the base of the calyx, at the oral end of the stalk (Figure 4J). Between ten to thirteen columnar ossicles, including the attachment disk, were observed in advanced cystideans. The columnar stalk ossicles were of different lengths (i.e., xenomorphic) at the cystidean stage with those in the median region longer than those from the apical and basal regions (Figure 5). The attachment disk is a modified columnar ossicle. It first appeared in the doliolaria, but typically lacked the well-defined central lumen of the other columnar ossicles (Figure 6A). Upon settlement, the disk developed rapidly (Figure 6B-C) and exhibited developmental plasticity: it was flat and disk-shape on flat surfaces, or developed into shapes that accommodated irregular surfaces (Figure 6D).
The young crinoid feeding podia first projected from inside the oral plates between 22 and 29 days post-fertilization, marking the start of the feeding pentacrinoid stage. At this stage the calyx (Figure 7A) was still composed of five oral plates (Figure 7B) and five basal plates (Figure 7C), but each oral plate was imbricated with its corresponding basal plate, at its respective wider end. The oral ends of the oral plates were narrowest and inwardly concave, which permitted closure of the oral cavity. The aboral end of the basal plates were narrowest at the junction with the most apical columnar ossicle (Figure 7D). As with the cystidean, columnar ossicles at the terminal ends (apical and basal) were shorter than those in the median region, indicating that new columnar ossicles developed adjacent to the calyx (Figure 7E) and not by intercalation. The oldest pentacrinoids cultured were 56 days old and developed no new type of ossicle from those that first appeared in the doliolaria. Pentacrinoids from 22 to 56 days old had between eleven and fourteen columnar ossicles, including the attachment disk. This variation was not due to parentage because three 56-day old sibling pentacrinoids from the same culture had eleven, twelve, and thirteen columnar ossicles.

The shape and size of plate and columnar ossicles varied not only among siblings but within an individual. Some doliolariae explored and settled onto the substrate before any stroma formed in either calyx plate or columnar ossicles. A chart of the diameters of oral and basal calyx plate ossicles between doliolariae, cystideans and early pentacrinoids (Figure S3) shows that i) the diameter of the plate ossicles varied greatly among larvae of similar age and ii) the average size of cystidean plate ossicles tended to be larger than those of unsettled doliolariae of the same age. However, the difference in ossicle sizes between doliolariae and cystideans may have been

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2 see Supplementary Files Figure S1: Selected ossicles of five sibling doliolariae at 9d7h old of the feather star *Florometra serratissima*.

3 see Supplementary Files Figure S2: The complete skeleton of a 9d7h old doliolaria of the feather star *Florometra serratissima* shows variability in ossicle shapes and sizes.

4 see Supplementary Files Figure S3: Ossicle diameter varied greatly in feather star *Florometra serratissima* and were larger in cystideans than in same age doliolariae.
influenced by the presence of coralline algae (see Figure S3 caption for more information). Although uncommon, an additional small calyx plate ossicle was found in five doliolariae and three cystideans. We could not always determine the location of these plates, but in three doliolariae and one cystidean, it was located between the basal plate and the column (Figure 8A-B), where one would find infrabasal plates in dicyclic species. Finally, one pentacrinoid possessed a sixth narrow basal plate (Figure 8C-D), positioned between other basal plates. The location suggests it was an anal plate.

Discussion

An interspecific comparison of ossicle development among crinoids shows abundant variation in the timing of appearance, the shape and the number of ossicles in larvae (Table 1). Less appreciated is the intraspecific variation documented here for *F. serratissima* and elsewhere for *A. bifida* (Clark 1921). Ossicles of *F. serratissima* first appeared variably between the initial doliolaria to very late doliolaria stage. In some cases we observed settlement and metamorphosis of doliolariae that had rudimentary spicules lacking stroma. Oral and basal plates began development as tri-, tetra-, or penta-radiate spicules. The shape and size of the ossicles varied within an individual and among sibling larvae. In contrast, among sea urchins and brittle stars, this variability is not observed in the development of the pluteus larval spicule, but rather, is more characteristic of the first spicules to form in the early juvenile rudiments of these two echinoderms (Hendler 1982; Yamashita 1985; Heyland and Hodin 2014).

