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Author(s): Delphine Haesaerts, Michel Jangoux, Patrick Flammang
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Adaptations to Benthic Development: Functional Morphology of the Attachment Complex of the Brachiolaria Larva in the Sea Star *Asterina gibbosa*

DELPHINE HAESAERTS[1], MICHEL JANGOUX[1,2], AND PATRICK FLAMMANG[2,*]

[1] *Université Libre de Bruxelles, Laboratoire de Biologie Marine, Académie universitaire Wallonie-Bruxelles, Brussels, Belgium; and* [2] *Université de Mons-Hainaut, Laboratoire de Biologie Marine, Académie universitaire Wallonie-Bruxelles, Mons, Belgium*

Abstract. The asteroid *Asterina gibbosa* lives all its life in close relation to the sea bottom. Indeed, this sea star possesses an entirely benthic, lecithotrophic development. The embryos adhere to the substratum due to particular properties of their jelly coat, and hatching occurs directly at the brachiolaria stage. Brachiolariae have a hypertrophied, bilobed attachment complex comprising two asymmetrical brachiolar arms and a central adhesive disc. This study aims at describing the ultrastructure of the attachment complex and possible adaptations, at the cellular level, to benthic development. Immediately after hatching, early brachiolariae attach by the arms. All along the anterior side of each arm, the epidermis encloses several cell types, such as secretory cells of two types (A and B), support cells, and sensory cells. Like their equivalents in planktotrophic larvae, type A and B secretory cells are presumably involved in a duo-glandular system in which the former are adhesive and the latter de-adhesive in function. Unlike what is observed in planktotrophic larvae, the sensory cells are unspecialized and presumably not involved in substratum testing. During the larval period, the brachiolar arms progressively increase in size and the adhesive disc becomes more prominent. At the onset of metamorphosis, brachiolariae cement themselves strongly to the substratum with the adhesive disc. The disc contains two main cell types, support cells and secretory cells, the latter being responsible for the cement release. During this metamorphosis, the brachiolar arms regress while post-metamorphic structures grow considerably, especially the tube feet, which take over the role of attachment to the substratum. The end of this period corresponds to the complete regression of the external larval structures, which also coincides with the opening of the mouth. This sequence of stages, each possessing its own adhesive strategy, is common to all asteroid species having a benthic development. In *A. gibbosa,* morphological adaptations to this mode of development include the hypertrophic growth of the attachment complex, its bilobed shape forming an almost completely adhesive sole, and the regression of the sensory equipment.

Introduction

Brachiolaria larvae, which are characterized by a specialized attachment complex comprising three larval arms (brachia) and an adhesive disc, occur in the life cycle of many asteroids whatever their nutritional mode (planktotrophy *versus* lecithotrophy) or their developmental habitat (pelagic, benthic, or intragonadal) (McEdward and Janies, 1993, 1997; Byrne, 1999; McEdward and Miner, 2001; Byrne *et al.,* 2003). Among the different development patterns, pelagic planktotrophy is traditionally considered to be the ancestral one (see, *e.g.,* McEdward and Janies, 1993, 1997). Successive evolutionary transitions would then have occurred to give rise to various types of nonfeeding larval development by (a) loss of larval feeding structures such as ciliated bands and functional gut, and gain of large, yolky eggs, followed by (b) loss of planktonic dispersal, and then (c) gain of parental brood protection. Recently, however, molecular phylogenetic studies conducted on the family Asterinidae (Hart *et al.,* 1997, 2004) showed that ordered transformations between the four modes of development comprising brachiolariae (*i.e.,* planktotrophic-pelagic, leci-

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* To whom correspondence should be addressed. Patrick.Flammang@umh.ac.be

thotrophic-pelagic, lecithotrophic-benthic, and lecithotrophic-intragonadal) could not be easily reconstructed, and that many parallel changes in larval form, habitat, and dispersal potential occurred. For example, in this family, benthic lecithotrophy evolved three times independently, in *Parvulastra* (*Patiriella*) *exigua,* in *Aquilonastra* (*Asterina*) *minor* and in the two sister species, *Asterina gibbosa* and *Asterina phylactica* (Hart *et al.,* 2004; Byrne, 2006). In these species, brachiolariae all possess a hypertrophied attachment complex (Ludwig, 1882; MacBride, 1896; Komatsu *et al.,* 1979; Marthy, 1980; Byrne, 1995, 2006). Larvae of *A. gibbosa* differ, however, from those of *A. minor* and *P. exigua* because their attachment complex develops as a sole-like structure rather than as a tripod-like structure as it is the case in the other two species.

Very little is known about the fine structure and functioning of the attachment complex in asteroids with benthic larvae; most ultrastructural and immunocytochemical information on this organ comes from planktotrophic larvae. In planktotrophic developers, the brachiolar arms provide temporary attachment to and sensory testing of the substratum during settlement, whereas the adhesive disc is involved in the permanent attachment that marks the onset of metamorphosis (Barker, 1977; Gondolf, 2000; Haesaerts *et al.,* 2003). In planktonic larvae, brachiolar arms are tipped with several sensory-secretory papillae, in which both the epidermis and the underlying nerve plexus are greatly thickened. The papillary epidermis encompasses support cells, serotonergic sensory cells, and secretory cells (Barker, 1978; Byrne *et al.,* 2001; Haesaerts *et al.,* 2005a). The latter function as a duo-glandular system with some cells acting as adhesive cells and some others as de-adhesive cells (Hermans, 1983; Flammang, 1996; Haesaerts *et al.,* 2005a). As for the adhesive disc, it is a rounded, concave structure lying between the brachiolar arms. It is made up of support cells, ciliated secretory cells, and neurons (Barker, 1978; Gondolf, 2000; Byrne *et al.,* 2001; Haesaerts *et al.,* 2005a). In benthic developers, on the other hand, hypertrophied growth of the brachiolar arms has been described as an adaptative character to benthic habitat, but it is not known if other adaptations occur at the cellular level.

The present work describes the morphogenesis and ultrastructure of the larval attachment complex in *Asterina gibbosa.* This common European asteroid produces large, yolky eggs that are laid in masses attached to the substratum through their jelly coat (Marthy, 1980; Crump and Emson, 1983). Eggs develop directly into lecithotrophic brachiolariae that, from hatching until metamorphosis, adhere to the seafloor by means of a hypertrophied attachment complex (Ludwig, 1882; MacBride, 1896; Marthy, 1980). This attachment complex, which has been described as bilobed or sole-like, appears to be unique among asteroid brachiolariae (Byrne, 2006). In this study, particular attention is paid to the different cell types that constitute the attachment complex of *A. gibbosa* and to possible adaptations, at the cellular level, to benthic development.

Material and Methods

Larval rearing

Specimens of *Asterina gibbosa* (Pennant, 1777) were collected intertidally at Roscoff (Brittany, France) in 2001, 2002, and 2003 during spring tides. They were kept in marine aquaria (14 °C, 33 psu) at the marine biology laboratory of the University of Mons-Hainaut. Each year, at the end of April, groups of about 60 individuals were transferred into a 10-l tank filled with unfiltered aerated seawater at room temperature. When maintained in such conditions, individuals spawned spontaneously after a few hours to a few days, depending on the group. Fertilized eggs were collected the day they were laid and transferred into large petri dishes at 14 °C and 21 °C. Dishes were filled with filtered seawater (0.22 μm). Water was changed daily, and the larvae were maintained to the juvenile stage.