More is known about the variation in columnar ossicle number among crinoid species. Doliolariae of *F. serratissima* possess ten or eleven columnar ossicles, and more are added following settlement. Including the attachment disk, from ten to thirteen columnar ossicles
were present in cystideans and from ten to fourteen in pentacrinoids. In all species compared, the new columnar ossicles in cystideans and pentacrinoids form at the base of the calyx (under the basal plates). Sea lilies generate (and regenerate) the stalk under the basal plate of the calyx, though in older individuals new columnar ossicles intercalate between older ones (Webster 1974; Breimer 1978; Simms 1989; Nakano et al. 2004). This intercalation procedure is common among Paleozoic crinoids (Moore et al. 1968; Brower 1974; Haude 1980). Small grains of biomineralized elements have been found between the doliolaria columnar ossicles of the comatulid *Tropiometra carinata* (Lamarck, 1816) but it is not clear if they are new intercalated ossicles or fragments of the main columnar ossicles (Mortensen 1920b). This standing variation in the number of columnar ossicles in culture may be maintained by selection, as feeding is considered the principal factor influencing a crinoid stalk length (Bottjer and Ausich 1986; Kitazawa et al. 2007). On the other hand, rather than anything to do with external selection, variation in columnar ossicle number may be related to developmental (or more generally organismal) internal coadaptive integration with a developmentally plastic attachment disk.

The attachment disk of *F. serratissima* was sometimes morphologically differentiated from the other columnar ossicles in doliolariae and invariably well developed and differentiated after larva settlement. Its shape developed according to the form of the substrate, to which the cystidean is attached. In *A. wilsoni*, the attachment disk is well developed at the doliolaria stage (Haig and Rouse 2008). In *O. japonicus*, the larva settles as early as 2 days post-fertilization, and the attachment disk is differentiated in the newly settled larva (Shibata et al. 2008). The attachment disk of the cystidean of the sea lily *M. rotundus* differs from that of the comatulids by its division into five plates (Amemiya et al. 2016). The maintenance of developmental plasticity of the attachment disk is presumably under strong adaptive selection.
In eight of sixty cases we observed an additional small plate in some doliolariae and cystideans of *F. serratissima*. It was positioned below the basal plates, at the position of an infrabasal plate. The sea lily *M. rotundus* is dicyclic because it has an additional circlet of five plates that form below the basals, called the infrabasals. The five infrabasals appear as early as 6.5 days post-fertilization in the sea lily cystidean, at the same time as the oral and basal plates (Amemiya et al. 2016). The calyx plates of *Antedon mediterranea* ( Lamarck, 1816) doliolaria are variable in form and include no visible infrabasal (Barbaglio et al. 2012) to five infrabasals (Clark 1921; Moore and Teichert 1978). In *O. japonicus*, the infrabasal plates appear at the cystidean stage and radials and an anal plate appear in the pentacrinoid, two weeks after metamorphosis, around 40 days post-fertilization (Shibata et al. 2008) (Table 1). The presence of the infrabasal plates in *A. bifida* is controversial. Whereas Lahaye and Jangoux (1987) did not find infrabasal in any pentacrinoids, Mortensen (1920a) identified three infrabasal plates forming a small ring hidden inside the basal plates of his *A. bifida* pentacrinoid cultures. Carpenter suggested that the comatulid *Atelocrinus balanoides* (Carpenter, 1881) have resorbed infrabasals (Clark 1915). We interpret these extra plates as vestigial structures. This interpretation is congruent with the classic scheme of crinoid plate homologies put forward by Moore and Teichert (1978), and the phylogenies of Ubaghs (1969) and Simms (1993) in which the dicyclic form is viewed as primitive. Some fossil specimens of the crinoid *Bactrocrinites*, dating from the Middle Devonian, exhibit anomalies by losing a complete basal circlet (McIntosh 1979), suggesting that the labile nature of crinoid plate development may be an ancient trait. Documenting the intraspecific variation in calyx plate number and position is central to the taxonomy of crinoids as these are traits are generally recognized as distinguishing features between families (Breimer 1978).

Here, we characterized ossicle development in the comatulid crinoid *Florometra serratissima* through the doliolaria, cystidean and pentacrinoid larval stages and show that crinoid ossicle
development is variable in form, number, and time of appearance of both calyx plates and
columnar ossicles. Fossils of Cambrian blastozoan echinoderms also exhibit high phenotypic
variability in the skeleton of the feeding appendages, in which the fundamental organization is
the same as crinoid arms (Zamora and Smith 2011). Standing variation of a population, and
probably also unusual variants, like occasional infrabasal plates, most probably play a key role in
the origin of phenotypes and species. This readiness with which crinoids and blastoids allow the
production of different kinds of variant is sometimes referred to as evolvability (Kirschner and
Gerhart 1998). Evolvability was arguably significant during the early evolution of echinoderms,
which was characterized by a rapid radiation and morphological disparity (Smith et al. 2013).