Morphological observations

Specimens of *A. gibbosa* were observed and photographed *in vivo* with a Leica MZ8 binocular microscope equipped with a Nikon Coolpix digital camera. For scanning electron microscopy (SEM), larvae were fixed in Bouin's fluid for 12 h, dehydrated in a graded ethanol series, dried by the critical point method (with CO_2 as transition fluid), mounted on aluminum stubs, coated with gold in a sputter coater, and observed with a JEOL JSM-6100 scanning electron microscope. Images were digitized with the SEMafore 3.0 Pro software (JEOL, Tokyo, Japan). Some additional larvae were fixed in glutaraldehyde and postfixed in osmium tetroxide (see below), a method that does not preserve the cuticle and thus reveals the underlying structures (Ameye *et al.,* 2000).

For light microscopy (LM) and transmission electron microscopy (TEM), specimens were fixed in 3% glutaraldehyde in cacodylate buffer (0.1 mol l^{-1}, pH 7.8, adjusted to an osmolality of 1030 mOsmol kg^{-1} with NaCl) for 30 min at 4 °C, rinsed in cacodylate buffer, and postfixed for 1 h in 1% osmium tetroxide in the same buffer. After a final buffer wash, they were dehydrated in a graded ethanol series and embedded in Spurr's resin. For LM analysis, semithin sections (1-μm) were cut with a Reichert OmU2 ultramicrotome equipped with a glass knife, stained with an equivolumic mixture of 1% Azur II and 1% methylene blue solutions, and observed and photographed with a Leitz Orthoplan light microscope equipped with a Leica DC 300F digital camera. For TEM analysis, ultrathin sections (70–80 nm) were cut with a Leica UCT ultramicrotome equipped with a diamond knife. Sections were contrasted with uranyl acetate and lead citrate and observed and photographed with

Table 1

Asterina gibbosa: *chronology of a lecithotrophic development*

Event	Stage	Length		Figure
		At 14°C	At 21°C	
Embryonic life	Zygote	0 h	0 h	1A
	8-cell stage	6 h	4 h	NI
	64-cell stage	24 h	8 h	NI
	Early blastula (wrinkled stage)	32 h	24 h	NI
	Late blastula	48 h	—[1]	NI
	Early gastrula	3 days	36 h	1B
	Late gastrula	4–5 days	48 h	1C
	Pre-hatching larva	7 days	3–4 days	1D
Larval life	Early brachiolaria (hatching larva)	9 days	4 days	1E, F
	Late brachiolaria	10–11 days	5 days	1G, 2A, 2C
	Competent brachiolaria	14 days	6–7 days	1H, 2D
Metamorphosis	Metamorphic individual	15 days	8 days	1I, J, K; 2E, F, G
Juvenile life	Exotrophic individual	—[2]	17 days	1L, 2H

NI indicates that the stage is not illustrated.

[1] Not recorded: at this temperature some stages were too short to be observed.

[2] Not reached: rearing was interrupted.

a Zeiss LEO 906 E transmission electron microscope equipped with a digital camera (Keen View Slow Scan CCD).

All morphological measurements are given as mean ± standard deviation.

Results

Brief survey on the development of Asterina gibbosa

The sea star *Asterina gibbosa* has a benthic lecithotrophic development whose chronology is outlined in Table 1. Fertilized eggs are bright orange with a diameter of 536 ± 47 μm ($n = 10$; Fig. 1A). They stick onto the substratum with an adhesive jelly coat. Early blastulae are highly wrinkled; late blastulae appear to be smoother. Early gastrulae show an enlarged blastopore whose aperture progressively reduces in older gastrulae (compare Fig. 1B and 1C). A few days later, the blastopore closes and a posterior annular groove appears on the early pre-hatching larva, delimiting the future anterior and posterior parts of the larva (Fig. 1D). Hatching occurs after four (21 °C) to nine (14 °C) days (Fig. 1E).

Newly hatched brachiolariae immediately attach to the substratum with their two asymmetrical and already functional brachiolar arms (Fig. 1F). From the tip of the longest arm to the posterior end of the larval body, early brachiolaria larvae have a length of about 600 μm (615 ± 62 μm; $n = 9$), which increases up to about 950 μm for late brachiolariae (944 ± 111 μm; $n = 13$). For late larvae, the longer brachiolar arm measures about 470 μm in length (473 ± 93 μm; $n = 5$) and the shorter one about 340 μm (337 ± 76 μm; $n = 5$); the ratio of larval body length to longer brachiolar arm length is about 2:1 (Figs. 1G, 2A). The hydropore is located dorsally at the base of the longer arm; it is a small orifice of about 10 μm in diameter. Brachiolariae are uniformly ciliated, without recognizable ciliated tracts. They are sedentary but not sessile: they can move over short distances (a few centimeters), either by alternatively attaching and detaching their arms or by swimming using their cilia.

Competent larvae have a prominent adhesive disc located between the two brachiolar arms, with the disc and the arms together forming the larval attachment complex (Figs. 1H, 2D). When fully developed, the disc has a mean diameter of 165 ± 21 μm ($n = 6$; Figs. 2D, 3D). Competent brachiolariae display a slight leftward flexion that coincides with appearance of the five hydrocoel lobes in the rudiment on the left hand side of the larva (Figs. 1H, 2C, 2D). The rudiment in a competent larva consists of five quintets of protuberances corresponding to the future tube feet and terminal tentacles of the juvenile (Fig. 2D). After 8 to 15 days, depending on the temperature, a larva enters metamorphosis by cementing itself to the substratum with its adhesive disc (Figs. 1I, 1J, 2E).

Metamorphosis lasts for at least 5 days (Table 1) during which the attachment complex progressively resorbs while the rudiment gains a sea-star shape (Figs. 1I, 1J, 2E, 2F). During that period, tube feet become functional and exert a

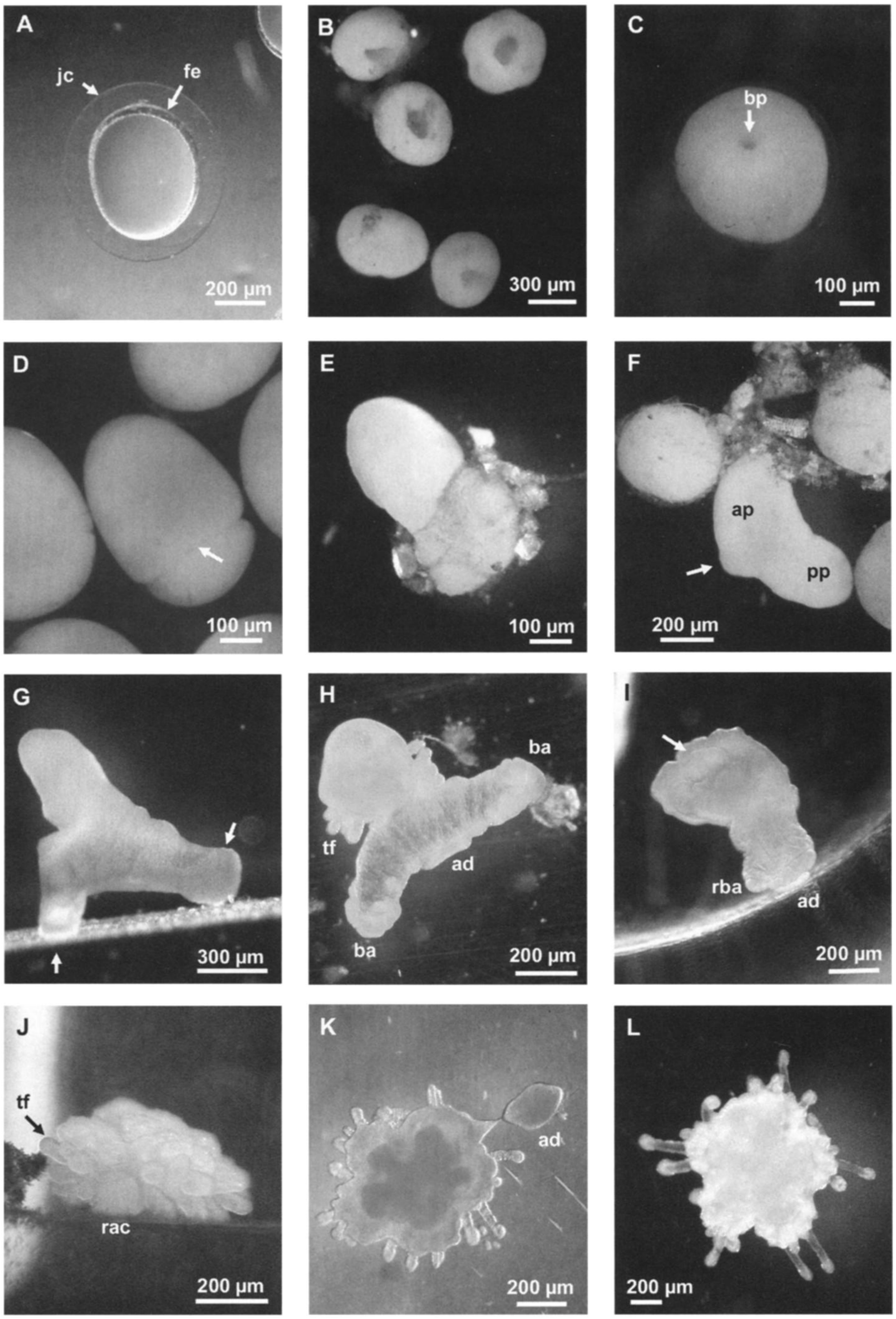

Figure 1. Light microscopy of the development of live specimens of *Asterina gibbosa.* (**A**) One-cell stage surrounded by the jelly coat (jc): fe, fertilization envelope. (**B**) Early gastrulae with a large blastopore. (**C**) Gastrulae with a reduced blastopore (bp). (**D**) Pre-hatching larva marked by a groove separating the future anterior and posterior parts (arrow). (**E**) Hatching larva, with particles still attached to the jelly coat. (**F**) Early brachiolaria with a truncated shape, attached to other embryos and particles; shorter brachiolar arm (arrow): ap, anterior part; pp, posterior part. (**G**) Brachiolaria with well-developed brachiolar arms (arrows); anterior part translucent while posterior part remains opaque. (**H**) Competent brachiolaria with a fully developed attachment complex (arms and disc) and developing tube feet on the left side of the larva: ad, adhesive disc; ba, brachiolar arms; tf, tube feet. (**I**) Early metamorphic larva (arrow indicates developing tube feet): ad, adhesive disc; rba, resorbing brachiolar arm. (**J**) Metamorphic larva: rac, resorbing attachment complex; tf, tube feet. (**K**) Metamorphic larva still attached to the substratum by the adhesive disc (ad). (**L**) Post-metamorphic individual.