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## Tables

### Table 1. Skeletal elements appearance from fertilization to pre-juvenile stage in five species of crinoids.

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- **F. serratissima** (data taken from Mladenov and Chia 1983; current work)
- **A. wilsoni** (data taken from Haig and Rouse 2008)
- **A. bifida** (data taken from Clark 1921; Lahaye and Jangoux 1987)
- **O. japonicus** (data taken from Shibata et al. 2008)

- Amemiya et al. (2016) refers to the stage at which an individual begins to feed with podia as a late cystidean, whereas here we regard it as an early pentacrinoid.

- In the present study, a few individuals possessed an eleventh plate ossicle at the position of the infrabasals. Clark (1921) reported infrabasals in stalked individuals of *A. bifida*, whereas Lahaye and Jangoux (1987) did not.

**Nota**
The data in this table were compiled from the publications in the top row. The black cells indicate the first larval stage at which a skeletal element was observed. The number of columnar ossicles includes the attachment disk. aur: auricularia (stalked crinoid only); dol: doliolaria; cys: cystidean; juv: juvenile (detachment from stalk, except stalked crinoid); pen: pentacrinoid.
Figure captions

**Figure 1.** Diagrammatic representation of ossicle development through larval stages of the feather star *Florometra serratissima*. Spicules, the earliest forms of the skeleton, appeared in the doliolaria and subsequently developed into ossicles (with at least one stroma). The accolades bracket the ossicle type from each of the three larval stages. The body drawings show the location of each ossicle type, which are colour coded.

**Figure 2.** Development and variation of plate ossicles of the feather star *Florometra serratissima* from doliolaria to cystidean. A: Tri-radiate early plate spicule from a 5d21h old doliolaria; B: Plates from a 4d5h old doliolaria; arrows point to early spicules not having a tri-radiate form, prior to their development into plates; C: Plate from a 7d old doliolaria; D: Plate from a 10d19h old doliolaria; E: Plate from a 7d7h old cystidean; F: 11d old doliolaria, the rectangles encompass the location of the five oral and five basal plates; G: Overlay of 11d2h old cystidean calyx with some visible plates. H to Q: Set of the ten plate ossicles from a single 8d7h old doliolaria. No two plates were identical, and basal plates were not distinct from oral plates. ant: anterior; bp: basal plate(s); op: oral plate(s); post: posterior.

**Figure 3.** SEM images of spicule growth in two oral plate ossicles of 22d old pentacrinoids of the feather star *Florometra serratissima*. A-B: Arrows show the direction of the growth of ossicles when the spicules elongated to form stroma of the stereom. They also grew in width (illustrated by the two-headed arrow in A).

**Figure 4.** The columnar ossicles from ten larvae of the feather star *Florometra serratissima* from different developmental time periods. A: Columnar spicules of a 5d5h old doliolaria, at the earliest ellipsoid shape (1) and semicircle shapes (2-3); B, C, D: Columnar ossicle(s) of 6d1h, 8d7h and 9d17h old doliolariae, respectively; E: Stalk portion of the same 9d17h old doliolaria; F: Location of stalk in a 11d3h old doliolaria; G: Location of the stalk (rectangle) and calyx (circle) in an overlay of a 10d2h old recently settled cystidean; H: The calyx has rotated into a nearly upright position over the stalk (rectangle) in this 7d7h old cystidean; I: Stalk of a 13d old cystidean with xenomorphic columnar ossicles; J: Lateral view of a 44d old cystidean shows that the new columnar ossicles are shorter than the median columnar ossicles. ad: attachment disk; ant: anterior; dor: dorsal region; post: posterior; ven: ventral region
**Figure 5.** Skeletal elements of a 43d old cystidean of the feather star *Florometra serratissima*. Some of the five oral and five basal plates are visible. Eleven columnar stalk ossicles and the attachment disk form the xenomorphic stalk of this cystidean. The ossicles in the median region are longer than those on either end. The junctions between the ossicles are not easily seen; instead the arrows point to the bulge in the middle of three ossicles. ad: attachment disk; bp.1 to 3: basal plates; c.1 to 11: columnar stalk ossicle starting at the apical region; op.1 to 3: oral plates

**Figure 6.** The attachment disk of the feather star *Florometra serratissima* is plastic, conforming to the substrate. A: An early form of the attachment disk of an 8d1h old doliolaria; B: Attachment disk and a partial stalk of a 11d4h old cystidean that had settled five days earlier; C: Overlay of a 15d old cystidean that had settled on a flattened substrate with its attachment disk having taken a flattened, enlarged disk-shape; D: This attachment disk of a 45d old pentacrinoid is in the form of an irregular cylinder and was attached to an irregularly shaped substrate.