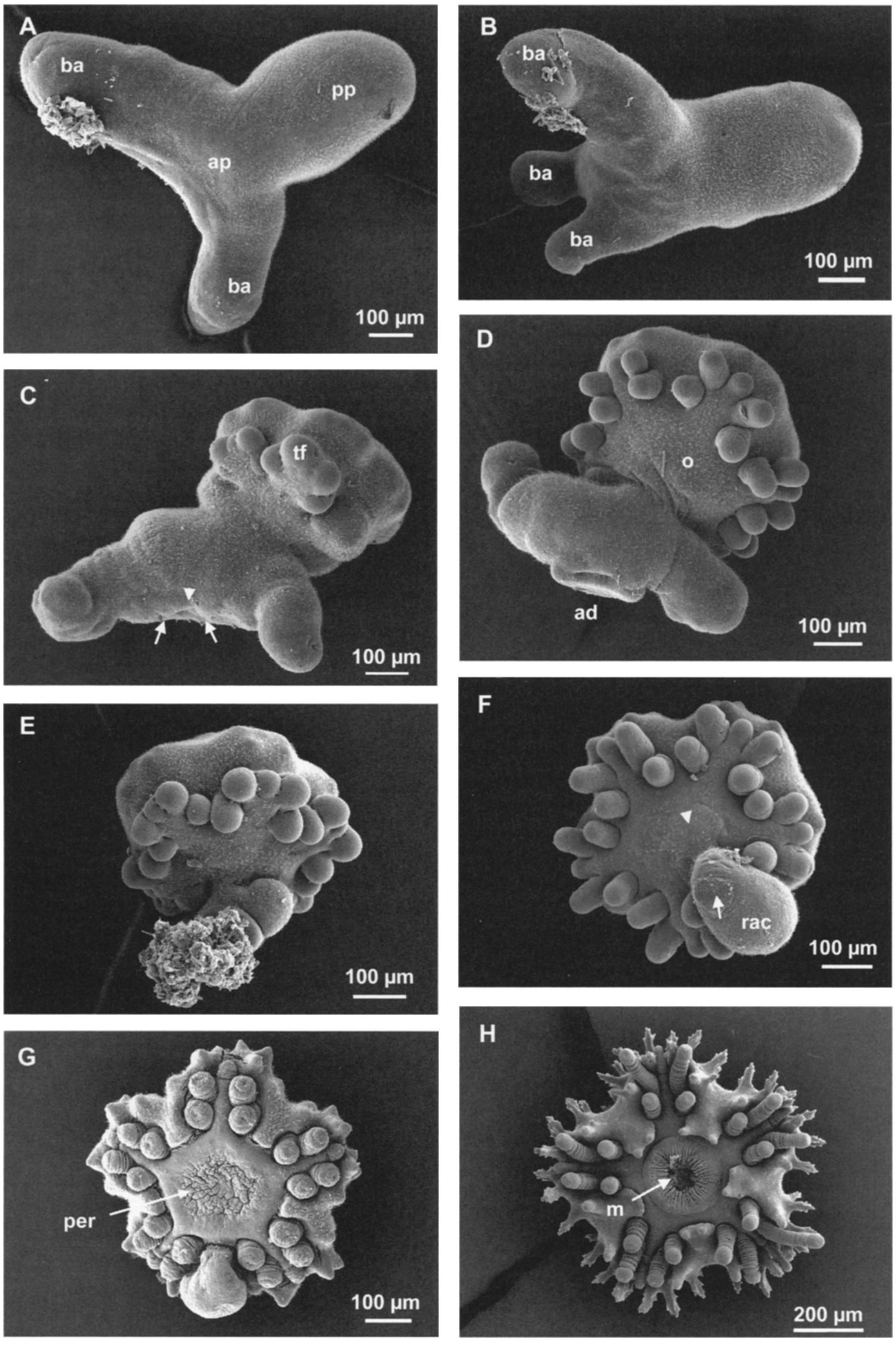

Figure 2. Scanning electron microscopy of the development of *Asterina gibbosa* during its perimetamorphic period. (**A**) Left side of a 2-arm brachiolaria (regular larval form) showing the hypertrophy of the brachiolar arms (ba): ap, anterior part; pp, posterior part. (**B**) View of a 3-arm brachiolaria (exceptional larval form). (**C**) Left side of a late brachiolaria. Arrowhead indicates the location of the disc, and arrows show the epidermal bulges around the disc: tf, tube feet. (**D**) Left side of a competent larva: ad, adhesive disc; o, oral side of the future adult. (**E**) Metamorphic larva attached by the disc to particles, brachiolar arms regressing. (**F**) Early metamorphic larva with a detached resorbing attachment complex (rac) showing the location of the adhesive disc (arrow). Arrowhead indicates the location of the mouth of the future adult. (**G**) Metamorphic larva with a resorbing attachment complex, functional tube feet, and a peristomium (per). (**H**) Juvenile with opened mouth (m).

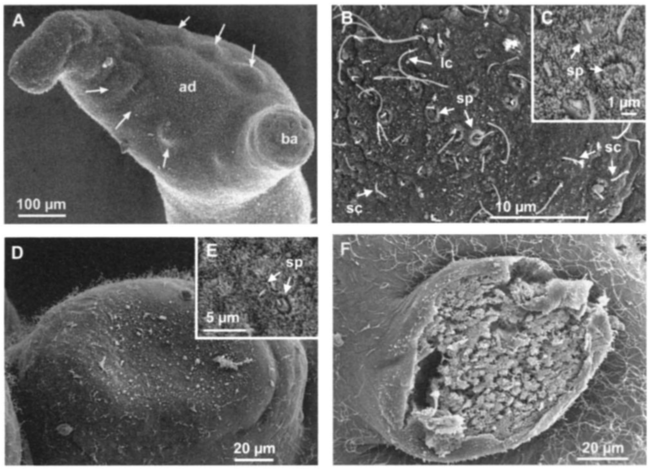

Figure 3. Scanning electron microscopy of the larval attachment complex of *Asterina gibbosa*. (**A**) Frontal view of the attachment complex. Arrows indicate the bulges along it: ba, brachiolar arm; ad, adhesive disc. (**B**) Detail of the secretory area of the brachiolar arms: lc, long cilium; sc, short cilium; sp, secretory pore. (**C**) Detail of the secretory pores (sp). (**D**) Detail of the adhesive disc showing cilia and short protuberances. (**E**) Detail of the secretory pores of the disc (sp). (**F**) Detail of the ruptured stalk of a regressing attachment complex.

pulling action on the attachment complex, which progressively transforms into a narrow stalk (Fig. 1K). In most individuals, the stalk finally breaks, leaving the disc cemented to the substratum. Freshly detached metamorphic individuals measure about 600 μm in diameter, and remnants of the attachment complex are still visible at the periphery of the oral surface (Fig. 2G, 3F). Acquisition of the mouth appears to coincide with the end of the resorption of the external larval structures, which marks the end of metamorphosis (Fig. 2F). Early juveniles measure about 1 mm in diameter (Fig. 1L). In our rearing conditions, these juveniles come up to the water-air interface, where they float upside down. This behavior lasts a few days, after which they re-adhere to the substratum owing to the adhesive properties of their tube feet and continue to grow.

External morphology of the attachment complex

The attachment complex roughly corresponds to the anterior part of the larval body. Most larvae have two brachiolar arms, but a few, representing less than 1% of the observed individuals, show three arms (Fig. 2B). Such larvae have one longer arm and two equal shorter arms. Brachiolar arm tips are covered by secretory patches characterized by secretory pores (Fig. 3B, C). Each pore is surrounded by a ring of microvilli from which protrudes a rather short cilium (2.1 ± 0.5 μm in length; $n = 4$). Pores with a cilium are irregularly interspersed between solitary cilia, which are either long and twisted (5.5 ± 0.8 μm in length; $n = 9$) or short and straight (2.4 ± 0.6 μm in length; $n = 4$) (Fig. 3B). Similar long and short solitary cilia are present over the larval body. The adhesive disc is covered by short cilia and small cuticular protuberances (Fig. 3D). A few pores with short cilia were also observed on the disc surface (arrows, Fig. 3E). The disc is surrounded by small bulges, which also occur all along the inner surface of the brachiolar arms (arrows, Figs. 2C, 3A). These bulges, like the secretory patches on the arm tips, are made up of pores, each with a cilium, intermingled with short and long solitary cilia.

Fine structure of the attachment complex

The wall of the attachment complex is made up of four tissue layers: an inner myomesothelium, a connective tissue layer, a subepidermal nerve plexus, and an outer epidermis covered by a cuticle (Figs. 4A, 5A). Spherical to ovoid yolk granules (up to 4 μm in diameter) were seen in most constitutive cells (Figs 4B, C, D and 5C, G, H). The mesothelium is made of myoepithelial cells and ciliated adluminal cells. The connective tissue layer is composed of an extracellular matrix containing a few collagen fibers and many mesenchymatous cells (Figs. 4A, 5A). The nerve plexus forms a thin sheath beneath the epidermis and is made up of a meshwork of neurites containing clear or dense-core vesicles (Figs. 4F, 5D, 5E).

Cells forming the monostratified epidermis of brachiolar arms are connected apically by junctional complexes made up of a distal zonula adherens and a proximal septate junction (Fig. 4B). Six cell types were identified at the arm tip: two types of secretory cells (A and B), cells with rod-shaped granules, support cells, vacuolated cells, and sensory cells. Type A secretory cells are narrow and present a scattered distribution (Fig. 4B, C). They are filled with ellipsoidal membrane-bound granules (1.2 ± 0.1 μm long by 0.8 ± 0.1 μm wide; $n = 6$) containing a homogeneous electron-dense material. These granules are easily distinguished from the yolk granules by their smaller size and higher electron density. They are expelled through an apical pore delimited by a ring of microvilli (Fig. 4B, C). Type A secretory cells bear a short cilium that protrudes through the apical secretory pore (Fig. 4C). These cilia correspond to the pore-associated cilia observed on scanning electron micrographs. Type B secretory cells are characterized by the occurrence of numerous small, dense-core spherical granules (0.24 ± 0.05 μm in diameter; $n = 24$) in their cytoplasm (Fig. 4B, D). They are not ciliated and are few in number. Cells with rod-shaped granules are more frequent (Fig. 4C). These are narrow cells with electron-dense rod-shaped granules measuring 0.45 ± 0.07 μm by 0.25 ± 0.03 μm ($n = 6$). Support cells are ciliated, contain vesicles of variable size and electron density, and are crossed by a narrow bundle of filaments connecting the basal and apical parts of the cell (Fig. 4G). Vacuolated cells are large, and their vacuoles are filled with loose, amorphous electron-lucent material. Sensory cells have electron-dense cyto-

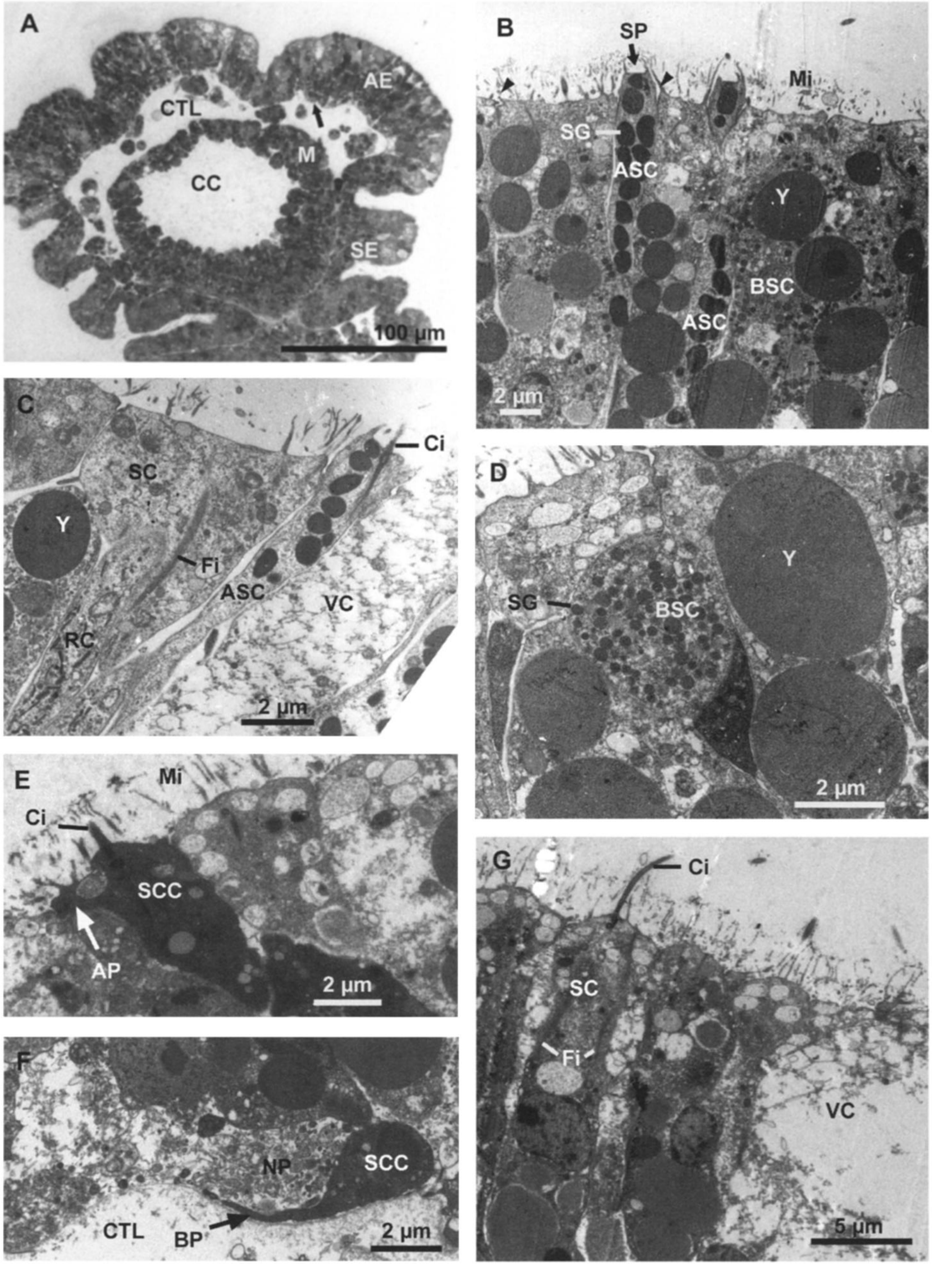

Figure 4. Fine structure of the brachiolar arms of the competent larva of *Asterina gibbosa* (light microscopy [LM] and transmission electron microscopy [TEM]). (**A**) Longitudinal section in a brachiolar arm (LM): AE, adhesive epidermis; CC, coelomic cavity; CTL, connective tissue layer; M, mesothelium; SE, stem epidermis. Arrow indicates the presence of the nerve plexus. (**B, C**) Section through a sensory secretory area in the arm (TEM): ASC, type A secretory cell; BSC, type B secretory cell; Ci, cilium; SC, support cell; Fi, fibers; Mi, microvilli; RC, cell containing rod-shaped granules; SG, secretory granule; SP, secretory pore; VC, vacuolated cell; Y, yolk granule. Arrowheads indicate the presence of zonula adherens. (**D**) Detail of a type B secretory cell (TEM): BSC, type B secretory cell, SG, secretory granule; Y, yolk granule. (**E**) Detail of the apical part of a sensory ciliated cell (TEM): AP, apical process; Ci, cilium; Mi, microvilli; SCC, sensory ciliated cell. (**F**) Section through the nerve plexus and the basal part of a sensory ciliated cell (TEM): BP, basal process; CTL, connective tissue layer; NP, nerve plexus; SCC, sensory ciliated cell. (**G**) Detail of the stem epidermis (TEM): Ci, cilium; Fi, fiber; SC, support cell; VC, vacuolated cell.

plasm with small vesicles of light-to-medium electron density (Fig. 4E). These are ciliated cells whose apical part develops processes that extend laterally over the neighboring cells (Fig. 4E), and whose basal part intimately contacts the underlying nerve plexus (Fig. 4F).