**Figure 7.** SEM images of ossicles of a 22d old pentacrinoid of the feather star *Florometra serratissima*. The soft tissue of the larva was removed. A: Calyx; B: Oral plate; C: Basal plate; D: The youngest columnar ossicle was located directly below the calyx. Plates in A through D are positioned with the oral end up. bp: basal plate; c.1 to c.5: first five columnar stalk ossicles starting at the calyx; op: oral plate

**Figure 8.** An additional ossicle was occasionally found in the doliolariae, cystideans and pentacrinoids of the feather star *Florometra serratissima*. A: An additional small plate (arrow) was found close to a columnar ossicle in 9d7h old doliolaria. B: An additional small plate (arrow) was found close to the basal plates in a 15d old cystidean. C-D: A sixth thin basal plate was found in 45 day old pentacrinoids (framed and arrow).

**Supplementary material**

**Table S1.** Timetable of developmental events of the feather star *Florometra serratissima*, from fertilization to pentacrinoid. Development begins by free spawning of gametes into the water (between 9.5 and 11.5°C) where fertilization takes place. These observations, which corroborate those of Mladenov and Chia (1983), were made on material derived from fertilization of...
gametes obtained from dissection and embryos reared in laboratory cultures for up to 6 months. Observations from this study are in italics.

**Figure S1.** Selected ossicles of five sibling doliolariae at 9d7h old of *Florometra serratissima*. These ossicles show the size and shape disparity within and among larvae. Each row (A to E) represents an individual larva. Columns a and b are plate ossicles, c are mid-columnar ossicles, d are those columnar ossicles from directly below the calyx, and e are attachment disk ossicles. Rarely, columnar ossicles were oblong-shaped and had small stroma (Bc-Bd). The attachment disk (column e) was also circular but lacked a central stroma. It was otherwise variable in shape, having formed in a manner that appeared to accommodate the substrate. Ossicle shape, developmental rate, and diameter varied substantially among larvae (see Figure S3). A complete complement of ossicles from a 9d7h larva, taken from the same culture as these five larvae, appears in Figure S2.

**Figure S2.** The complete skeleton of a 9d7h old doliolaria of *Florometra serratissima* shows variability in ossicle shapes and sizes. See Figure S1 to see the variation among five 9d7h old doliolariae. The skeleton consists of ten calyx plates (A to J) and ten columnar ossicles (K to P). At this stage, some plates had numerous complete stroma (E to H) whereas others lacked them (B). K to P: The columnar ossicles formed complete, closed circles with radially projecting spicules that assured the oral-aboral imbrication of the adjoining ossicles. Two columnar ossicles remain imbricated in K1-2. The columnar ossicles immediately under the calyx were flatter with poorly developed radial spicules (P). This individual did not possess an attachment disk.

**Figure S3.** Ossicle diameter in *Florometra serratissima* was greater in cystideans than in the same age doliolariae. The maximum dimension of oral and basal calyx plate ossicles were measured and are shown for doliolariae (\(n_{\text{larvae}} = 16; \ n_{\text{ossicles}} = 115\)), cystideans (\(n_{\text{larvae}} = 15; \ n_{\text{ossicles}} = 124\)) and pentacrinoids (\(n_{\text{larvae}} = 7; \ n_{\text{ossicles}} = 52\)). The time period is days post-fertilization. Some skeletal elements were simple spicules (ossicles lacking stroma). Larvae cultured in the presence and absence of coralline algae were pooled. The coralline algae appeared to increase settlement rate in cultures and likely accelerated ossicle development. It is important to note that for the larvae represented in this graphic, 15 of the 16 doliolariae were cultured without coralline algae and 14 of the 15 cystideans were cultured with coralline algae. This is due to the earlier settlement of larvae in presence of coralline algae and the difficulty to culture older doliolariae.
in presence of the algae. We cannot therefore conclude if the cystideans have significantly larger ossicles due to metamorphosis, presence of algae, or both.
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