The epidermis of the bulges observed on the inner surface

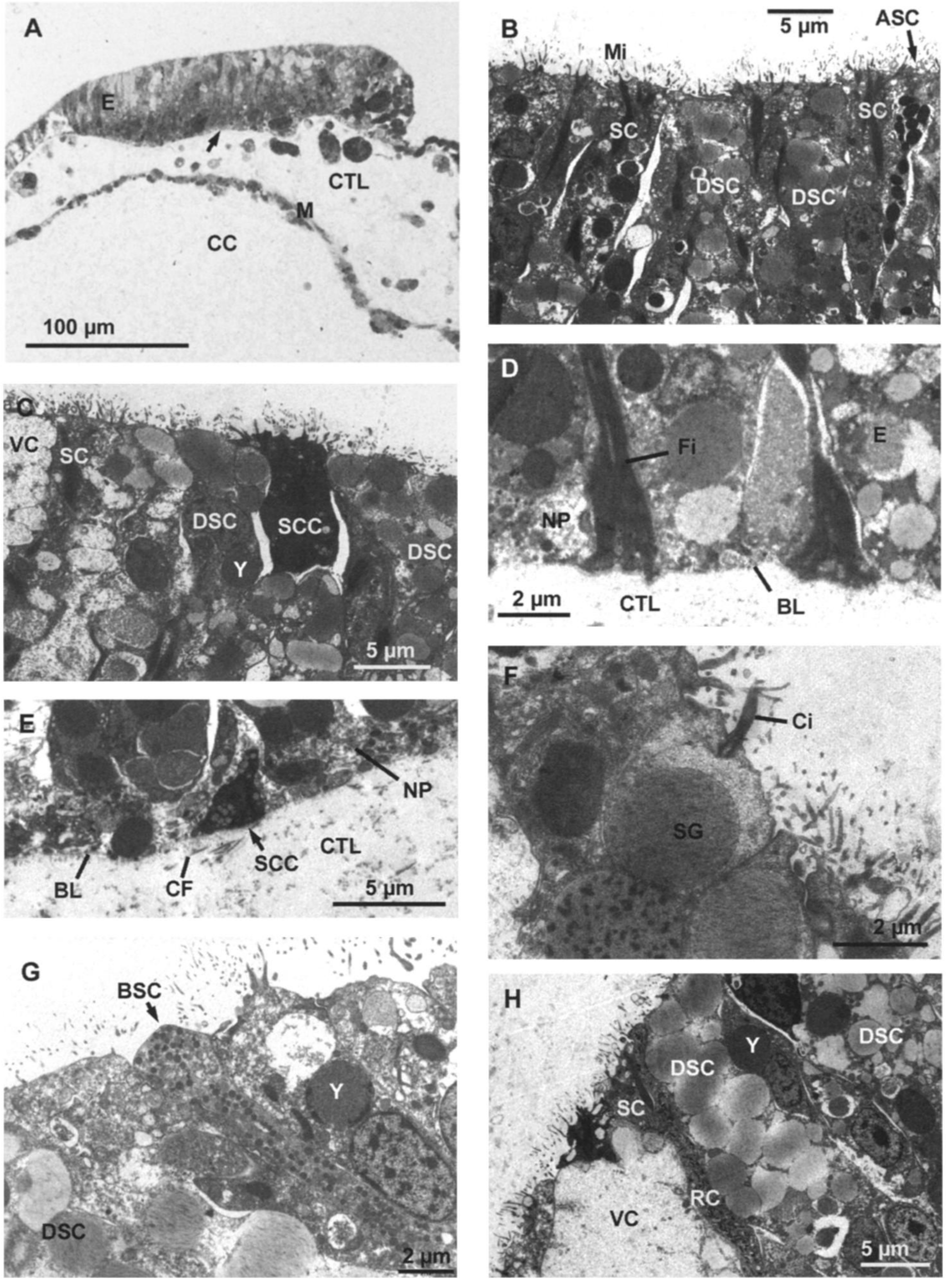

Figure 5. Fine structure of the disc of the competent brachiolaria of *Asterina gibbosa* (light microscopy [LM] and transmission electron microscopy [TEM]). (**A**) Longitudinal section through the disc (LM): CC, coelomic cavity; CTL, connective tissue layer; E, epidermis; M, mesothelium. Arrow indicates the nerve plexus. (**B**, **C**) Longitudinal sections through the disc epidermis (TEM): ASC, type A secretory cell; DSC, disc secretory cell; Mi, microvilli; SC, disc support cell; SCC, sensory ciliated cell; VC, vacuolated cell; Y, yolk granule. (**D**) Detail (TEM) of the basal epidermis (E) showing the thin subepidermal nerve plexus (NP) and fibers (Fi) from support cells that anchor to the basal lamina (BL): CTL, connective tissue layer. (**E**) Detail (TEM) of the subepidermal nerve plexus (NP) and a sensory ciliated cell (SCC): BL, basal lamina; CF, collagen fiber; CTL, connective tissue layer. (**F**) Detail of a secretory granule (SG) from a disc secretory cell (TEM): Ci, cilium. (**G**) Detail (TEM) of a type B secretory cell (BSC): DSC, disc secretory cell; Y, yolk granule. (**H**) Longitudinal section through the disc epidermis (TEM): DSC, disc secretory cell; RC, cell containing rod-shaped granules; SC, disc support cell; VC, vacuolated cell; Y, yolk granule.

of arms and around the disc has the same structure as that of the arm tip. The rest of the arm epidermis (stem epidermis) consists only of support cells, vacuolated cells, and sensory cells. Sensory cells are, however, more numerous in the vicinity of the arm secretory cells.

At the level of the adhesive disc, the epidermis is made up of two main cell types that cover most of the disc surface: disc secretory cells (type D cells) and disc support cells (Fig. 5B, C, H). Disc secretory cells bear a short cilium and house numerous large spherical membrane-bound granules (2.8 ± 0.6 μm; $n = 16$) whose fibrous content has a woven appearance and is of medium electron density (Fig. 5F, G). Disc support cells contain a conspicuous bundle of filaments up to 2 μm in diameter running from the base to the apex of the cell (Fig. 5B, D). These bundles are much thicker than those in the support cells of the arms. Other cell types (such as type A and type B secretory cells and sensory cells) also occur in the disc epidermis, though much less frequently than type D cells and support cells.

Discussion

The attachment complex of benthic brachiolaria larvae

The attachment complex of *Asterina gibbosa* has a typical bilobed shape due to its two asymmetrically sized brachiolar arms. This unique organization differs from the typical conformation of the complex consisting of a long anterior and two shorter lateral brachiolar arms surrounding the adhesive disc, as it occurs in planktotrophic brachiolariae such as those of *Coscinasterias calamaria* and *Stichaster australis* (Barker, 1978), *Patiriella regularis* (Byrne and Barker, 1991), and *Asterias rubens* (Gondolf, 2000; Haesaerts *et al.,* 2005a). This arrangement also differs from that of the triradiate tripod complex of other benthic developers, in which the three arms are equally developed (Byrne, 1995). However, this bilobed shape seems to have evolved from an ancestral larval type with three brachiolar arms; indeed, we observed a typical three-arm attachment complex in a few larvae. This phenomenon was recurrent in our rearing conditions and was already pointed out by Luwig (1882) and McBride (1898), who compared the "occasional bifurcation" of the smallest lobe with the three-arm attachment complex of other asteroids. It can be hypothesized, therefore, that this smallest lobe resulted from fusion of the two lateral arms present in the ancestral-type brachiolaria. Yet there is no indication whether the immediate ancestor was a benthic or a planktonic lecithotrophic brachiolaria. A benthic tripod form seems unlikely, as it would require reversal from equally sized to unequally sized arms. The evolution of a bilobed attachment complex thus seems specific to larvae of the Atlantic *Asterina,* which interestingly were found by molecular methods to be sister to all Pacific asterinids possessing tripod larvae (Hart *et al.,* 1997, 2004; Byrne, 2006).

In addition to its peculiar shape, the attachment complex of *A. gibbosa* is also very large and occupies all the anterior part of the larva. This is a characteristic shared by other benthic developers—for example, *Aquilonastra* [*Asterina*] *minor* (Komatsu *et al.,* 1979); *Parvulastra* [*Patiriella*] *exigua* (Byrne, 1995); and *Leptasterias ochotensis similispinis* (Kubo, 1951). Generally, the brachiolar arms are well-developed or hypertrophied, while the diameter of the disc does not exceed the range of size found in planktotrophic developers (Haesaerts *et al.,* 2005a).

Although the brachiolar arms of *A. gibbosa* do not possess the well-defined domelike papillae characteristic of the arms of planktotrophic larvae (Barker, 1978; Byrne and Barker, 1991; Haesaerts *et al.,* 2005a), they have, however, large secretory areas specialized in temporary attachment. These areas, which occur not only at the tip of the arms but also all along the anterior surface of the attachment complex, are characterized by the presence of secretory cells named types A and B. These cells are also scattered in the adhesive disc epidermis. Goto (1898) already described the occurrence of unicellular glands all over the anterior surface of the larval attachment complex of *A. gibbosa,* including the adhesive disc. According to his description, these are likely to correspond to type A secretory cells. Type A and type B cells share many morphological similarities with their equivalents in the brachiolar arms of the planktotrophic larvae where secretory cells form a duo-gland adhesive system that is responsible for temporary adhesion (Flammang, 1996; Haesaerts *et al.,* 2005a). Concerning type A cells (adhesive cells), the morphology of their secretory pores with a short cilium, and the ultrastructure of their secretory granules (contents, size and shape) remain remarkably constant from one species to another. In other benthic developers, the presence of such cells can easily be inferred from their characteristic pores with associated cilium at the surface of the brachiolar arms (see, *e.g., P. exigua;* Byrne *et al.,* 2001). In pelagic developers, the secretion released by type A cells is muccopolysaccharidic and cross-reacts with antibodies raised against the temporary adhesive of the tube feet (Haesaerts *et al.,* 2005a). On the other hand, type B cells are typical echinoderm de-adhesive cells (Flammang, 1996). The occurrence of a duo-gland adhesive system in the larval arms of benthic developers is also confirmed by their behavior. Indeed, in *A. gibbosa,* larvae are able to move over short distances by repeatedly attaching and detaching their arms, as do the tripod larvae of *P. exigua* (Byrne, 1995). The occurrence of type A and B secretory cells in the adhesive disc epidermis indicates that this structure is involved not only in permanent attachment but also in temporary attachment before metamorphosis, an ability already observed by MacBride (1896). The very wide distribution of type A and type B cells over the brachiolar arms and the adhesive disc increases the adhesive surface area in contact with the substratum. The resulting sole-like attachment presumably provides more efficient adhesion for the organism, thus reducing the possibility that the larva will be dislodged by water motion before metamorphosis. The larvae of *A. gibbosa,* like other benthic brachiolariae (Byrne, 1995), spend 4 to 6 days attached temporarily by the brachiolar arms, contrary to planktotrophic brachiolariae that remain in this phase of temporary attachment for not more than one hour, after which they either resume swimming or attach by the disc and metamorphose (Barker, 1977).

The main function of the disc is to cement the larva to the substratum during metamorphosis. The disc secretory cells (type D cells) dominate in the disc epidermis and are responsible for permanent attachment. Ultrastructurally the secretory granules of the type D cells are remarkably similar to those of asteroids with planktotrophic larvae (Barker, 1978; Haesaerts *et al.,* 2005a), suggesting that the secretion of type D cells is probably proteinaceous (Barker, 1978).

In *A. gibbosa,* the sensory cells observed in the secretory areas of the brachiolar arms and in the adhesive disc were identical to those distributed all over the larval epidermis. This is different from what is seen in planktotrophic brachiolariae, in which a unique type of serotonergic sensory cell, presumably involved in the detection of cues associated with the substratum, appears to be restricted to the papillae of the brachiolar arms (Barker, 1978; Chee and Byrne, 1999; Haesaerts *et al.,* 2005a). Once a suitable substratum is found, the stimulation of these specialized cells presumably triggers the behavioral sequence leading to larval fixation and metamorphosis (Barker, 1978; Haesaerts *et al.,* 2005a). In *A. gibbosa,* the sensory cells of the attachment complex differ in ultrastructure from the ones found in the papillae of planktotrophic larvae. Moreover, their homogeneous distribution over the entire larval body suggests that they are unrelated to settlement or metamorphosis, which is not surprising since larvae of this species move only short distances; in fact, they develop and metamorphose on the same spot where the fertilized eggs are laid by the adults, making the search for a suitable metamorphosis site unnecessary. The loss of the sensory cells required for surface testing is significant from the evolutionary point of view. Indeed, McEdward and Janies (1997) proposed that, contrary to the evolutionary transition from feeding to nonfeeding development which would be irreversible because it involves marked changes in larval morphology, the transition between pelagic and benthic development would be an ecological change independent of changes in morphogenesis and thus should be reversible. Regression of the sensory equipment in *A. gibbosa* suggests that reversal from benthic lecithotrophy to pelagic lecithotrophy would probably be more difficult than previously thought.

Regression of sensory abilities does not mean regression of the nervous system altogether and, in *A. gibbosa,* the nerve plexus associated with the attachment complex is well developed, as is the case in other benthic larvae (Byrne *et al.,* 2001). In the larvae of *P. exigua,* for example, an extensive peptidergic nerve plexus was demonstrated in the attachment complex, especially at the level of the adhesive disc (Byrne *et al.,* 2001).

Characteristics of asteroid benthic development

During its ontogeny, *A. gibbosa* keeps an almost uninterrupted relation with the substratum on which it lives. We have discerned four benthic developmental stages, each relying on particular adhesive structures or organs: (1) the embryonic stage, during which the embryos are attached to the substratum in clusters by their jelly coat; (2) the brachiolaria stage, when the brachiolar arms provide temporary adhesion; (3) the metamorphic stage, which corresponds to the fixation by the adhesive disc; and (4) the post-metamorphic stage, when the first tube feet take over the role of attachment to the substratum, while enabling locomotion. This sequence of stages, each possessing its own adhesive strategy, seems to be characteristic of all asteroid species having a benthic development (Komatsu *et al.,* 1979; Byrne, 1995). In *A. gibbosa,* a turbulent channel flow apparatus has been used to evaluate the attachment strength of three of these developmental stages (Haesaerts *et al.,* 2005b). The results showed that the flows needed to dislodge brachiolariae attached by the arms and postmetamorphic individuals attached by the tube feet are similar and much lower than the flow needed to detach metamorphic individuals attached by the disc. The larval fixation is considered to be a permanent attachment because the disc remains cemented when the organism detaches and assumes a free benthic life. Such a feature is also observed in planktotrophic larvae (Gemmill, 1914; Strathmann, 1978; Barker, 1978).

The attachment complex of *A. gibbosa* presents some characteristics typical of the benthic mode of development, but it also has some unique characteristics. The hypertrophic development of the complex is a feature that evolved independently several times in phylogenetically distant asteroid lineages that have benthic development, while the bilobed shape seems to have evolved only once, in the genus *Asterina* (Kubo, 1951; Komatsu *et al.,* 1979; Byrne, 1995; 2006). At the ultrastructural level, the wide distribution of the secretory cells involved in temporary attachment to form an almost complete adhesive sole at the anterior surface of the attachment complex (see above) is presumably correlated to this bilobed shape. The lack of sensory cells involved in the detection of settlement-associated cues, on the other hand, is described for the first time in a benthic developer. More ultrastructural data from species with tripod larvae are needed to determine whether this feature has also evolved in other benthic developers.

Another common feature of the benthic development pattern is the floating behavior of juveniles. In our rearing conditions, we observed this behavior a few days after metamorphosis when the juveniles went to the water surface upside down. Such behavior was previously observed in other asteroid species with benthic development—*i.e.* in *Asterina phylactica, A. minor, P. exigua,* and *Patiriella pseudoexigua*—and suggested to be a means of dispersal (Marthy, 1980; Soliman and Nojima, 1984; Byrne, 1995; Chen and Chen, 1992). This juvenile rafting is likely to be an important mechanism for enhancing gene flow in species having an entirely benthic development (Byrne, 1995; Waters and Roy, 2004).

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Literature Cited

Ameye, L., R. Hermann, Ph. Dubois, and P. Flammang. 2000. Ultrastructure of the echinoderm cuticle after fast-freezing/freeze substitution and conventional chemical fixations. *Microsc. Res. Tech.* **48:** 385-393.

Barker, M. F. 1977. Observations on the settlement of the brachiolaria larvae of *Stichaster australis* (Verrill) and *Coscinasterias calamaria* (Gray) (Echinodermata: Asteroidea) in the laboratory and on the shore. *J. Exp. Mar Biol. Ecol.* **30:** 95–108.

Barker, M. F. 1978. Structure of the organs of attachment of brachiolaria larvae of *Stichaster australis* (Verrill) and *Coscinasterias calamaria* (Gray) (Echinodermata. Asteroidea) *J. Exp Mar Biol. Ecol* **33:** 1–36.

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.

Byrne, M. 1999. Echinodermata. Pp. 940–954 in *Encyclopedia of Reproduction,* J. Knobil and J Neill eds. Academic Press, New York

Byrne, M. 2006. Life history diversity and evolution in the Asterinidae. *Integr. Comp. Biol.* **46:** 243–254

Byrne, M., and M. F. Barker, 1991. Embryogenesis and larval development of the asteroid *Patiriella regularis* viewed by light and scanning electron microscopy *Biol Bull.* **180:** 332–345.

Byrne, M., P. Cisternas, and D. Koop. 2001. Evolution of larval form in the sea star genus *Patiriella:* conservation and change in the larval nervous system *Dev. Growth Differ.* **43:** 459–468

Byrne, M., M. W. Hart, A. Cerra, and P. Cisternas. 2003. Reproduction and larval morphology of broadcasting and viviparous species in the *Cryptasterina* species complex *Biol. Bull.* **205:** 285–294

Chee, F., and M. Byrne. 1999. Serotonin-like immunoreactivity in the brachiolaria larvae of *Patiriella regularis. Invertebr. Reprod. Dev.* **36:** 111-115.

Chen, B. Y., and C. P. Chen. 1992. Reproductive cycle, larval development, juvenile growth and population dynamics of biology of *Patiriella pseudoexigua* (Echinodermata: Asteroidea) in Taiwan. *Mar. Biol.* **113:** 271-280.

Crump, R. G., and R. H. Emson. 1983. The natural history, life history and ecology of the two British species of *Asterina. Field Stud.* **5:** 867–882.

Flammang, P. 1996. Adhesion in echinoderms. Pp 1–60 in *Echinoderm Studies,* Vol 5, M Jangoux and J. M Lawrence, eds. Balkema, Rotterdam.

Gemmill, J. F. 1914. The development and certain points in the adult structure of the starfish *Asterias rubens,* L. *Philos. Trans. R. Soc. Lond. B* **205:** 213–294.

Gondolf, A. L. 2000. Light and scanning electron microscopic observations on the developmental biology of the common starfish *Asterias rubens* Linné (Echinodermata: Asteroidea). *Ophelia* **52:** 153–170

Goto, S. 1898. Some points in the metamorphosis of *Asterina gibbosa. J. Sci. Coll. Imp. Univ. Tokyo.* Vol XII, Pt III.

Haesaerts, D., M. Jangoux, and P. Flammang. 2003. Study of the perimetamorphic period of the sea star *Asterias rubens* by scanning electron microscopy. Pp. 155–159 in *Echinoderm Research 2001,* J.-P. Féral and B David, eds. Swets & Zeitlinger, Lisse.

Haesaerts, D., M. Jangoux, and P. Flammang. 2005a. The attachment complex of the brachiolaria larvae of the starfish *Asterias rubens* L. (Echinodermata): an ultrastructural and immunocytochemical study. *Zoomorphology* **124:** 67–78.

Haesaerts, D., J. A. Finlay, M. E. Callow, J. A. Callow, Ph. Grosjean, M. Jangoux, and P. Flammang. 2005b. Evaluation of the attachment strength of individuals of *Asterina gibbosa* (Asteroidea, Echinodermata) during the perimetamorphic period. *Biofouling* **21:** 229–235.

Hart, M. W., M. Byrne, and M. J. Smith. 1997. Molecular phylogenetic analysis of life-history evolution in asterinid starfish. *Evolution* **51:** 1848–1861

Hart, M. W., S. L. Johnson, J. A. Addison, and M. Byrne. 2004. Strong character incongruence and character choice in phylogeny of sea stars of the Asterinidae. *Invertebr Biol.* **123:** 343–356.

Hermans, C. O. 1983. The duo-gland adhesive system. *Oceanogr. Mar. Biol. Annu. Rev.* **21:** 281–339.

Komatsu, M., Y. T. Kano, H. Yoshizawa, S. Akabane, and C. Oguro. 1979. Reproduction and development of the hermaphroditic sea-star, *Asterina minor* Hayashi *Biol. Bull* **157:** 258–274.

Kubo, K. 1951. Some observations of the development of the sea-star *Leptasterias ochotensis smilispinus* (Clark) *J. Fac. Sci. Hokkaido Univ. Ser. VI Zool.* **10:** 97–105.

Ludwig, H. L. 1882. Entwicklungs Geschichte der *Asterina gibbosa* Forbes *Z. Wiss. Zool.* **37:** 1–98.

MacBride, E. W. 1896. The development of *Asterina gibbosa. Q J. Microsc. Sci.* **38:** 339- 411.

Marthy, H. J. 1980. Etude descriptive du développement de l'oeuf d' *Asterina gibbosa* (Echinoderme, Asteride) et son intérêt en embryologie expérimentale. *Vie Milieu* **30:** 75–80.

McEdward, L. R., and D. A. Janies. 1993. Life cycle evolution in asteroids: What is a larva? *Biol. Bull.* **184:** 255–268.

McEdward, L. R., and D. A. Janies. 1997. Relationships among development, ecology, and morphology in the evolution of echinoderm larvae and life cycles. *Biol. J. Linn Soc.* **60:** 381–400.

McEdward, L. R., and B. G. Miner. 2001. Larval and life cycle patterns in echinoderms *Can. J. Zool.* **79:** 1125–1170

Soliman, E. S., and S. Nojima. 1984. Some observations on dispersal behavior of the early juvenile of the sea star, *Asterina minor. Publ. Amakusa Mar. Biol. Lab. Kyushu Univ.* **7:** 81–93.

Strathmann, R. R. 1978. Larval settlement in echinoderms. Pp. 235–246 in *Settlement and Metamorphosis of Marine Invertebrate Larvae,* F. S. Chia and M. E. Rice, eds. Elsevier, New York.

Waters, J. M., and M. S. Roy. 2004. Out of Africa: the slow train to Australia. *Syst. Biol.* **53:** 18–24.